

Rodrigo de Oliveira Cavagna

AVALIAÇÃO DA FREQUÊNCIA E IMPACTO CLÍNICO DE MUTAÇÕES “DRIVERS” E ACIONÁVEIS EM
CERCA DE 1000 PACIENTES COM CÂNCER DE PULMÃO DE CÉLULAS NÃO PEQUENAS DO
HOSPITAL DE CÂNCER DE BARRETOS (HOSPITAL DE AMOR)

“Tese apresentada à Banca de Defesa ao
Programa de Pós-Graduação da Fundação Pio
XII – Hospital de Câncer de Barretos para
obtenção do Título de Doutor em Ciências da
Saúde”

Área de concentração: Oncologia Molecular
Orientador: Prof. Dr. Rui Manuel Reis
Coorientador: Prof. Dr. Letícia Ferro Leal
Coorientador: Prof. Dr. Miguel Angel Molina-
Vila

Barretos – São Paulo

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LISTA DE ABREVIATURAS

IARC	<i>International Agency for Research on Cancer</i>
INCA	<i>Instituto Nacional do Câncer</i>
CPPC	<i>Câncer de Pulmão de Pequenas Células</i>
CPNPC	<i>Câncer de Pulmão de não Pequenas Células</i>
PET-CT	<i>Positron Emission Tomography–Computed Tomography</i>
AJCC	<i>American Joint Committee on Cancer</i>
PS ECOG	<i>Eastern Cooperative Oncology Group Performance Status</i>
TKI	<i>Tyrosine Kinase Inhibitor</i>
EGFR	<i>Epidermal Growth Factor Receptor</i>
ALK	<i>Anaplastic Lymphoma Kinase</i>
BRAF	<i>V-raf Murine Sarcoma Viral Oncogene Homolog B</i>
MEK	<i>Mitogen-activated protein kinase kinase</i>
ROS1	<i>ROS Proto-Oncogene 1, Receptor Tyrosine Kinase</i>
PD-L1	<i>Programmed death-ligand 1</i>
PD-1	<i>Programmed death 1</i>
TP53	<i>Tumor Protein P53</i>
KRAS	<i>Kirsten Rat Sarcoma Virus</i>
STK11	<i>Serine/Threonine Kinase 11</i>
PIK3CA	<i>Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha</i>
CDKN2A	<i>Cyclin Dependent Kinase Inhibitor 2A</i>
MET	<i>Mesenchymal Epithelial Transition</i>
ERBB2/HER2	<i>erb-b2 receptor tyrosine kinase 2</i>
RET	<i>Rearranged During Transfection</i>
NTRK	<i>Neurotrophic Receptor Tyrosine Kinase</i>
TCGA	<i>The Cancer Genome Atlas</i>
DNA	<i>Deoxyribonucleic acid</i>
AKT1	<i>AKT Serine/Threonine Kinase 1</i>
NRAS	<i>Neuroblastoma Rat Sarcoma Virus</i>
RAS	<i>Rat Sarcoma Virus</i>
FOXL2	<i>Forkhead Box L2</i>

<i>GNA11</i>	<i>G Protein Subunit Alpha 11</i>
<i>GNAQ</i>	<i>G Protein Subunit Alpha Q</i>
<i>PDGFRA</i>	<i>Platelet Derived Growth Factor Receptor Alpha</i>
<i>KIT</i>	<i>KIT Proto-Oncogene, Receptor Tyrosine Kinase</i>
<i>RNA</i>	<i>Ribonucleic acid</i>
<i>WT</i>	<i>Wild-type</i>
<i>SNC</i>	Sistema Nervoso Central

LISTA DE SÍMBOLOS

%	Porcentagem
Δex	<i>Exon Skipping</i>
<	Menor

RESUMO

Cavagna, RO. Avaliação da frequência e impacto clínico de mutações *drivers* e acionáveis em cerca de 1000 pacientes com câncer de pulmão de células não pequenas do Hospital de Câncer de Barretos (Hospital de Amor). **Tese (Doutorado)**. Barretos: Hospital de Câncer de Barretos; 2025.

JUSTIFICATIVA: A identificação de alterações acionáveis em câncer de pulmão é imprescindível para a sua conduta. A presença destas alterações auxilia na determinação da agressividade da doença e seu prognóstico, além de direcionar o tratamento do paciente. Contudo, os dados acerca do perfil mutacional dos pacientes com câncer de pulmão no Brasil ainda são escassos, assim como seu impacto clínico. **OBJETIVO:** Avaliar a frequência e impacto das alterações moleculares (mutações e fusões genicas) “drivers” em cerca de 1000 pacientes com câncer de pulmão de células não pequenas do Hospital de Câncer de Barretos (atual Hospital de Amor).

METODOLOGIA: Foram avaliados pacientes diagnosticados na unidade de Barretos (n=1057) e da unidade de Porto Velho (n=76), além de 60 pacientes do Laboratório Pangaea (Barcelona, Espanha). O painel *TruSight Tumor 15* foi utilizado para análise de mutações, e o painel customizado *nCounter* foi empregado para a detecção de fusões génicas. A determinação da ancestralidade genética foi realizada com um painel de 46 marcadores para as populações europeia, asiática, africana e ameríndia (categorizadas em tercís). Ensaio de viabilidade de celular foi empregado em linhagens para avaliar o papel da sobre-expressão de *NTRK* na resposta ao Entrectinib. Para a associação das características clinicopatológicas com as alterações *drivers*, serão utilizados o teste Exato de Fisher, qui-quadrado e regressão logística, e *Kaplan-Meier* com o teste de *Log-Rank* e a regressão de Cox, para análise de sobrevida.

RESULTADOS: A maioria dos pacientes era originária da região Sudeste (65,2%), e 71,7% apresentavam uma proporção de ancestralidade europeia. Implementamos uma metodologia multiplex de RNA (*nCounter*) para detecção de fusões em *ALK/RET/ROS1/NTRK1/2/3* e *METexΔ14* em amostras parafinadas. No geral, 87% dos pacientes apresentavam pelo menos uma alteração “driver”, sendo o gene *TP53* o mais comumente mutado (57,9%), seguido pelos genes *KRAS* (25,3%), *EGFR* (20,5%), fusões de *ALK* (5,9%), *PIK3CA* (3,3%), *ERBB2* (2,9%), *BRAF* (2,5%) e *METΔex14* (1,4%). Os genes *NRAS*, fusões de *RET*, fusões de *ROS1*, *KIT* e fusões de *NTRK1-3* foram mutados em menos de 1% dos pacientes. Não foram encontradas mutações nos genes *AKT1*, *FOXL2*, *GNA11*, *GNAQ*, *PDGFRA* e *NTRK2*. De forma geral, as fusões foram identificadas em 16% dos pacientes, e as foram associadas a pacientes que nunca fumaram, e no subgrupo que recebeu terapias-alvo apresentou uma sobrevida global mediana melhor em comparação com aqueles que não receberam estas terapias. Evidenciamos que a variante, G12C do gene *KRAS* (alvo do fármaco Sotorasib), estava presente em 9% dos casos, e inserções no exon 20 do gene *ERBB2* (alvo do fármaco (Trastuzumab-Deruxtecan) se encontrou em 0,8% dos casos. Em casos de estadiamento inicial, observamos mutações no *EGFR* em 17,3% dos casos, e a positividade para PD-L1 (36,7%) foi associada ao tabagismo, perda de peso e estágios mais avançados da doença (IIB/IIIA). Quando avaliamos especificamente os pacientes que nunca fumaram (n=119), verificamos um perfil distinto, sendo *EGFR*, o gene mais frequentemente mutado (48,7%), seguido do gene *TP53* (39,5%), *ALK* (10,9%), e *ERBB2* (7,6%). A análise multivariada revelou várias associações independentes: presença de mutações no

TP53 foi associada a homens, fumantes ou ex-fumantes, histologia não adenocarcinoma, metástase no sistema nervoso central (SNC) e baixa ancestralidade europeia; a presença de mutações no *EGFR* foi associada a mulheres, nunca fumantes, metástase no SNC e baixa ancestralidade europeia; a presença de mutações no *KRAS* foi associada a fumantes ou ex-fumantes e histologia de adenocarcinoma; a presença de fusões de *ALK* foi associada a pacientes jovens que nunca fumaram; mutações no *PI3KCA* foram associadas a pacientes idosos e histologia escamosa; e mutações no *BRAF* foram associadas a mulheres. Em um subconjunto de pacientes (n=366), observamos a associação entre a presença de alterações no gene *EGFR* e no gene *TP53*. Além da presença de fusões de *NTRK1,2,3*, avaliamos também a superexpressão de mRNA. Verificamos que a sobreexpressão de *NTRK* (sem fusões) variou entre 6,6 e 10,8% dos casos, e que estes apresentavam um pior prognóstico. Ainda mostramos que linhas celulares (sem fusões) com altos níveis de *NTRK* (>30) apresentaram valores de IC50 significativamente menores para entrectinibe (~1 µM) em modelos 2D e 3D em comparação com aquelas com baixa expressão. **CONCLUSÃO:** O câncer de pulmão em pacientes brasileiros é molecularmente heterogêneo, com 87% apresentando alterações oncogênicas, que variam em função da ancestralidade dos pacientes. *TP53* é o gene mais frequentemente mutado, seguido por *EGFR* (20%, predominante em não fumantes) e *KRAS* (25%, com G12C em 10%). Alterações acionáveis como fusões em *ALK*, *RET*, *ROS1*, *NTRK* e *METΔex14* ocorrem em cerca de 10% dos casos. Os dados *in vitro* sugerem que a sobreexpressão de *NTRK*, mesma na ausência de fusão, é sensível a terapia alvo (anti-*NTRK*). Esse perfil molecular diversificado tem implicações no prognóstico e manejo clínico.

PALAVRAS CHAVE: Câncer de pulmão de não pequenas células; Perfil Molecular; alterações acionáveis; fusões gênicas; patogenicidade, Brasil, miscigenação.

ABSTRACT

Cavagna, RO. Evaluation of the frequency and clinical impact of driver and actionable mutations in approximately 1,000 patients with non-small cell lung cancer from the Barretos Cancer Hospital (Hospital de Amor). **Thesis (PhD's degree)**. Barretos: Barretos Cancer Hospital; 2025.

BACKGROUND: The identification of actionable alterations in lung cancer is crucial for its management. The presence of these alterations aids in determining disease aggressiveness, prognosis, and guiding patient treatment. However, data on the mutational profile of lung cancer patients in Brazil remain scarce, such as the information on their clinical impact.

AIMS: To evaluate the frequency and impact of molecular driver alterations (mutations and gene fusions) in approximately 1,000 patients with non-small cell lung cancer (NSCLC) treated at the Barretos Cancer Hospital (now Hospital de Amor).

METHODOLOGY: Patients diagnosed at the Barretos unit (n=1,057) and Porto Velho unit (n=76), along with 60 patients from the Pangaea Laboratory (Barcelona, Spain), were included. The TruSight Tumor 15 panel was used for mutation analysis, and the custom nCounter panel was employed for gene fusion detection. Genetic ancestry was determined using a panel of 46 markers for European, Asian, African, and Native American populations (categorized into tertiles). Cellular viability assays were performed on cell lines to evaluate the role of *NTRK* overexpression in response to entrectinib. The associations between clinicopathological characteristics and driver alterations were analysed using Fisher's exact test, chi-square, and logistic regression. Survival analysis was performed using Kaplan-Meier with the log-rank test and Cox regression. **RESULTS:** Most patients were from the Southeast region (65.2%), and 71.7% had a predominance of European ancestry. A multiplex RNA methodology (nCounter) was implemented for detecting *ALK/RET/ROS1/NTRK1/2/3* and *METΔex14* fusions in paraffin-embedded samples. Overall, 87% of patients presented at least one driver alteration, with *TP53* being the most frequently mutated gene (57.9%), followed by *KRAS* (25.3%), *EGFR* (20.5%), *ALK* fusions (5.9%), *PIK3CA* (3.3%), *ERBB2* (2.9%), *BRAF* (2.5%), and *METΔex14* (1.4%). Genes such as *NRAS*, *RET* fusions, *ROS1* fusions, *KIT*, and *NTRK1-3* fusions were mutated in less than 1% of patients. No mutations were identified in *AKT1*, *FOXL2*, *GNA11*, *GNAQ*, *PDGFRA*, and *NTRK2*. Fusions were identified in 16% of patients, primarily in non-smokers, and the subgroup receiving targeted therapies had a better median overall survival compared to those who did not. The *KRAS* G12C variant (targeted by sotorasib) was present in 9% of cases, and *ERBB2* exon 20 insertions (targeted by trastuzumab-deruxtecan) in 0.8%. Among early-stage cases, *EGFR* mutations were found in 17.3%, and PD-L1 positivity (36.7%) was associated with smoking, weight loss, and advanced disease stages (IIB/IIIA). In non-smoking patients (n=119), *EGFR* was the most frequently mutated gene (48.7%), followed by *TP53* (39.5%), *ALK* (10.9%), and *ERBB2* (7.6%). Multivariate analysis revealed independent associations: *TP53* mutations were linked to male patients, smokers or former smokers, non-adenocarcinoma histology, central nervous system (CNS) metastases, and lower European ancestry. *EGFR* mutations were associated with female patients, non-smokers, CNS metastases, and lower European ancestry. *KRAS* mutations

were linked to smokers or former smokers and adenocarcinoma histology. *ALK* fusions were associated with younger, non-smoking patients. *PIK3CA* mutations were linked to older patients and squamous histology, while *BRAF* mutations were associated with female patients. In a subset of patients (n=366), an association between *EGFR* and *TP53* alterations was observed. For *NTRK1*, 2, and 3 fusions, mRNA overexpression was also evaluated. *NTRK* overexpression (without fusions) was found in 6.6–10.8% of cases and was associated with worse prognosis. Additionally, cell lines (without fusions) with high *NTRK* expression (>30) exhibited significantly lower IC50 values for entrectinib (~1 µM) in 2D and 3D models compared to those with low expression. **CONCLUSION:** Lung cancer in Brazilian patients is molecularly heterogeneous, with 87% exhibiting oncogenic alterations, which vary based on patient ancestry. *TP53* is the most frequently mutated gene, followed by *EGFR* (20%, predominant in non-smokers) and *KRAS* (25%, with G12C in 10%). Actionable alterations such as *ALK*, *RET*, *ROS1*, *NTRK* fusions, and *MET*Δex14 occur in approximately 10% of cases. In vitro data suggest that *NTRK* overexpression, even in the absence of fusions, is sensitive to targeted therapy (anti-*NTRK*). This diverse molecular profile has implications for prognosis and clinical management.

KEYWORDS: Non-small cell lung cancer; Molecular profile; actionable alterations; gene fusions; pathogenicity; Brazil; mixed populations.

1 INTRODUÇÃO

1.1 Epidemiologia

De acordo com a Agência Internacional de Pesquisa em Câncer (IARC, do inglês *International Agency for Research on Cancer*), a estimativa global para o ano de 2022 foi de 2,5 milhões (12,4%) de novos casos de câncer de pulmão, com cerca de 1,8 milhões (18,7%) de mortes, tornando este o tipo tumoral mais incidente e mortal no mundo (Figura 1)¹.

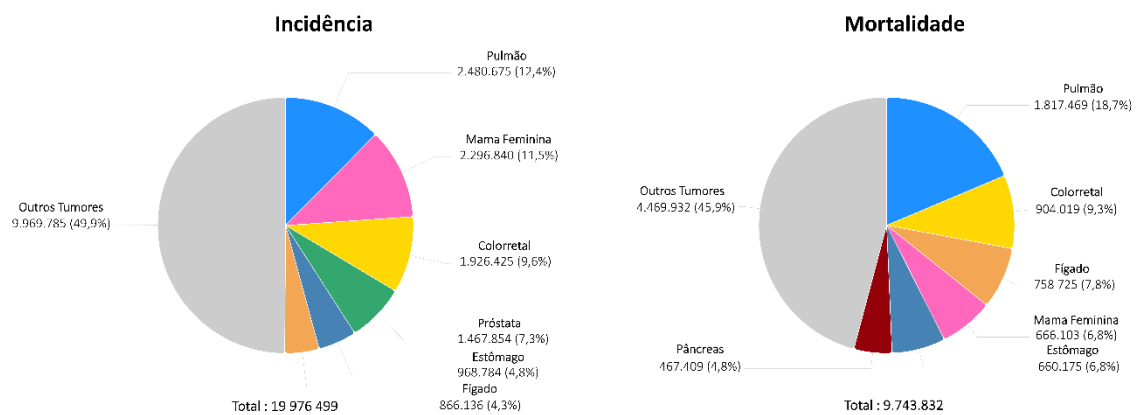


Figura 1 - Incidência e Mortalidade para os tipos de câncer mais incidentes e que levam a óbito globalmente de acordo com o site GLOBOCAN e Bray et al., 20241. Adaptado do site gco.iarc.fr.

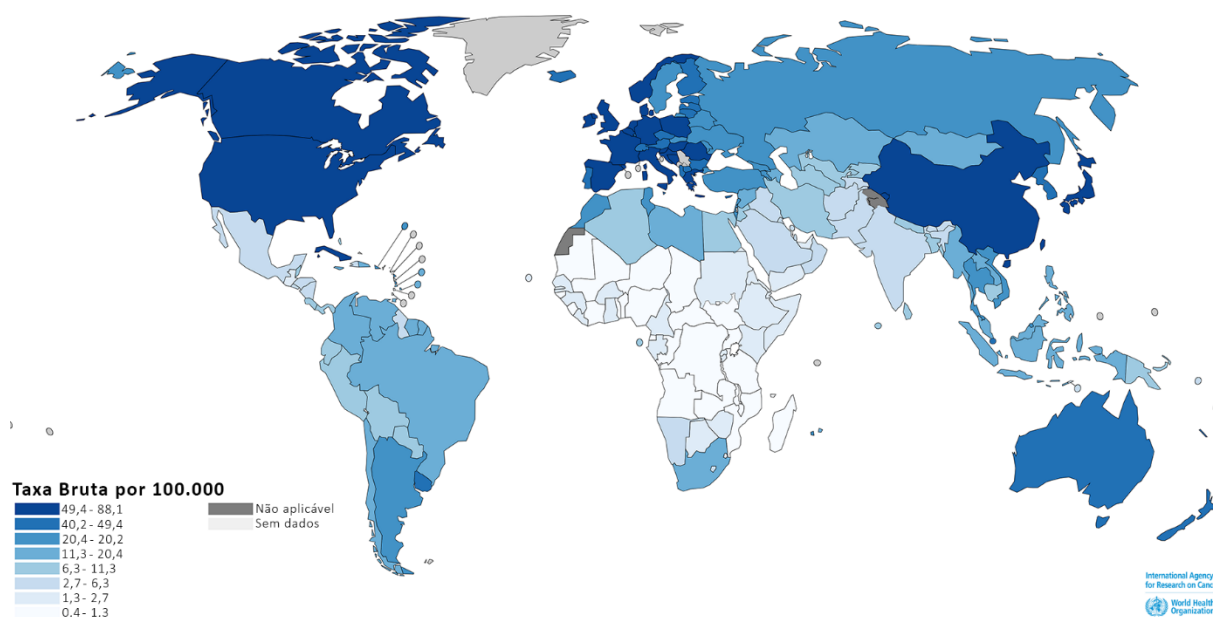
A incidência do câncer de pulmão varia geograficamente de acordo com o histórico de uso de tabaco e qualidade do ar da região, além de fatores como sexo e idade. Pacientes mais velhos tem mais chances de desenvolverem câncer de pulmão por conta da idade mais avançada, sendo 70 anos a idade média dos pacientes diagnosticados com esse tumor. Historicamente, homens são mais propícios a desenvolverem o câncer de pulmão comparado às mulheres, devido maior consumo de tabaco nessa população². Nos países desenvolvidos, a incidência da doença e as taxas de mortalidade são mais altas que nos países em desenvolvimento (Figura 2), reflexo da exposição ao tabaco tanto no passado quanto nos dias atuais^{1,3}.

No Brasil, foi estimado para o triênio de 2023-2025 (Figura 3) cerca de novos 18020 casos de câncer de pulmão para homens e 14540 novos casos para mulheres de acordo com o Instituto Nacional do Câncer (INCA)⁴. De acordo com o Globocan, o câncer de pulmão é o 4º mais incidente e o câncer que mais leva a óbito no Brasil⁵.

No Hospital de Amor de Barretos (antigo Hospital de Câncer de Barretos), o câncer de pulmão foi o terceiro tumor mais frequente em homens e o quarto mais frequente em mulheres no ano de 2022, representando 509 novos casos admitidos em ambos os sexos (https://infogram.com/rhc_hcb). Cerca de 73% dos pacientes admitidos se encontravam em estadiamento IV, sendo mais da metade dos pacientes admitidos sem diagnóstico prévio e sem nenhum tratamento. De acordo com o Departamento de Registro Hospitalar da instituição, somente 1% dos pacientes admitidos em estadiamento IV estarão vivos após 5 anos, e mesmo em estadiamentos mais iniciais, menos de 50% dos pacientes estão vivos após o mesmo período de 5 anos (https://infogram.com/rhc_hcb).

a)

Taxa de incidência por 100.000 habitantes, ambos os sexos, em 2022



b)

Taxa de Mortalidade por 100.000 habitantes, ambos os sexos, em 2022

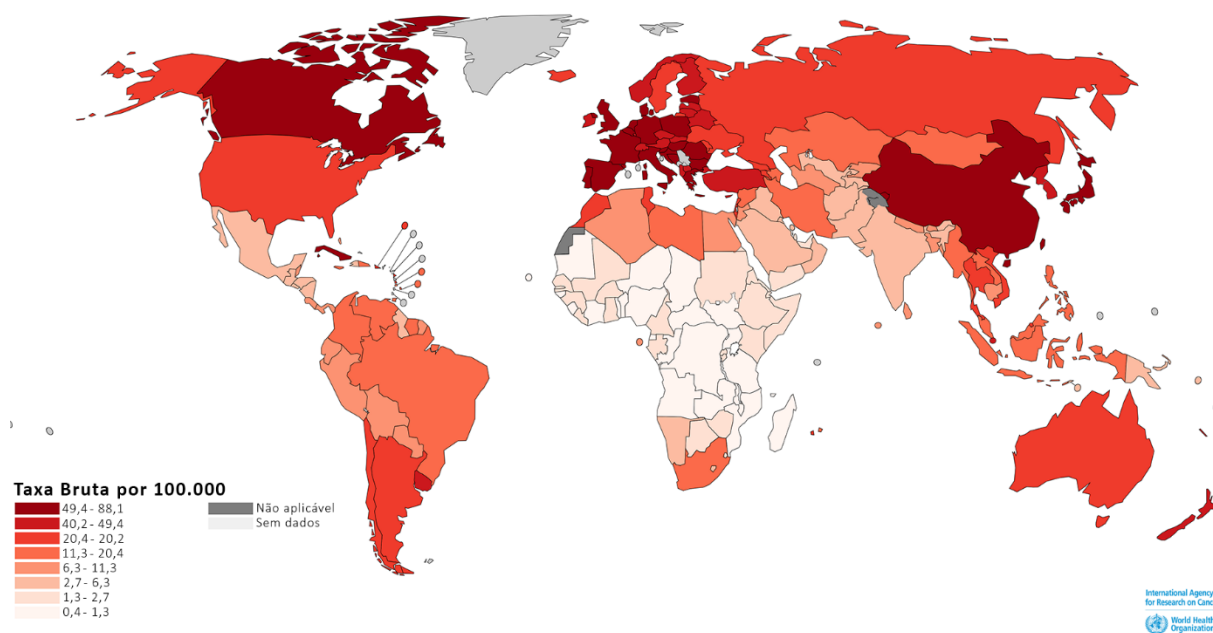


Figura 2 - Taxa de incidência e mortalidade do câncer de pulmão por 100.000 habitantes por país; a) representação da taxa de incidência; b) representação da taxa de mortalidade. **Fonte:** <https://gco.iarc.fr/today/>

Distribuição proporcional dos dez tipos de câncer mais incidentes estimados para 2023 por sexo, exceto pele não melanoma*

Localização Primária	Casos	%	Homens	Mulheres	Localização Primária	Casos	%
Próstata	71.730	30,0%			Mama feminina	73.610	30,1%
Cólon e reto	21.970	9,2%			Cólon e reto	23.660	9,7%
Traqueia, brônquio e pulmão	18.020	7,5%			Colo do útero	17.010	7,0%
Estômago	13.340	5,6%			Traqueia, brônquio e pulmão	14.540	6,0%
Cavidade oral	10.900	4,6%			Glândula tireoide	14.160	5,8%
Esôfago	8.200	3,4%			Estômago	8.140	3,3%
Bexiga	7.870	3,3%			Corpo do útero	7.840	3,2%
Laringe	6.570	2,7%			Ovário	7.310	3,0%
Linfoma não Hodgkin	6.420	2,7%			Pâncreas	5.690	2,3%
Fígado	6.390	2,7%			Linfoma não Hodgkin	5.620	2,3%

*Números arredondados para múltiplos de 10.

Figura 3- Distribuição dos dez tipos de câncer mais incidentes estimados para 2023 por sexo, exceto pele não melanoma⁶.

1.2 Fatores de Risco

O câncer de pulmão se tornou a principal causa de morte por câncer que poderia ser evitada, uma vez que o tabagismo é o principal fator etiológico. Cerca de 85% dos casos de câncer de pulmão ocorrem pacientes tabagistas. O risco de desenvolver câncer de pulmão aumenta de acordo com o número de maços de cigarros por dia e o número de anos que a pessoa fuma. Este risco também é elevado para pessoas que são fumantes passivos, ou seja, que estão em contato com as substâncias liberadas pela fumaça sem serem fumantes, comparado com aquelas pessoas que não são expostas ao tabaco⁷. Além dos cigarros, outros produtos também estão associados ao desenvolvimento do câncer de pulmão como o amianto, cigarros eletrônicos, narguilé, a qualidade do ar e a poluição nele presente, agrotóxicos utilizados em plantações e exposição ao radônio^{2,7}. Estes fatores podem contribuir para o número de casos de câncer de pulmão em pacientes não fumantes, que tem aumentado significativamente.

Além dos riscos supracitados, estima-se que cerca de 18% dos casos de câncer de pulmão tenham um componente hereditário, tendo a variante germinativa R337H no gene *TP53* conhecida como mutação fundadora na população Brasileira, presente em cerca de 5% dos casos de câncer de pulmão no Brasil⁸⁻¹⁰. Além do mais, mutações germinativas no gene *EGFR* tem sido descritas em casos de câncer de pulmão, inclusive a mutação T790M, associada à resistência a TKI, em cerca de 1% dos casos¹¹. A mutação T790M foi descrita como a mutação germinativa mais comum no gene *EGFR* em casos de câncer de pulmão, tendo um provável

efeito fundador na região sudeste dos Estados Unidos há cerca de dois séculos atrás¹². Além da mutação T790M, as mutações germinativas V769M, V834L, G724S e R776H, sendo essa última com alta penetrância relatada em contexto de câncer de pulmão familiar¹².

1.3 Histologia

O câncer de pulmão é classificado histologicamente em dois tipos principais: câncer de pulmão de pequenas células (CPPC), que compreende 10 a 15% dos casos de câncer de pulmão, e câncer de pulmão de não pequenas células (CPNPC), que compreende 80 a 85% dos casos de câncer do pulmão^{13,14}. O CPNPC é classificado de acordo com o tipo de célula que deu origem ao câncer, sendo, classicamente, dividido em três subtipos: adenocarcinoma, carcinoma de células escamosas e carcinoma de grandes células, sendo o adenocarcinoma o subtipo mais comum^{15,16}. De acordo com dados do Hospital de Amor de Barretos, cerca de 46% dos novos casos admitidos são da histologia adenocarcinoma, e cerca de 33% da histologia escamosa¹⁷.

1.3.1. Adenocarcinoma

De acordo com a Organização Mundial de Saúde, o adenocarcinoma de pulmão pode ser dividido entre adenocarcinoma mucinoso e não mucinoso, sendo o primeiro o subtipo mais frequente¹⁶. O adenocarcinoma não mucinoso é caracterizado pela evidência morfológica ou por imuno-histoquímica de diferenciação glandular, podendo apresentar os subtipos lepidico, acinar, papilar, micropapilar e sólido (Figura 4)¹⁶. Por outro lado, o adenocarcinoma invasivo mucinoso apresenta células com morfologia caliciforme ou colunar celular com abundante mucina intracitoplasmática (Figura 4). Outros tipos de adenocarcinoma menos comuns são os coloide, fetal e entérico¹⁶.

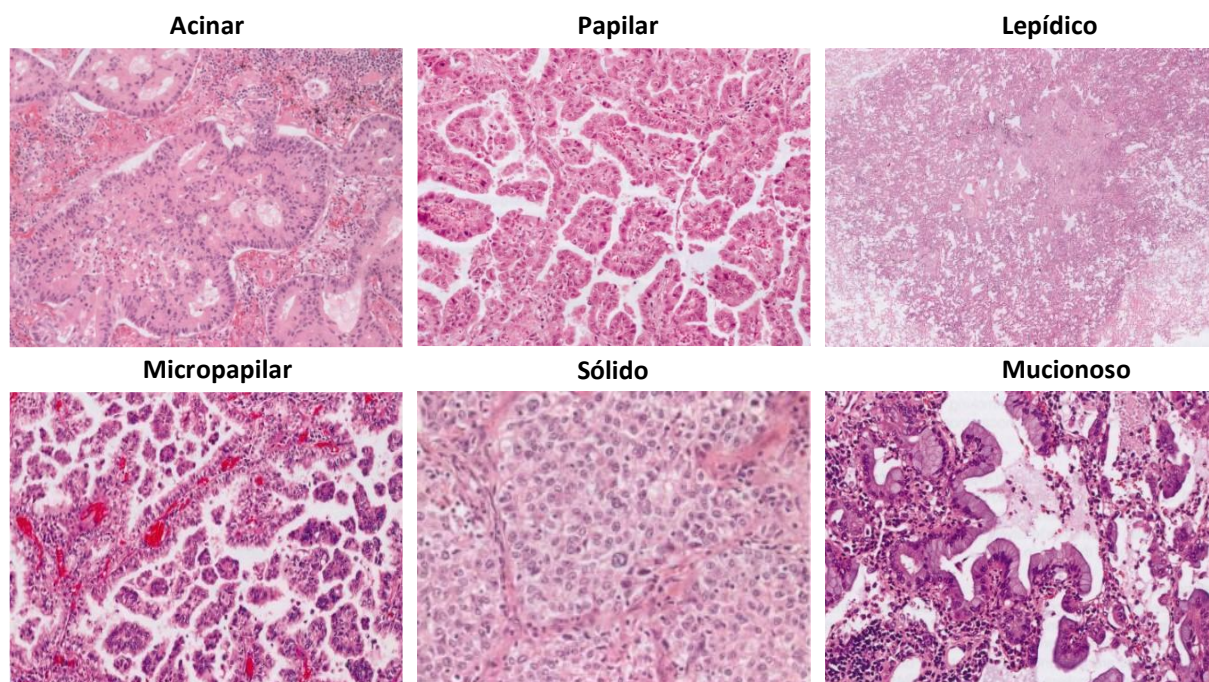


Figura 4- Representação dos subtipos histológicos acinar, papilar, lepidico, micropapilar, sólido e mucinoso. **Fonte:** Thoracic Tumours 5th edition¹⁶

1.3.2. Carcinoma de Células Escamosas

O carcinoma de células escamosas é caracterizado pela presença de queratinização nas células, pontes intracelulares ou por marcadores imuno-histoquímicos de diferenciação celular, além mais frequente em pacientes que são ex-fumantes ou fumantes pesados, representando cerca de 90% casos de carcinoma de células escamosas em pulmão^{16,18}. Pode apresentar os subtipos queratinizante, não-queratinizante e basalóide (Figura 5)¹⁶.

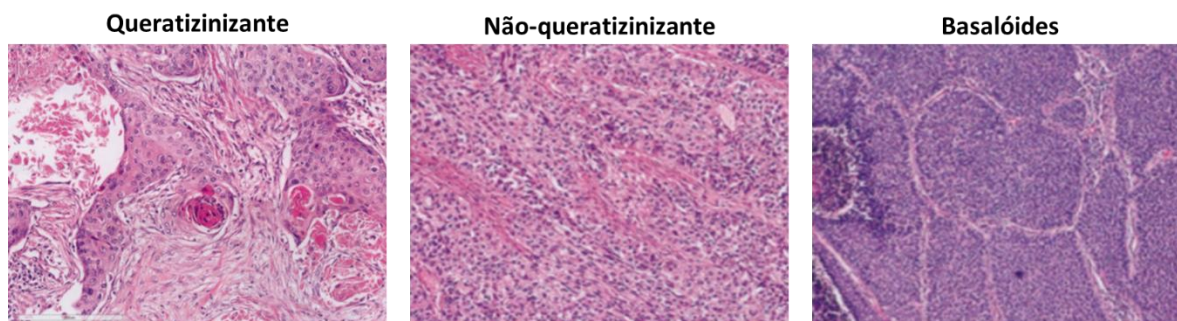


Figura 5- Representação dos subtipos histológicos do câncer escamoso, sendo o queratinizante, não-queratinizante e basalóides. **Fonte:** Thoracic Tumours 5th edition¹⁶

1.3.3. Outros tipos histológicos

Além dos tipos histológicos adenocarcinoma e células escamosas, o câncer de pulmão de não pequenas células também pode apresentar uma mistura de componentes entre esses dois tipos, sendo classificado como adenoescamoso¹⁶. O câncer de pulmão de não pequenas células também pode ser classificado como carcinoma de grandes células, um tumor indiferenciado com falta de características citológicas, imuno-histoquímicas e histoquímicas de pequenas células, adenocarcinoma carcinoma de células escamosas. O diagnóstico para o tipo histológico de grandes células não pode ser feito por biópsias, sendo necessário a avaliação em material proveniente de ressecções cirúrgicas¹⁶. Ademais, este tipo histológico pode apresentar diferenciação neuroendócrina, sendo classificado tumor neuroendócrino de grandes células¹⁶.

1.4 Diagnóstico, Prognóstico e Tratamento

Para diagnóstico de câncer de pulmão, os exames utilizados são: Tomografia computadorizada, broncoscopia e/ou biópsia transtorácica. Para confirmação do diagnóstico, é obrigatória a avaliação patológica, a partir de uma biópsia ou após procedimentos cirúrgicos para retirada de partes ou de todo o tumor. A avaliação microscópica da biópsia ou peça cirúrgica determinará a classificação histológica do tumor, além de auxiliar a determinar a extensão da invasão⁷.

Para o estadiamento da doença, são utilizados o *PET scan* (PET-CT, do inglês, *Positron Emission Tomography–Computed Tomography*) – que permite a avaliação do metabolismo tumoral por captação de glicose podendo identificar possíveis lesões metastáticas – e a ressonância magnética de encéfalo¹⁹. O estadiamento da doença é um importante fator prognóstico para os pacientes com câncer de pulmão. Para o estadiamento do tumor, são seguidas as recomendações da AJCC (do inglês *American Joint Committee on Cancer*), que classifica os tumores em estágios 0, I, II, III e IV de acordo com as características do tumor primário (T), invasão linfonodal (N) e a presença de metástase (M)²⁰.

Menos de um terço dos casos de câncer de pulmão são diagnosticados em estágios iniciais da doença, quando há opções de tratamento curativo^{21,22}. Consequentemente, a taxa de sobrevida em 5 anos para pacientes é extremamente baixa^{23–25}. No estado de São Paulo, de acordo com dados da Secretaria Estadual de Saúde, apenas cerca de 8,8% dos pacientes

diagnosticados com câncer de pulmão eram de estágio I (2000-2005)²⁶. No Hospital de Câncer de Barretos, mais de 70% dos casos admitidos já se encontram em estado avançado da doença, de acordo com os dados da própria instituição, sendo a sobrevida em 5 anos dos pacientes diagnosticados entre os anos 2000 e 2015 com tumores iniciais inferior a 50% e dos pacientes diagnosticados com tumores metastáticos inferior a 2%²⁷.

O diagnóstico precoce da doença, o bom estado clínico do paciente e ser do sexo feminino são considerados fatores prognósticos favoráveis^{28,29}. Outros fatores prognósticos importantes são a idade ao diagnóstico do paciente, a perda de peso e a *performance status* (PS ECOG, do inglês, *Eastern Cooperative Oncology Group Performance Status*)^{30,31}. Pacientes com um PS ECOG mais alto (3 e 4) tem uma pior sobrevida se comparado aos pacientes com PS ECOG 0, 1 e 2 ao diagnóstico, influenciados diretamente por sua saúde já debilitada e muitas vezes sem condições de receber tratamentos mais agressivos como a quimioterapia³². A perda de peso e a perda de massa corporal dos pacientes com câncer de pulmão nos últimos 6 meses antecedendo ao diagnóstico também está associada a uma pior sobrevida destes pacientes^{30,33}.

Há três principais tipos de tratamentos recomendados para pacientes com câncer de pulmão, cirurgia, radioterapia e terapia sistêmica³⁴. De modo geral, a cirurgia é recomendada como opção de tratamento curativo para aqueles pacientes que são diagnosticados em estágios I ou II da doença³⁵. A cirurgia no paciente com câncer de pulmão pode ser dividida em pneumectomia, segmentectomia e lobectomia, a depender da extensão e localização do tumor. Pacientes que são diagnosticados com estágios mais avançado da doença também podem ser recomendados para cirurgia para remoção de linfonodos metastáticos³⁴.

Em relação aos tratamentos não cirúrgicos, a radioterapia é uma das alternativas para o tratamento de doenças localmente avançadas, geralmente combinada com o uso de quimioterapia, como uma terapia definitiva para pacientes com estágios iniciais da doença, como procedimento pré-operatório ou pós-operatório para pacientes indicados para cirurgia, como terapia para recorrências limitadas do tumor ou metástases ou como tratamento paliativo para pacientes com doenças incuráveis³⁴.

A quimioterapia com intenção paliativa, um tipo de terapia sistêmica, é recomendada para pacientes que são diagnosticados com doença avançada (estágio IV) e que possuem um bom *score* de *performance status*. Para estes pacientes, a quimioterapia baseada em platina é benéfica^{34,36-38}. Para pacientes em estágios mais iniciais da doença e que são elegíveis para

cirurgia, o uso de quimioterapia adjuvante pode ser recomendado³⁴. Outra modalidade de tratamento sistêmico para pacientes com câncer de pulmão avançado é a terapia alvo, sendo os inibidores de tirosina quinase (TKIs; do inglês, *Tyrosine Kinase Inhibitors*) o tipo de tratamento mais utilizado para pacientes com alterações genéticas para as quais existem inibidores específicos. O uso da medicina personalizada, principalmente com o uso de TKIs, trouxe um aumento na sobrevida global dos pacientes com adenocarcinoma de pulmão, como, por exemplo, o uso de inibidores anti-EGFR, anti-ALK e, até mesmo, os anti-MEK e anti-BRAF^{34,39}. O tratamento com inibidores é baseado nas alterações moleculares de genes acionáveis, sendo necessária a análise molecular do tumor para o manejo mais apropriado do paciente³⁹.

Mais recentemente, a imunoterapia com inibidores de *checkpoints* imunológicos surge como uma alternativa de tratamento sistêmico importante para pacientes com doença metastática e que não possuem as alterações genéticas em genes como *EGFR*, *BRAF*, *ALK* e *ROS1*⁷. Para indicação de uso da imunoterapia é recomendado determinar o nível de expressão de PD-L1. No caso do tratamento com Pembrolizumab – um inibidor de PD1 - é necessário uma alta expressão de de PD-L1 (superior a 50%)⁴⁰.

As terapias sistêmicas supracitadas, que inclui quimioterapia, imunoterapia e terapia alvo, podem ser administradas no contexto de neoadjuvância antes da cirurgia com o objetivo de reduzir tumores, melhorar os resultados cirúrgicos e aumentar a sobrevida em pacientes com câncer⁴¹. O uso de terapias em neoadjuvância combinadas, como quimioterapia e imunoterapia, melhoram significativamente o prognóstico dos pacientes. Entretanto, toxicidades graves, como neutropenia e pneumonite, e a dificuldade de determinar-se qual paciente terá benefício, são um grande desafio para o uso do tratamento sistêmico neoadjuvante. A elegibilidade para terapia neoadjuvante geralmente se dá em pacientes em estágios I-III ressecáveis e, em tratamentos com terapias alvos, aqueles pacientes que possuem mutações específicas⁴¹.

1.5 Alterações Moleculares

O câncer de pulmão é uma doença geneticamente heterogênea caracterizada uma ampla diversidade de alterações moleculares. Essa heterogeneidade se reflete em variações clínicas e

moleculares que impactam diretamente no prognóstico e tratamento, e, por isso, se faz fundamental a identificação do perfil molecular dos pacientes⁴². É possível distinguir essas alterações moleculares entre duas categorias principais: alterações *drivers* e *passengers*. As mutações *drivers* são aquelas que desempenham um papel direto no desenvolvimento e progressão do câncer, conferindo vantagem seletiva às células tumorais⁴³. Já as mutações *passengers* são consideradas alterações secundárias, que não contribuem diretamente para o crescimento tumoral, mas ocorrem como consequência da instabilidade genômica do câncer⁴³. Dentre as mutações *drivers*, algumas são consideradas acionáveis, ou seja, podem ser alvos de terapias personalizadas. Essas alterações genéticas específicas permitem o desenvolvimento de tratamentos dirigidos, como inibidores de tirosina quinase, que têm revolucionado o manejo de pacientes com câncer de pulmão ao melhorar a resposta ao tratamento e a sobrevida⁴⁴.

Os principais genes *drivers* mais frequentemente mutados no câncer de pulmão são o *TP53* (54%), *KRAS* (23%), *EGFR* (23%), *STK11* (11%), *PIK3CA* (6%), *CDKN2A* (6%) *BRAF* (5%), *MET* (4%), *ERBB2* (3%), além de fusões nos genes *ALK* (3%), *RET* (2%), *ROS1* (1%), *NTRK1,2 e 3* (<1%)⁴⁵. Dentre as mutações *drivers* no câncer de pulmão tem-se as mutações *acionáveis*, principalmente as que são alvos de terapias personalizadas⁴⁶. Essas incluem as alterações nos éxons 18,19,20 e 21 do gene *EGFR* (23%), mutações no gene *BRAF*, especialmente a mutação V600E, e inserções no éxon 20 do gene *ERBB2* (1%)⁴⁷. No gene *KRAS*, apenas a mutação G12C, que ocorre em 9% dos casos, é considerada *acionável*⁴⁸. Além disso, as fusões gênicas, particularmente as envolvendo o gene *ALK* (3%), e as alterações de *METΔex14* (4%) também figuram entre as mutações *drivers* acionáveis, sendo fundamentais para a escolha de terapias específicas e eficazes⁴⁶.

Na rotina clínica do Hospital de Amor de Barretos, os pacientes diagnosticados com câncer de pulmão, principalmente com histologia de adenocarcinoma, são indicados para a realização de testes moleculares pelo Departamento de Diagnóstico Molecular da instituição, onde o *status* mutacional para os genes *EGFR*, *KRAS*, *BRAF* e *ERBB2* são avaliados e disponibilizados para o médico do paciente.

Mutações no gene *EGFR* são utilizadas como marcadores para direcionamento do tratamento com TKIs, tais como erlotinib e gefitinib. Pacientes com adenocarcinoma de pulmão acometidos por estas mutações tem um aumento significativo da sobrevida global após o tratamento com TKI pela eficiência que as drogas tem em inibir o efeito causado pela presença de determinada mutação, agindo como um inibidor/supressor tumoral⁴⁹, fazendo com que

mutações no gene *EGFR* sejam as alterações genéticas com maior relevância clínica⁵⁰. Contudo, algumas mutações, como a *EGFR* p.T790M e as inserções no *exon* 20, estão associadas à resistência para erlotinib e gefitinib^{29,49,51}. Para esses pacientes carreadores da mutação p.T790M é recomendado o uso de Osimertinib para o tratamento³⁴. Os pacientes com doença avançada, com inserções no *exon* 20 identificadas, e com progressão da doença após uso de quimioterapia, podem se beneficiar com uso de Mobocertinib⁵².

Mutações no gene *KRAS* são associadas com um pior prognóstico dos pacientes com adenocarcinoma de pulmão, o que se deve ao fato de serem associadas a tumores com fenótipos mais agressivos^{29,30,50,51}. Apesar do gene *KRAS* ter sido descoberto há mais de 30 anos, nenhuma terapia alvo para estas alterações tem sido aplicada no manejo destes pacientes. Contudo, muitos estudos tem juntado esforços e tentado encontrar formas de tratar os pacientes com mutações no gene *KRAS*^{53–55}. Recentemente, os fármacos Sotorasib e Adagrasib surgiram como alternativa para o tratamento de pacientes carreadores da mutação p.Gly12Cys no gene *KRAS*⁵⁶. Estes inibidores bloqueiam a mutação p.Gly12Cys em um estado inativo, interrompendo os sinais das proteínas mutadas e prevalecendo somente das proteínas inalteradas^{57–59}. Em estudo de fase I, cerca de 1/3 dos pacientes tratados com Sotorasib apresentaram resposta objetiva e cerca 90% apresentaram doença controlada⁶⁰. Com relação ao Adagrasib, em estudos de fase I e II, 94% dos pacientes tratados apresentaram doença controlada⁵⁸. Buscando prever possíveis resistências ao tratamento dos pacientes com as drogas Sotorasib e Adagrasib, um estudo avaliou in vitro possíveis alterações de resistência⁶¹. Foi evidenciado pelo estudo que as mutações Y96D/S conferem resistência tanto ao Sotorasib quanto ao Adagrasib, sendo necessária uma abordagem diferente para os pacientes carreadores da mutação p.Gly12Cys em *KRAS* que também possuam alterações no códon Y96⁶¹.

Mutações no códon V600 do gene *BRAF* são identificadas em cerca de 2% dos casos de adenocarcinoma de pulmão, enquanto mutações fora do códon V600 são identificadas em cerca de 8% dos casos. A presença de mutações V600 no gene *BRAF* possui papel importante no manejo dos pacientes, pois pacientes carreadores dessas mutações são indicados a tratamento com drogas alvo anti-BRAF e anti-MEK^{62,63}.

O gene *ERBB2* (*HER2*) encontra-se mutado em cerca de 2-4% dos casos de câncer de pulmão de não pequenas células e amplificado em cerca de 2% dos casos. Pacientes de câncer de pulmão carreadores de mutações no gene *ERBB2* tendem a um pior prognóstico, especialmente em pacientes diagnosticados em estadiamento avançado, possivelmente pela

resistência ao tratamento com quimioterapia causada pela presença de mutações nesse gene⁶⁴. Ainda não há uma terapia alvo para pacientes carreadores de alterações neste gene. Contudo, ele mostra-se como um potencial alvo terapêutico para o futuro⁶⁵.

O gene supressor tumoral *TP53* é o gene mais comumente mutado em tumores de câncer de pulmão, estando presente em cerca de 46% dos casos de adenocarcinoma de pulmão e 80-90% nos casos de câncer de pulmão de células escamosas de acordo com o TCGA (do inglês, *The Cancer Genome Atlas*)^{63,66}. O impacto clínico nos pacientes com *TP53* mutado não é bem esclarecido em câncer de pulmão. As mutações no gene *TP53* são identificadas ao longo de todas as regiões do gene, sendo o domínio de ligação do DNA o mais mutado^{67,68}. Os efeitos causados pelas diversas mutações no gene *TP53* são variados, desde perda de expressão até ganho de função. Devido aos distintos efeitos funcionais, a categorização das mutações identificadas tem se mostrado necessária para as análises dos impactos causados nos pacientes carreadores de mutações no gene *TP53*⁶⁹⁻⁷¹. Em 2007, Poeta *et al* propuseram classificar as mutações identificadas no gene *TP53* em disruptivas e não disruptivas de acordo com local e a troca de aminoácido^{69,70}. As mutações no gene *TP53* também podem ser classificadas de acordo com seu efeito em truncadas e não-truncadas. Um estudo recente analisou o impacto em pacientes com câncer de pulmão entre as duas formas de classificação dessas mutações, observando que os pacientes com mutações truncadas no gene *TP53* possuem uma pior sobrevida comparado aos pacientes sem mutações e carreadores de mutações não truncadas⁷¹. De igual forma, utilizando a classificação em disruptiva e não disruptiva, os autores também observaram uma pior sobrevida para os pacientes com mutações disruptivas nos tumores mais iniciais⁷¹.

O gene *AKT1* é um oncogene conhecido que promove a mobilidade e proliferação celular, a partir da via de sinalização de *PIK3CA*^{72,73}. De acordo com a literatura, alterações nesse gene são identificadas em diversos tumores sólidos, com uma frequência de cerca de 1% em tumores de pulmão, 8% em tumores de mama, 6% em tumores de colorretal e 5% em tumores de bexiga⁷³.

O gene *PIK3CA* é um gene codificador para a subunidade de proteína catalítica da família da fosfatidilinositol-3 quinase (PI3Ks), via envolvida em vários tipos de tumores⁷⁴. Apesar de bem descrito o papel desse gene em vários tipos tumorais, o papel prognóstico das mutações identificadas ainda é controverso⁷⁵. De acordo com o TCGA, o gene *PIK3CA* encontra-se mutado

em cerca de 7% dos casos de adenocarcinoma de pulmão e em cerca de 6% de todos os casos de câncer de pulmão de não pequenas células pela base de dados do cBioportal⁷⁶.

O gene *NRAS* pertence à família RAS e sua desregulação pode levar a tumorigenese. É frequentemente mutado em tumores sólidos, com uma frequência de 13% a 25% em casos de melanoma, cerca de 10% em casos de leucemia mieloide, 2% a 5% em casos de câncer colorretal, cerca de 1% em carcinomas hepatocelulares, cerca de 6% em tumores de tireoide e em torno de 1% em casos de câncer de pulmão⁷⁷. O códon 61 do gene *NRAS* é o mais frequentemente mutado, representando cerca de 90% das alterações⁷³.

O gene *FOX2L* é membro da classe FOXL de fatores de transcrição, que são essenciais para a diferenciação e função ovariana. De acordo com buscas na base de dados PubMed (pubmed.ncbi.nlm.nih.gov/) utilizando os termos *FOX2L* e *Lung Cancer*, não há muitas informações sobre o papel deste gene nos tumores de pulmão. Contudo, ao se buscar pelo gene *FOX2L* na base de dados do cBioportal (<https://www.cbioportal.org/>), encontra-se uma frequência de cerca de 4% desde gene com alterações (amplificações e mutações)⁷⁸. Outros genes, *GNA11* e *GNAQ* se encontram alterados em cerca de 1% e o gene *PDGFRA* se encontra alterado em cerca de 6% das amostras de pulmão, o gene *KIT* encontra-se mutado em cerca de 2% dos casos presente na base de dados cBioportal⁷⁹.

Fusões gênicas originam-se a partir da união de dois genes por translocações estruturais, possuindo propriedades oncogênicas e podendo agir como alterações *drivers* em diversos tipos de tumores. O papel oncogênico das fusões gênicas pode se dar pela alteração da regulação de um dos genes envolvidos na fusão, geralmente um proto oncogene ligado a um promotor de alta expressão^{80,81}.

Translocações do gene *ALK* são identificadas em cerca de 5% dos casos de adenocarcinoma de pulmão e raramente são identificados em pacientes com câncer de pulmão de células escamosas^{63,82}. Estes pacientes são sensíveis a inibidores anti-ALK, como o crizotinib^{49,83}. Contudo, quando presentes em pacientes com câncer de pulmão de células escamosas, as drogas alvos desenvolvidas para adenocarcinoma de pulmão são ineficazes⁶⁶.

Translocações do gene *ROS1* estão presentes em 1% dos casos de adenocarcinoma de pulmão e estão associadas à pacientes mais jovens e que nunca fumaram. Atualmente, sua importância clínica é devido aos pacientes portadores destas alterações serem tratados com uso de crizotinib, que mostra-se eficaz nestes pacientes com fusões do gene *ROS1*⁶⁵.

Translocações do gene *RET* são identificadas em cerca de 1-2% dos casos de câncer de pulmão de células não pequenas⁸⁴. O uso de TKIs - cabozantinib, vandetanib e lenvatinib - como tratamento para os pacientes com câncer de pulmão de células não pequenas carreadores de translocações no gene *RET* tem apresentado respostas parciais ao tratamento, variando de acordo com o gene parceiro identificado⁸⁵.

Translocações nos três genes da família NTRK (*NTRK1/2/3*) estão envolvidos em diversos tipos de tumores sólidos como glioblastoma pediátrico, câncer colorretal, câncer de pulmão de células não pequenas, dentre outros, e são considerados como alterações importantes no desenvolvimento tumoral⁸⁶. Uso das drogas inibidoras entrectinib e larotrectinib em ensaios clínicos têm demonstrado taxas de respostas satisfatórias, além de aumento na sobrevida dos pacientes, mostrando a potencialidade destas drogas em pacientes carreadores de translocações do genes *NTRK*^{87,88}.

O gene *MET* é um oncogene envolvido na sobrevivência, proliferação, migração e invasão das células cancerígenas. Alterações no exon 14 do gene *MET* são diversas e são identificadas em cerca de 2,7% dos pacientes com câncer de pulmão de não pequenas células nos Estados Unidos, sendo 70% destes pacientes com tumor de adenocarcinoma. O uso de crizotinib é o recomendado para pacientes com alto nível de amplificação no gene *MET*⁸⁹. Para os pacientes carreadores de deleção do exon 14 do gene *MET* (*MET*Δex14) é recomendado o uso de capmatinib e cabozantinib⁹⁰.

1.6 Impacto Clínico do Perfil Molecular

O perfil molecular do tumor dos pacientes com câncer de pulmão de células não pequenas, obtido tanto por amostras de DNA tumoral quando de RNA, tem papel importante na medicina personalizada. A medicina personalizada faz uso de alterações presentes no tumor para individualizar a prevenção, o diagnóstico, prognóstico e tratamento, inserindo os dados do perfil molecular do tumor dentro da tomada de decisão nas práticas médicas⁹¹. As alterações no gene *EGFR* em pacientes com câncer de pulmão de não pequenas células foram as primeiras alterações genéticas com maior relevância clínica com capacidade para mudar o curso da doença⁵⁰. Além das alterações no gene *EGFR*, outras alterações são clinicamente relevantes, como por exemplo as translocações do gene *ALK*, sendo que os pacientes carreadores desta

alteração podem ser beneficiados do uso com Crizotinib (Figura 6)⁹². Nota-se que uma grande parcela das alterações em câncer de pulmão é desconhecida, com provável impacto clínico.

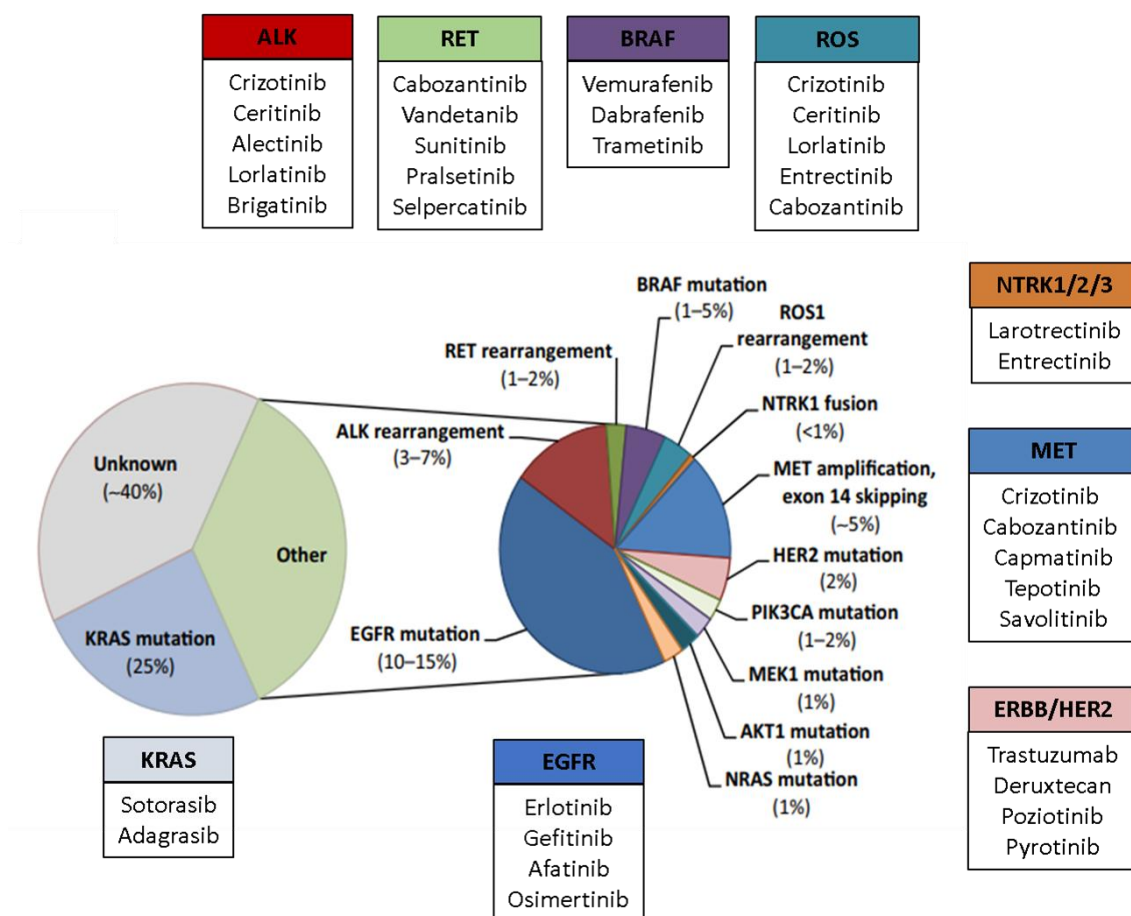


Figura 6 - Alterações moleculares “drivers” e acionáveis mais frequentes em adenocarcinoma de pulmão e principais terapias alvos. Figura adapta de Lin et al, 2016 e Ruiz-Cordero & Devine, 2020^{90,93}.

1.7 Perfil molecular divergente entre populações

A frequência identificada entre os pacientes com CPNPC variam de acordo com a etnia do paciente, como por exemplo as diferentes frequências identificadas para os genes *KRAS* e *EGFR* em pacientes europeus e asiáticos⁹⁴. No Brasil, onde tem-se uma população extremamente miscigenada, pode-se observar associação entre a presença de alterações no gene *EGFR* e pacientes com uma alta ancestralidade genética asiática³⁰. Contudo, no Brasil, as informações referentes a frequência mutacional em câncer de pulmão é escassa, enquanto em outras regiões do mundo temos uma frequência bem descrita (Figura 7)⁹⁴. Recentemente, foi demonstrado em estudos com pacientes do Hospital de Amor de Barretos uma possível associação entre as mutações no gene *KRAS* e os pacientes com ancestralidade nativa

americana⁴⁸. Em outro estudo do grupo, foi demonstrado a associação entre a presença de mutações no *EGFR* e pacientes com alta proporção de ancestralidade asiática³⁰.

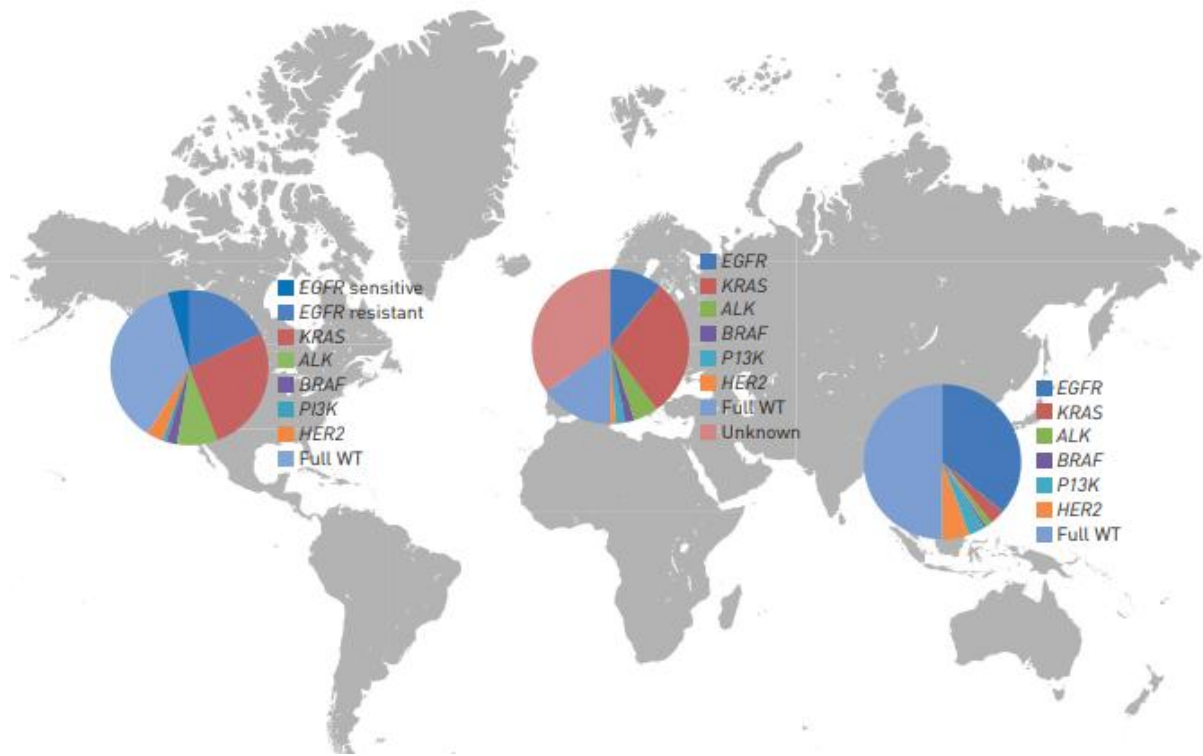


Figura 7 - Distribuição dos principais genes mutados em câncer de pulmão em diversas populações⁹⁴.

2 JUSTIFICATIVA

O perfil molecular dos tumores de câncer de pulmão de não pequenas células tem impacto direto no manejo clínico dos pacientes. As alterações concomitantes mostram-se extremamente importantes no manejo dos pacientes.

Apesar do perfil molecular para os pacientes com câncer de pulmão ser bem elucidado em países da Europa e nos Estados Unidos, boa parte das alterações moleculares ainda são pouco exploradas no Brasil.

No Brasil, há também a particularidade da sua população ser altamente miscigenada, fator que pode alterar a frequência com que determinadas alterações são identificadas. Com base nessas características, torna-se de suma importância a análise de ancestralidade genética dos pacientes brasileiros para associação com as características do perfil molecular.

3 OBJETIVOS

3.1 Objetivo Geral

Avaliar a frequência e impacto das alterações moleculares (mutações e fusões genicas) “drivers” em cerca de 1000 pacientes com câncer de pulmão de células não pequenas tratados no Hospital de Câncer de Barretos.

3.2 Objetivos Específicos

- I. Analisar a frequência de variantes identificadas por painel “targeted” em pacientes com câncer de pulmão de células não pequenas;
- II. Classificar as variantes identificadas com relação a patogenicidade e importância clínica.
- III. Avaliar a presença de translocações dos genes *ALK*, *RET*, *ROS1*, *NTRK1/2/3*, e *METΔex14*.
- IV. Avaliar a frequência e impacto clínico das variantes do gene *TP53*.
- V. Associar o perfil mutacional com a ancestralidade genética dos pacientes.
- VI. Correlacionar os dados moleculares com os dados clínicos e patológicos e o tempo médio de sobrevida dos pacientes com câncer de pulmão de células não pequenas.
- VII. Comparar o perfil molecular entre os pacientes nunca fumantes e os pacientes fumantes.
- VIII. Avaliar *in vitro*, o impacto terapêutico de sobre-expressão de *NTRKs*.

4 MATERIAIS E MÉTODOS

Os materiais e métodos, bem como o delineamento do estudo e informações sobre os pacientes se encontram descritos em seus respectivos capítulos presentes na seção resultado desta tese.

5 RESULTADOS

Os resultados da presente tese foram divididos em subcapítulos, associados aos trabalhos publicados ou em fase final de submissão:

- 1- Caracterização de mutações no gene *TP53* e seu impacto clínico em pacientes brasileiros diagnosticados com adenocarcinoma de pulmão.
- 2- Frequência da mutação p.Gly12Cys no gene *KRAS* em pacientes brasileiros
- 3- Frequência de inserções no éxon 20 do gene *ERBB2* em pacientes brasileiros
- 4- Implementação de nova metodologia de detecção de fusões dos genes *NTRK1,2*, e 3 e sua frequência em pacientes diagnosticados com câncer de pulmão não pequenas células e validação
- 5- Frequência de fusões gênicas e *METΔex14* em pacientes diagnosticados com câncer de pulmão no Hospital de Câncer de Barretos
- 6- Frequência de mutações no gene *EGFR* e expressão de PD-L1 em pacientes com Câncer de Pulmão de não Pequenas Células diagnosticados em estadiamentos iniciais.
- 7- Perfil molecular de pacientes nunca fumantes diagnosticados com adenocarcinoma de pulmão
- 8- Perfil Molecular dos pacientes com Câncer de Pulmão de não Pequenas Células
- 9- Análise funcional da sobre-expressão dos genes *NTRK* em câncer de pulmão e seu impacto resposta a terapia *in vitro*.

5.1 Caracterização de mutações no gene *TP53* e seu impacto clínico em pacientes brasileiros diagnosticados com adenocarcinoma de pulmão.

Este artigo avaliou a frequência de mutações somáticas no gene *TP53*, seu impacto funcional e clínico, além da associação com a ancestralidade genética.

Disruptive and Truncating *TP53* Mutations Are Associated with African-Ancestry and Worse Prognosis in Brazilian Patients with Lung Adenocarcinoma

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Keywords

TP53 · Disruptive/truncating mutations · Lung adenocarcinoma · Brazil · Genetic ancestry

Abstract

Introduction: *TP53* is the most frequently mutated gene in lung tumors, but its prognostic role in admixed populations, such as Brazilians, remains unclear. In this study, we aimed to evaluate the frequency and clinicopathological impact of *TP53* mutations in non-small cell lung cancer (NSCLC) patients in Brazil. **Methods:** We analyzed 446 NSCLC patients from Barretos Cancer Hospital. *TP53* mutational status was evaluated through targeted next-generation sequencing (NGS) and the variants were

biologically classified as disruptive/nondisruptive and as truncating/nontruncating. We also assessed genetic ancestry using 46 ancestry-informative markers. Analysis of lung adenocarcinomas from the cBioportal dataset was performed. We further examined associations of *TP53* mutations with patients' clinicopathological features. **Results:** *TP53* mutations were detected in 64.3% ($n = 287/446$) of NSCLC cases, with a prevalence of 60.4% ($n = 221/366$) in lung adenocarcinomas. *TP53* mutations were associated with brain metastasis at diagnosis, tobacco consumption, and higher African ancestry. Disruptive and truncating mutations were associated with a younger age at diagnosis. Additionally, cBioportal dataset revealed that *TP53* mutations were associated with younger age and Black skin color. Patients harboring disruptive/truncating *TP53*

mutations had worse overall survival than nondisruptive/nontruncating and wild-type patients. **Conclusion:** *TP53* mutations are common in Brazilian lung adenocarcinomas, and their biological characterization as disruptive and truncating mutations is associated with African ancestry and shorter overall survival.

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Introduction

The tumor suppressor gene *TP53* is the most recurrent mutant gene in human malignancies and is known to play a key role in lung tumors [1, 2]. The gene encodes the p53 protein, which is crucial for maintaining general cellular mechanisms, like DNA damage, oncogenic signaling suppressing, and response to cellular injury and stress [3–5]. The p53 protein comprises three major domains: the transactivation domain, DNA binding, and C-terminal [5]. Although the mutations can occur along the whole coding region, they are predominantly found in the DNA-binding domain [5, 6]. While most mutations result in the loss of protein expression, some can lead to gain-of-function mutations, which may contribute to cancer aggressiveness [7].

To better understand the functional effects of amino acid replacements on the p53 protein, various structure-based studies have classified the *TP53* mutations as disruptive and nondisruptive, depending on the location of the variants and the predicted changes in amino acid [8, 9]. A first report on surgically resected head and neck squamous cell carcinoma showed that patients harboring *TP53* mutations presented with shorter overall survival (OS) than wild-type patients, with disruptive mutations being associated with even worse outcomes than nondisruptive mutations and wild-type patients [8]. In contrast, in advanced NSCLC patients, those with nondisruptive *TP53* mutations exhibited a shorter OS than those with disruptive and wild-type patients [10]. Another recent classification categorizes *TP53* mutations as either truncating or nontruncating, with lung cancer patients harboring truncating *TP53* mutations experiencing shorter OS than those with nontruncating and wild-type patients [11].

The frequency and clinical impact of *TP53* mutations in lung cancer patients are scarce in admixed populations, such as Brazil's. Therefore, our goal was to evaluate the prevalence and profile of *TP53* mutations and their association with genetic ancestry, molecular, and clinicopathological features in a cohort of Brazilian NSCLC patients.

Materials and Methods

Study Population

We evaluated a retrospective cohort of 446 Brazilian NSCLC patients who were diagnosed and recommended for routine molecular testing (*EGFR*, *KRAS*, *BRAF*, and *ERBB2*) at Barretos Cancer Hospital (Barretos, São Paulo, Brazil), between 2018 and 2020. The clinicopathological and molecular data were collected from patients' medical records. The Institutional Review Board approved the study protocol (CAAE 05744712.3.0000.5437) and waived written informed consent due to the study's retrospective nature.

DNA Isolation

DNA was isolated from FFPE (formalin-fixed paraffin-embedded) tumor tissue using the commercial kit QIAamp DNA Micro Kit (Qiagen, Hilden, Germany) as described [12]. DNA concentration and purity were evaluated by NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA) and by Qubit 2.0 Fluorometer (Thermo Fisher Scientific) with Qubit dsDNA HS assay kit (Thermo Fisher Scientific).

TP53 Mutational Status

The mutational analysis of the entire *TP53* (NM_000546) coding region was assessed by NGS using the TruSight Tumor 15 panel (Illumina, San Diego, CA, USA) on the MiSeq instrument, according to the manufacturer's instruction as previously described [13]. The read alignment and variant calling were performed with BaseSpace BWA Enrichment version 2.1 (Illumina, San Diego, CA, USA) and Sophia DDM[®] software version 4.2 (Sophia Genetics SA, Saint Sulpice, Switzerland). Variants were filtered out according to the following criteria: intronic (except splicing variants), synonymous single nucleotide variants (SNVs), populational frequency >1%, poorly read depth <×500, allele frequency <5%, and nonpathogenic variants according to Sophia DDM[®]. All retained variants were manually reannotated using the online tool wANNOVAR-<https://wannovar.wglab.org/> [14].

Classification of *TP53* Variants

The *TP53* mutations were further biologically grouped as disruptive and nondisruptive mutations and as truncating and nontruncating mutations as reported [8, 10, 11].

Genetic Ancestry Evaluation

The genetic ancestry background was performed in a subset of patients ($n = 312$) using a set of 46 ancestry-informative markers, INDELs, selected based on their high information content for European (EUR), African (AFR), Asian (ASN), and Native American (AME) population groups as described [12].

In silico Analysis

We obtained the *TP53* mutational status and clinical data for lung adenocarcinoma patients from the publicly available cBioportal online database [15, 16, 19]. We selected only the lung adenocarcinoma cases ($n = 3,750$) using the cBioportal online filters, excluding entries with no self-declared skin color data and manually checked and removed any duplicate entries, leading to 547 lung adenocarcinoma cases included for statistical analysis.

Statistical Analysis

For statistical analysis, the percentage was used to describe categorical variables, and medians were used to describe continuous variables. As previously described, individual ancestry proportions were defined as categorical variables and divided into tertiles (low, intermedium, and high) [17]. For univariate analysis, the Fisher's exact test, χ^2 test, and multinomial logistic regression were used for the association analysis between the presence of *TP53* mutations and the clinicopathological and molecular data. The multinomial logistic regression test was used to investigate the independent association of clinicopathological and molecular data with the *TP53* mutational status. The log-rank test and the Kaplan-Meier curves were used to analyze patients' OS. The Cox regression method was used to investigate the association of clinicopathological data to the outcome (death). All tests were made in the software IBM SPSS Statistics Version 22 (IBM, Armonk, Nova York, USA) with a limit of statistical significance of 0.05.

Results

Characterization of Patients' Clinicopathological Features

We evaluated a series of 446 NSCLC patients (Table 1). Of them, 366/446 (82.0%) were lung adenocarcinoma, 22/446 (5.0%) were squamous cell carcinomas, and 58/446 (13.0%) had other NSCLC histologies, including not otherwise specified, adenosquamous, neuroendocrine, and large cell. Adenocarcinomas showed *TP53* variants in 60.4% of cases, while squamous cell carcinomas and other NSCLC histologies showed *TP53* mutations in 81.8% and 82.8% of cases, respectively (Table 1).

Since adenocarcinomas constituted the majority of cases, we further evaluated this histological subtype for the role of *TP53* and genetic ancestry. The cohort's median age was 64.0 years, 53.0% ($n = 192/366$) were male, and 59.6% ($n = 218/366$) were of self-reported white skin color. Concerning tobacco consumption, 70.2% ($n = 257/366$) were patients who were current or former smokers, and 12.6% ($n = 46/366$) were diagnosed with Eastern Cooperative Oncology Group performance status (ECOG PS) 3–4. Finally, 69.7% ($n = 255/366$) were diagnosed in an advanced stage of the disease, and from these patients, 23.5% ($n = 86/366$) were diagnosed with CNS metastasis (Table 1).

TP53 Status and Molecular Analysis

Of the 366 lung adenocarcinoma patients, 221 (60.4%) had *TP53* mutations, including 201 with one variant and 20 with two or more variants (see online suppl. Tables 1, 2; for all online suppl. material, see www.karger.com/doi/10.1159/000530587). A total of 249 *TP53* mutations were

identified, with the majority (78.2%) located in the p53 DNA-binding domain. The most frequently mutated codon was 273 (5.6%, 14/249), followed by codon 337 (4.8%, 12/249). Then, we compared the identified *TP53* mutations in our series with the IARC *TP53* database and found 8.8% ($n = 22/249$) of unreported variants (Fig. 1b; online suppl. Tables 1, 2).

We further classified the *TP53* status as disruptive mutations (26.2%; $n = 96/366$), nondisruptive mutations (34.2%; $n = 125/366$), and wild-type (39.6%; $n = 145/366$; online suppl. Table 2). Additionally, we also classified the *TP53* status as truncating mutations (17.2%; $n = 63/366$), nontruncating mutations (43.2%; $n = 158/366$), and wild-type (39.6%; $n = 145/366$; online suppl. Table 2).

The mutational status of *TP53* was associated with mutations in the *EGFR* and *KRAS* genes. Among the 366 adenocarcinomas evaluated, 14.5% ($n = 53/366$) were mutant *TP53/EGFR* patients, 14.8% ($n = 54/366$) were mutant *TP53/KRAS* patients, and 31.1% ($n = 114/366$) exhibited only *TP53* mutation(s) (Fig. 1a). We evaluated the genetic ancestry in a subset of 312 patients with lung adenocarcinoma and found the following proportions: 71.4% EUR, 14.6% AFR, 6.4% ASN, and 7.6% AME (Fig. 2).

Association of *TP53* and Adenocarcinoma Patients' Ancestry and Clinicopathological Features

TP53 mutational status was further associated with adenocarcinoma patients' clinicopathological features (online suppl. Table 3; Fig. 1). Univariate analysis revealed that disruptive mutations were associated with younger patients, stage IV disease, and worse performance status at diagnosis. Moreover, both nondisruptive and disruptive mutations were associated with patients who were former or current smokers, as well as those with CNS metastasis at diagnosis (online suppl. Table 3; Fig. 1). The disruptive mutations were also associated with the solid subtype adenocarcinoma, lower EUR, higher AFR, high ASN, and higher AME ancestry proportions. No association was observed between *TP53* mutations and *EGFR* and *KRAS* mutation status (online suppl. Table 3; Fig. 1). Concerning the classification of *TP53* mutations in truncating and nontruncating, we observed that the truncating *TP53* mutations were associated with younger age at diagnosis, loss of weight $\leq 10\%$ prior 6 months to diagnosis, and CNS metastasis at diagnosis (online suppl. Table 3; Fig. 1). Both truncating and nontruncating *TP53* mutations were associated with current and former smokers, worse performance status, and higher AFR and AME ancestry proportions (online suppl. Table 3; Fig. 1).

Table 1. Clinicopathological and molecular description of Brazilian NSCLC patients

Characteristics	Parameters	NSCLC (n = 446)					
		adenocarcinoma (n = 366)		squamous cell (n = 22)		others* (n = 58)	
Age**	Median (range)	64.0	(27–88)	62.5	(39–77)	65.0	(31–94)
	≤64 years	192	(52.5)	14	(63.6)	27	(46.6)
	>64 years	174	(47.5)	8	(36.4)	31	(53.4)
Sex	Female	172	(47.0)	5	(22.7)	20	(34.5)
	Male	192	(53.0)	17	(77.3)	38	(65.5)
Self-reported skin color	White	218	(59.6)	18	(81.8)	36	(62.0)
	Nonwhite	77	(21.0)	2	(9.1)	11	(19.0)
	Missing	71	(19.4)	2	(9.1)	11	(19.0)
Smoking status	Never	103	(28.2)	6	(27.2)	7	(12.1)
	Quitter	120	(32.8)	8	(36.4)	19	(32.8)
	Current	137	(37.4)	8	(36.4)	25	(43.0)
	Missing	6	(1.6)	0	(0.0)	7	(12.1)
Disease stage at diagnosis***	I/II	50	(13.7)	11	(50.0)	2	(3.4)
	III	47	(12.8)	7	(31.8)	9	(15.5)
	IV	255	(69.7)	4	(18.2)	42	(72.5)
	Missing	14	(3.8)	0	(0.0)	5	(8.6)
ECOG PS	0	59	(16.1)	5	(22.7)	5	(8.6)
	1	144	(39.3)	13	(59.1)	19	(32.8)
	2	73	(20.0)	0	(0.0)	13	(22.4)
	3/4	46	(12.6)	0	(0.0)	7	(12.1)
	Missing	44	(12.0)	4	(18.2)	14	(24.1)
Metastasis at diagnosis	No	97	(26.5)	18	(81.8)	11	(19.0)
	Yes, CNS	86	(23.5)	0	(0.0)	15	(25.9)
	Yes, others	161	(44.0)	3	(13.6)	26	(44.8)
	Missing	22	(6.0)	1	(4.5)	6	(10.3)
EGFR status	Wild-type	277	(75.7)	18	(81.8)	51	(87.9)
	Mutant	83	(22.7)	0	(0.0)	7	(12.1)
	Missing	6	(1.6)	4	(18.2)	0	(0.0)
KRAS status ^a	Wild-type	260	(71.0)	10	(45.5)	46	(79.3)
	Mutant	97	(26.5)	2	(9.0)	12	(20.7)
	Missing	9	(2.5)	10	(45.5)	0	(0.0)
TP53 status	Wild-type	145	(39.6)	4	(18.2)	10	(17.2)
	Mutant	221	(60.4)	18	(81.8)	48	(82.8)

n, number of patients. Figures are numbers with percentages in parentheses unless indicated otherwise.

*Adenosquamous carcinoma, NOS (not otherwise specified), large cells, and neuroendocrine carcinoma.

**Age was categorized into two groups considering the median age of the entire series as the cutoff.

***According to AJCC 7th edition. ^aPreviously reported [17].

Additionally, we evaluated the independent association between *TP53* mutations and patients' clinical and molecular features (Table 2). The multinomial logistic regression analysis revealed an independent association of disruptive and truncating mutations with younger patients at diagnosis, CNS metastasis at diagnosis, and higher proportions

of AFR ancestry. Furthermore, disruptive mutations were independently associated with current smokers, and truncating mutations were independently associated with high AME ancestry proportions (Table 2). On the other hand, the nondisruptive and nontruncating mutations had an independent association with patients who were former

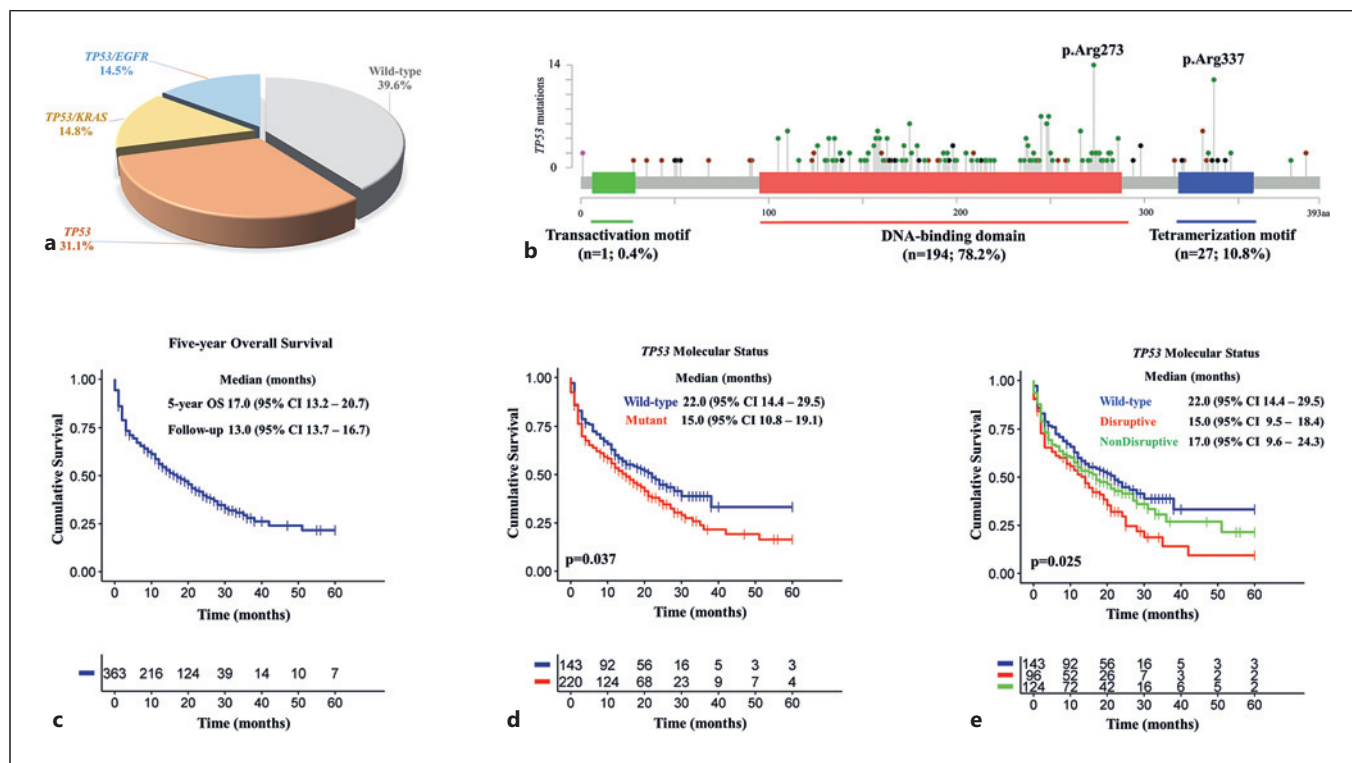


Fig. 1. **a** Frequency of *TP53* mutations and co-occurrence with *EGFR* and *KRAS* mutations in the lung adenocarcinoma samples included in the study ($n = 366$). **b** *TP53* mutations ($n = 249$) identified in lung adenocarcinomas. Mutations are represented by color as follows: missense ($n = 176$) as green; nonsense ($n = 30$) as black; INDELs (insertion or deletions; $n = 32$) as red; and start loss ($n = 2$) as purple.

Splicing mutations (p.?) ($n = 9$) are not represented. Figure constructed with Mutation Mapper from cBioportal – cbioportal.org. **c** Five-year OS of lung adenocarcinoma patients ($n = 366$). **d** Five-year OS of mutant *TP53* patients versus wild-type *TP53* patients. **e** Five-year OS of *TP53* disruptive patients versus *TP53* nondisruptive patients versus wild-type patients.

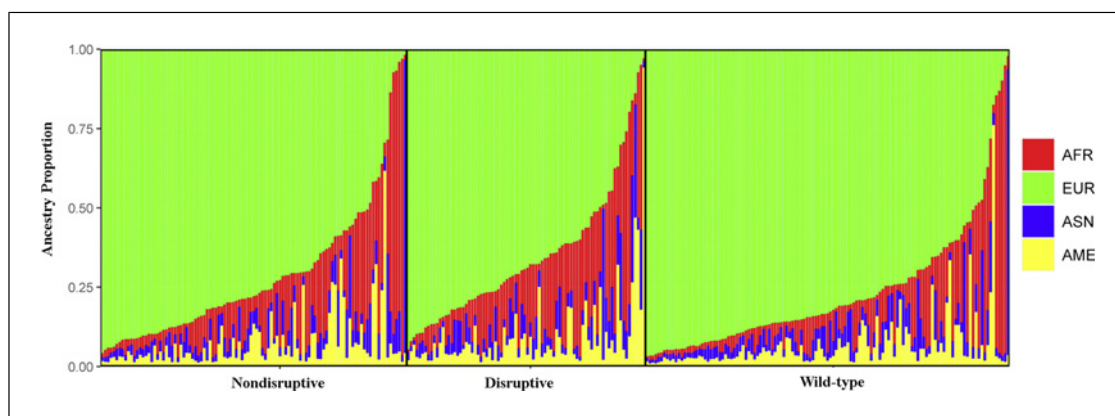


Fig. 2. Genetic ancestry proportions from lung adenocarcinomas ($n = 312$). Figure shows the ancestry proportions divided as nondisruptive, disruptive, and wild type in the x axis and the ancestry proportion of the populations for each individual patient in the y axis.

Table 2. Multinomial logistic regression analysis of *TP53* mutations

Characteristics	Nondisruptive <i>TP53</i> mutations				Disruptive <i>TP53</i> mutations				Nontruncating <i>TP53</i> mutations				Truncating <i>TP53</i> mutations			
	OR		95% CI		p value		OR		95% CI		p value		OR		95% CI	
	lower	upper	lower	upper	lower	upper	lower	upper	lower	upper	lower	upper	lower	upper	lower	upper
Age*																
≤64 years	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
>64 years	0.699	0.386	1.263	0.235	0.235	0.429	0.223	0.825	0.011	0.878	0.502	1.537	0.649	Ref	0.110	0.572
Smoking																
Never	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Quitter	2.862	1.346	6.087	0.006	0.051	2.343	0.996	5.509	0.051	2.173	1.101	4.290	0.025	1.940	0.751	5.009
Current	3.380	1.545	7.396	0.002	0.028	2.618	1.110	6.175	0.028	2.762	1.394	5.474	0.004	2.127	0.855	5.290
Metastasis at diagnosis																
No/others	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
CNS ¹	2.292	1.115	4.708	0.024	0.006	2.876	1.358	6.089	0.006	2.252	1.140	4.447	0.019	3.207	1.302	7.038
African ancestry																
Low	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Intermediate	1.977	0.996	3.922	0.051	0.038	2.386	1.049	5.426	0.038	2.379	1.242	4.559	0.009	1.894	0.730	4.912
High	2.165	1.037	4.518	0.040	<0.0001	5.202	2.303	11.753	<0.0001	3.688	1.860	7.313	<0.0001	3.811	1.499	9.685
Native American ancestry**																
Low	-	-	-	-	-	-	-	-	-	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Intermediate	-	-	-	-	-	-	-	-	-	0.750	0.396	1.244	0.378	1.940	0.740	5.086
High	-	-	-	-	-	-	-	-	-	1.436	0.731	2.822	0.293	3.596	1.366	9.464
KRAS status***																
Wild-type	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	-	-	-	-	-	-	-
Mutant	0.422	0.209	0.852	0.016	0.727	0.876	0.418	1.836	0.727	-	-	-	-	-	-	-

n, number of patients; OR, odds ratio; 95% CI, 95% confidence interval; p value, significance of multinomial regression logistic test; Ref, reference group (TP53 wild-type patients); CNS, central nervous system. Significant associations are indicated in bold. *Age was categorized into two groups considering the average age of the entire series as the cutoff. **Native American ancestry was significant only for truncating classification. ***KRAS status was significant only for disruptive/nondisruptive classification.

Table 3. Logistic regression analysis of *TP53* mutations from cBioportal online dataset ($n = 547$)

Characteristics	Parameters	<i>TP53</i> mutations			
		OR	95% CI		<i>p</i> value
			lower	upper	
Age	≤64 years	Ref	Ref	Ref	Ref
	>64 years	0.592	0.40	0.86	0.007
Self-reported skin color	White	Ref	Ref	Ref	Ref
	Black/African	1.89	1.02	3.52	0.042
	Other	0.78	0.20	3.01	0.788

n, number of patients; OR, odds ratio; 95% CI, 95% confidence intervals; *p* value, significance of regression logistic test; Ref, reference group. Age was categorized into two groups considering the average age of the entire series as the cutoff. Significant associations are indicated in bold.

and current smokers, CNS metastasis at diagnosis, and higher proportions of AFR ancestry. Additionally, non-disruptive *TP53* mutations were associated with wild-type *KRAS* mutational status (Table 2).

To validate our findings in an independent cohort, we used 547 lung adenocarcinoma patients with self-declared race and *TP53* status from the public cBioportal database (Table 3). The univariate analysis showed an association of *TP53* mutations with younger patients (online suppl. Table 4). The multivariate analysis showed an independent association of *TP53* mutations with Black/African patients and younger age at diagnosis (Table 3).

Association of TP53 Mutations and Adenocarcinoma Patients' Survival

Next, we evaluated the clinical and molecular features associated with patients' survival (Table 4). The median 5-year OS (Fig. 1c) in our cohort of lung adenocarcinoma patients was 17 months (95% CI: 13.2–20.7 months), and the median follow-up was 13 months (95% CI: 13.7–16.7). Univariate analysis revealed several clinical features associated with patients' outcomes, such as weight loss before 6 months to diagnosis, performance status, disease stage of diagnosis, metastasis at diagnosis, *EGFR*, and *TP53* mutational status (Table 4). The mutant *TP53* patients had worse OS compared with wild-type patients ($p = 0.037$; Fig. 1d), and among the *TP53* classification groups, the disruptive group had worse OS compared with the wild-type and nondisruptive groups ($p = 0.025$; 15.0 months, 95% CI: 9.5–18.4, 22.0 months, 95% CI: 14.4–29.5, and 17.0 months, 95% CI: 9.6–24.3, respectively; Fig. 1e). Moreover, patients harboring truncating *TP53* mutations had worse OS compared with wild-type patients and patients harboring nontruncating mutations ($p = 0.038$; 14.0 months, 95%

CI: 9.3–18.6, 22.0 months, 95% CI: 14.4–29.5, 17.0 months, 95% CI: 11.2–22.71, respectively; online suppl. Fig. 2). The multivariate Cox regression analysis showed that only worse performance status ($p < 0.0001$), the presence of CNS metastasis at diagnosis ($p < 0.0001$), and *EGFR* wild-type patients ($p < 0.0001$) were independently associated with a higher risk of death (Table 4).

Discussion

In the present study, we addressed the frequency and role of *TP53* mutations in Brazilian NSCLC patients. *TP53* mutations were present in 60% of adenocarcinomas and >80% of other histologies. The functional classification revealed the association of *TP53* disruptive and truncating mutations with tobacco exposure, higher African genetic ancestry, younger age, and CNS metastasis at diagnosis. We also showed that *TP53* disruptive and truncating mutations confer a worse OS for lung adenocarcinoma patients.

Worldwide, the frequency of *TP53* mutations in lung adenocarcinoma tumors ranges from 29% to 60%, possibly due to variations in methodology, histological subtypes, and ethnic populations [1, 11, 18, 20–29]. In 2014, the TCGA reported that about 45% of lung adenocarcinoma patients harbor *TP53* mutations [1]. A study with lung adenocarcinoma patients from North America and Germany reported that 50% of patients were harboring *TP53* mutations [28]. Recently, two studies reported that 36.0% of East Asian and American NSCLC patients harbor *TP53* mutations [21, 22]. In Brazil, Mascarenhas and colleagues reported 53.6% ($n = 275/513$) of NSCLC patients harboring *TP53* mutations [23], similar to our study. The frequency of *TP53* mutations in Brazilian NSCLC patients seems higher

Table 4. Prognostic factors for OS for lung adenocarcinoma patients

Characteristics	Parameters	n	Univariable analysis				Multivariable analysis		
			event, n	time, months		p value	HR	95% CI	p value
				median	95% CI ¹				
Age*	≤64 years	191	119	17.00	12.24–21.74	0.652	–	–	–
	>64 years	172	102	17.00	12.04–21.95				
Sex	Female	170	99	21.00	16.52–25.47	0.085	–	–	–
	Male	193	122	21.00	7.37–16.62				
Self-declared race	White	218	134	17.00	12.90–21.10	0.248	–	–	–
	Nonwhite	76	51	11.00	0.14–21.85				
Smoking status	Never	102	60	22.00	19.42–24.57	0.285	–	–	–
	Former	119	84	13.00	8.22–17.77				
	Current	136	73	12.00	8.26–15.74				
Loss of weight**	No	114	45	36.00	19.04–52.95	<0.0001	–	–	–
	Yes, ≤10	103	76	11.00	6.24–15.76				
	Yes, >10	75	53	9.00	4.95–13.04				
ECOG PS	0	57	9	–	–	<0.0001	Ref	Ref	Ref
	1	144	92	17.00	11.52–22.47		4.89	2.39–9.99	<0.0001
	2	73	19	6.00	2.80–9.19		7.25	3.44–15.28	<0.0001
	3/4	45	7	2.00	0.403–3.597		9.54	4.38–20.78	<0.0001
Stage at diagnosis***	I/II	49	6	60.00	9.90–110.09	<0.0001	–	–	–
	III	47	20	30.00	10.23–49.76				
	IV	254	184	12.00	9.24–14.75				
Metastasis at diagnosis	No	96	26	60.00	38.49–81.50	<0.0001	Ref	Ref	Ref
	CNS	85	68	8.00	4.51–11.48		4.52	2.68–7.63	<0.0001
	Others	161	112	13.00	9.35–16.64		3.28	2.05–5.26	<0.0001
Asian ancestry	Low	105	54	23.00	13.78–32.21	0.086	–	–	–
	Intermedium	102	66	15.00	9.63–20.36				
	High	102	64	12.00	6.48–17.51				
African ancestry	Low	106	59	17.00	7.13–26.86	0.113	–	–	–
	Intermedium	102	55	21.00	13.68–28.31				
	High	101	70	13.00	9.02–16.97				
European ancestry	Low	102	65	12.00	5.45–18.54	0.132	–	–	–
	Intermedium	103	64	16.00	10.53–21.46				
	High	104	55	22.00	10.74–33.25				
Native American ancestry	Low	107	60	21.00	12.18–29.81	0.216	–	–	–
	Intermedium	104	67	13.00	8.38–17.61				
	High	98	57	21.00	12.35–29.64				
KRAS status ^a	Wild-type	258	158	19.00	14.54–23.45	0.104	–	–	–
	Mutant	97	58	10.00	2.92–17.07				
EGFR status	Wild-type	276	172	12.00	9.21–14.78	0.009	Ref	Ref	Ref
	Mutant	82	45	24.00	20.60–27.39				

Table 4 (continued)

Characteristics	Parameters	<i>n</i>	Univariable analysis				Multivariable analysis		
			event, <i>n</i>	time, months		<i>p</i> value	HR	95% CI	<i>p</i> value
				median	95% CI ¹				
<i>TP53</i> status	Wild-type	143	77	22.00	14.48–29.51	0.025	Ref	Ref	Ref
	Disruptive	96	68	14.00	9.50–18.49		1.147	0.79–1.66	0.471
	Nondisruptive	124	76	17.00	9.68–24.31		1.090	0.75–1.57	0.644
	Wild-type	143	76	22.00	14.48–29.51	0.038	Ref	Ref	Ref
	Nontruncating	157	97	17.00	11.28–22.71		1.125	0.802–1.578	0.601
	Truncating	63	46	14.00	9.30–18.69		1.121	0.730–1.722	0.495

Univariable analysis was performed using the Kaplan-Meier plot and the log-rank test. Multivariable analysis was performed using the Cox regression test, and only variables with $p \leq 0.2$ in the univariable analysis and statistically significant in the final model were described. Apart from *TP53*, the multivariable analysis only retained prognostic factors significant at 0.05 level. *n*, number of patients; HR, hazard ratio; 95% CI, 95% confidence interval; *p* value, significance of Kaplan-Meier plot and log-rank test for univariable analysis and significance of Cox regression test for multivariable analysis; Ref, reference group. *Age was categorized into two groups considering the average age of the entire series as the cutoff. **Loss of weight in percentage of total body weight. ***According to AJCC 7th edition; ECOG PS, Eastern Cooperative Oncology Group performance status; CNS, central nervous system. Significant associations are indicated in bold. ¹Previously reported [17].

than in other populations. This fact can be due to Brazilian ethnicity. Saulsberry et al. [30] described that genetic ancestry is an indicator of the inheritance of DNA. Evaluating genetic ancestry background in admixture populations is crucial for stratifying populational groups and a better understanding of genetic diversity [30].

Our study evaluated the genetic ancestry of a Brazilian series of NSCLC cases and identified that *TP53* mutation frequency was significantly higher in patients with African ancestry. Our in silico analysis corroborated this observation, which showed that *TP53* mutations were associated with African-Americans compared with white patients. Similar to our results, a study reported that rates of *TP53* mutations are significantly higher in African American (89.0%; $n = 16/17$) than in Caucasian (56.0%; $n = 10/18$), Hispanic (43.0%; $n = 9/21$), and Asian (40.0%; $n = 25/62$) gastric patients [31]. Therefore, the distinct ethnicity can justify, at least in part, the higher frequency of *TP53* mutations in the Brazilian population of NSCLC patients. Nevertheless, other studies addressed this issue, and no association between *TP53* mutations and ancestry has also been reported [26, 32].

We classified the *TP53* mutations as disruptive or nondisruptive due to the distinct biological outcomes of *TP53* mutations [5, 8, 10, 33]. We observed that disruptive mutations were associated with younger age at diagnosis. Both disruptive and nondisruptive *TP53* mutations were associated with CNS metastasis at diagnosis and African ancestry. We also classified the *TP53* mutations as truncating and nontruncating [11]. Thus,

we observed that truncating mutations were associated with younger age at diagnosis and high native American genetic ancestry background, and nontruncating mutations were associated with current and quitter smokers. Both truncating and nontruncating mutations were associated with CNS metastasis at diagnosis and higher African genetic ancestry background. The association between *TP53* mutations and CNS metastasis had also been previously reported [34–36]. Additionally, we observed that former and current smoker patients were independently associated with *TP53* mutations, which had also been previously reported [37, 38].

Most *TP53* mutations occur in the DNA-binding domain of the gene, and hot spots are in codons 175, 245, 248, 249, 273, and 282 [39]. We identified hot spot mutations in codons 273 (5.6%; $n = 14/249$) and 337 (4.8%; $n = 12/249$). Interestingly, the mutation p.(Arg337His) is a known founder mutation in the Brazilian population for its higher prevalence, mainly in the south and southeast regions [40]. In Brazilian NSCLC patients, the frequency of p.(Arg337His) mutation ranges from 3.5% to 8.9%, and some studies suggest a higher frequency in mutant *EGFR* patients [41–43]. This germline mutation is associated with lower penetrance in the context Li-Fraumeni syndrome [44]. In our study, we evaluated the presence of *TP53* in tumor tissue, and we could not confirm its germline nature. Nevertheless, for the patients harboring the *TP53* p.(Arg337His) mutation, genetic counseling and potential confirmation of its germline nature will be offered.

Regarding outcome, patients harboring *TP53* mutations showed a shorter OS than wild-type patients. Several studies have associated the *TP53* mutations in patients with lung cancer with a worse prognosis [5, 10, 11, 45–47]. The prognostic value of disruptive and nondisruptive *TP53* mutations was evaluated in 318 Spanish advanced NSCLC tumors harboring *EGFR* mutations [10]. Patients harboring nondisruptive mutations revealed worse overall and progression-free survival than patients harboring disruptive mutations and wild-type patients [10]. Our results showed a worse OS in patients harboring disruptive mutations than in patients harboring nondisruptive mutations and wild-type patients. We included patients with early-stage disease, independent of *EGFR* status, and evaluated the whole coding region by a targeted panel in NGS. Yet, they only analyzed the DNA-binding domain (exons 5–8) using high-resolution melting approach [10]. Recently, the prognostic value of *TP53* mutations categorized into disruptive/nondisruptive and *TP53* truncating/nontruncating was evaluated in 6,297 NSCLC patients [11]. In patients with localized disease, the mutant *TP53* patients classified as truncating *TP53* had worse OS than the nontruncating *TP53* and wild-type patients. Similarly, the mutant *TP53* patients classified as disruptive also had worse OS than nondisruptive and wild-type patients [11]. From patients with advanced disease, we observed no significant difference between groups considering both classifications of *TP53* mutations [11]. However, patients' OS was similar regardless of classification, with truncating and disruptive mutations associated with worse OS [11].

Conclusion

In conclusion, our study reported that *TP53* mutations are common in Brazilian lung adenocarcinoma patients. Their biological characterization in disruptive and truncating mutations is associated with African ancestry and shorter OS.

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Statement of Ethics

The Institutional Review Board at Barretos Cancer Hospital approved the study protocol (CAAE 05744712.3.0000.5437) and

waived written informed consent due to the study's retrospective nature. All procedures were performed following the Helsinki Declaration.

Conflict of Interest Statement

Rodrigo de Oliveira Cavagna, Icaro Alves Pinto, Flávia Escremim de Paula, Gustavo Noriz Berardinelli, Débora Sant'Anna, Iara Vidigal Santana, Eduardo Caetano Albino da Silva, Vinicius Duval da Silva, José E. Miziara, Artur Jacinto, Augusto Antoniazzi, and Miguel A. Molina-Vila have no conflicts of interest to declare. Josiane Mourão Dias – research grants for clinical trials from AbbVie, Amgen, AstraZeneca, Bristol-Myers Squibb, GSK, Janssen, Lilly, Merck, MSD, Novartis, Pfizer, Roche, Sanofi, and Xcovery; honoraria for presentations from AstraZeneca and Sanofi; and support for attending meetings and/or travel from Amgen, Boehringer Ingelheim, Janssen, and Sanofi. Pedro De Marchi – research grant from AstraZeneca do Brasil; honoraria for presentations from AstraZeneca do Brasil; support for attending meetings and/or travel from AstraZeneca do Brasil; and advisory board from AstraZeneca do Brasil. Letícia Ferro Leal – research grant from AstraZeneca do Brasil. Rui Manuel Reis – research grant for translational research from MSD; research grant from AstraZeneca do Brasil; and associate editor of Pathobiology Journal.

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Conception: Rodrigo de Oliveira Cavagna, Miguel Angel Molina-Vila, Letícia Ferro Leal, Rui Manuel Reis. Data analysis and interpretation: Rodrigo de Oliveira Cavagna, Letícia Ferro Leal, Rui Manuel Reis, Débora Sant'Anna, Icaro Alves Pinto, Flávia Escremim de Paula, and Gustavo Noriz Berardinelli. Preparation of the manuscript: Rodrigo de Oliveira Cavagna, Letícia Ferro Leal, and Rui Manuel Reis. Revision of the clinical data: José Elias Miziara, Josiane Mourão Dias, Pedro de Marchi, Alexandre Jacinto, and Augusto Antoniazzi. Revision of the histology: Iara Santana, Vinicius Duval da Silva, and Eduardo Caetano Albino da Silva. Supervision: Rui Manuel Reis.

Data Availability Statement

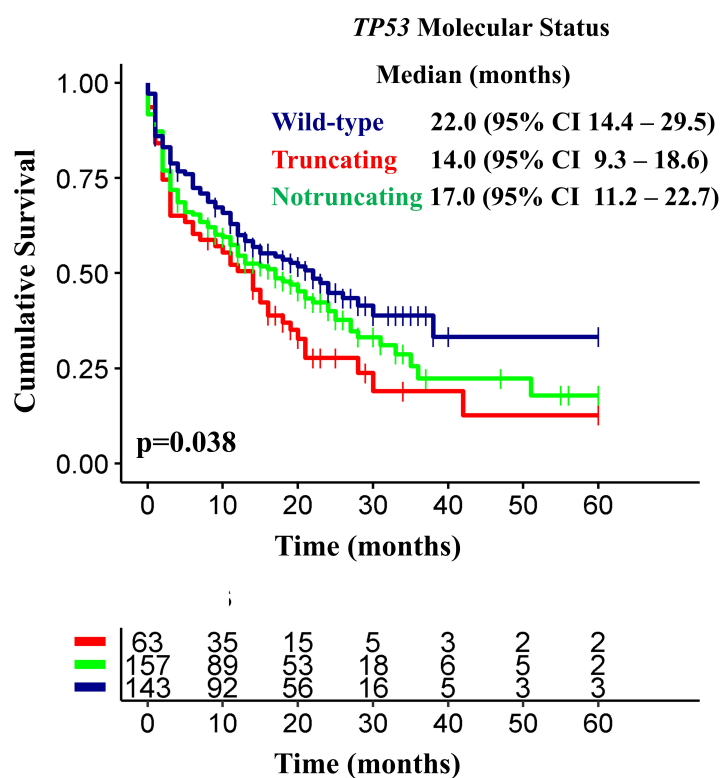
All data generated or analyzed during this study are included in this article and its supplementary material files. Further inquiries can be directed to the corresponding author.

References

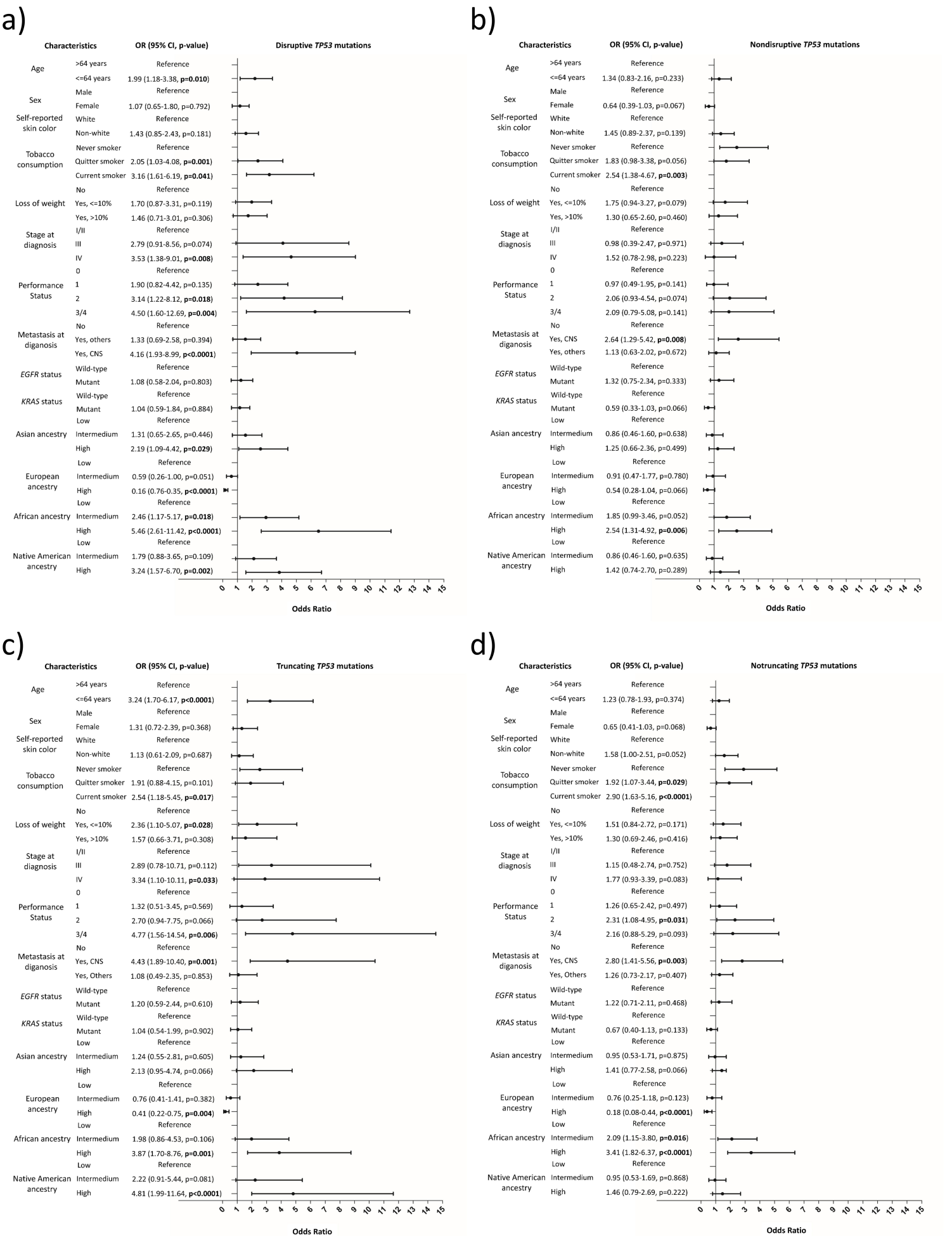
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Supplementary Figure 2 - Overall survival of mutant *TP53* patients classified as truncating and others mutation.



Supplementary Figure 1 - Forest plot showing association between both classifications of *TP53* mutations and clinical and molecular data. The univariate analysis was performed using the multinomial logistic regression test.



Supplementary Tables

Supplementary Table 1 - Description and frequency of *TP53* mutations identified for each lung adenocarcinoma patient (n=221).

ID	c.	p.	Type	Classification#1	Classification#2
6	c.475G>C	p.(Ala159Pro)	Missense	Nondisruptive	Notruncating
9	c.446C>G	p.(Ser149Cys)	Missense	Nondisruptive	Notruncating
	c.472C>G	p.(Arg158Gly)	Missense	Nondisruptive	Notruncating
10	c.809T>C	p.(Phe270Ser)	Missense	Nondisruptive	Notruncating
12	c.742C>T	p.(Arg248Trp)	Missense	Disruptive	Notruncating
15	c.1010G>A	p.(Arg337His)	Missense	Nondisruptive	Notruncating
17	c.818G>T	p.(Arg273Leu)	Missense	Nondisruptive	Notruncating
20	c.1010G>A	p.(Arg337His)	Missense	Nondisruptive	Notruncating
26	c.631A>G	p.(Thr211Ala)	Missense	Nondisruptive	Notruncating
28	c.466C>G	p.(Arg156Gly)	Missense	Nondisruptive	Notruncating
34	c.819_841del	p.(Val274Profs*24)	Frameshift	Disruptive	Truncating
37	c.991C>T	p.(Gln331*)	Nonsense	Disruptive	Truncating
38	c.841G>C	p.(Asp281His)	Missense	Nondisruptive	Notruncating
46	c.817C>T	p.(Arg273Cys)	Missense	Nondisruptive	Notruncating
47	c.488delA	p.(Tyr163Serfs*7)	Frameshift	Disruptive	Truncating
49	c.994-2A>T	p.(?)	Splice_site	Disruptive	Truncating
50	c.473G>T	p.(Arg158Leu)	Missense	Nondisruptive	Notruncating
51	c.586C>T	p.(Arg196*)	Nonsense	Disruptive	Truncating
52	c.84_86delAAA	p.(Glu28_Asn29delinsAsp)	Deletion/Insertion	Nondisruptive	Notruncating
55	c.1010G>A	p.(Arg337His)	Missense	Nondisruptive	Notruncating
	c.748_750delCCC	p.(Pro250del)	Deletion/Insertion	Nondisruptive	Notruncating

#1, Disruptive / Nondisruptive[14,16]; #2, Truncating / Notruncating[17].

Continued Supplementary Table 1 - Description and frequency of *TP53* mutations identified for each lung adenocarcinoma patient (n=221).

ID	c.	p.	Type	Classification#1	Classification#2
56	c.499C>T	p.(Gln167*)	Nonsense	Disruptive	Truncating
	c.102del	p.(Leu35Cysfs*9)	Frameshift	Disruptive	Truncating
57	c.415A>T	p.(Lys139*)	Nonsense	Disruptive	Truncating
61	c.857A>T	p.(Glu286Val)	Missense	Nondisruptive	Notruncating
65	c.469G>T	p.(Val157Phe)	Missense	Nondisruptive	Notruncating
68	c.569delC	p.(Pro190Leufs*57)	Frameshift	Disruptive	Truncating
70	c.396_397delGA	p.(Lys132Asnfs*16)	Frameshift	Disruptive	Truncating
71	c.742C>T	p.(Arg248Trp)	Missense	Disruptive	Notruncating
72	c.128delT	p.(Leu43*fs*1)	Frameshift	Disruptive	Truncating
73	c.388C>G	p.(Leu130Val)	Missense	Nondisruptive	Notruncating
74	c.1010G>A	p.(Arg337His)	Missense	Nondisruptive	Notruncating
75	c.1010G>A	p.(Arg337His)	Missense	Nondisruptive	Notruncating
76	c.503A>G	p.(His168Arg)	Missense	Nondisruptive	Notruncating
86	c.707A>G	p.(Tyr236Cys)	Missense	Nondisruptive	Notruncating
92	c.818G>T	p.(Arg273Leu)	Missense	Nondisruptive	Notruncating
97	c.818G>A	p.(Arg273His)	Missense	Nondisruptive	Notruncating
98	c.363_375deL	p.(?)	Frameshift	Disruptive	Truncating
99	c.1010G>A	p.(Arg337His)	Missense	Nondisruptive	Notruncating
100	c.751A>T	p.(Ile251Phe)	Missense	Nondisruptive	Notruncating
102	c.394A<T	p.(Lys132*)	Nonsense	Disruptive	Truncating
108	c.329G>T	p.(Arg110Leu)	Missense	Nondisruptive	Notruncating

#1, Disruptive / Nondisruptive[14,16]; #2, Truncating / Notruncating[17].

Continued Supplementary Table 1 - Description and frequency of *TP53* mutations identified for each lung adenocarcinoma patient (n=221).

ID	c.	p.	Type	Classification#1	Classification#2
109	c.782_782+18del	p.(?)	Splice_site	Disruptive	Truncating
111	c.535C>T	p.(His179Tyr)	Missense	Disruptive	Notruncating
115	c.1010G>T	p.(Arg337Leu)	Missense	Nondisruptive	Notruncating
118	c.584T>C	p.(Ile195Thr)	Missense	Disruptive	Notruncating
119	c.797G>A	p.(Gly266Glu)	Missense	Nondisruptive	Notruncating
122	c.592G>T	p.(Glu198*)	Nonsense	Disruptive	Truncating
123	c.536A>G	p.(His179Arg)	Missense	Nondisruptive	Notruncating
124	c.825T>G	p.(Cys275Trp)	Missense	Nondisruptive	Notruncating
127	c.811G>A	p.(Glu271Lys)	Missense	Nondisruptive	Notruncating
130	c.818G>T	p.(Arg273Leu)	Missense	Nondisruptive	Notruncating
131	c.734G>T	p.(Gly245Val)	Missense	Nondisruptive	Notruncating
133	c.1006G>T	p.(Glu336*)	Nonsense	Disruptive	Truncating
134	c.820G>T	p.(Val274Phe)	Missense	Nondisruptive	Notruncating
135	c.473G>T	p.(Arg158Leu)	Missense	Nondisruptive	Notruncating
136	c.490A>T	p.(Lys164*)	Nonsense	Disruptive	Truncating
	c.473G>T	p.(Arg158Leu)	Missense	Nondisruptive	Notruncating
147	c.536A>G	p.(His179Arg)	Missense	Nondisruptive	Notruncating
149	c.148_149del	p.(Ile50*)	Nonsense	Disruptive	Truncating
151	c.151G>T	p.(Glu51*)	Nonsense	Disruptive	Truncating
153	c.814G>A	p.(Val272Met)	Missense	Nondisruptive	Notruncating
157	c.991 dupC	p.(Gln331Profs*6)	Frameshift	Disruptive	Truncating
	c.1132T>C	p.(Ser378Pro)	Missense	Nondisruptive	Notruncating
	c.1155dupC	p.(Lys386Glnfs*6)	Frameshift	Disruptive	Truncating

#1, Disruptive / Nondisruptive[14,16]; #2, Truncating / Notruncating[17].

Continued Supplementary Table 1 - Description and frequency of *TP53* mutations identified for each lung adenocarcinoma patient (n=221).

ID	c.	p.	Type	Classification#1	Classification#2
159	c.746G>T	p.(Arg249Met)	Missense	Disruptive	Notruncating
160	c.991C>T	p.(Gln331*)	Nonsense	Disruptive	Truncating
164	c.747G>T	p.(Arg249Ser)	Missense	Disruptive	Notruncating
165	c.733G>T	p.(Gly245Cys)	Missense	Disruptive	Notruncating
167	c.818G>T	p.(Arg273Leu)	Missense	Nondisruptive	Notruncating
170	c.818G>T	p.(Arg273Leu)	Missense	Nondisruptive	Notruncating
172	c.730G>T	p.(Gly244Cys)	Missense	Disruptive	Notruncating
175	c.652G>A	p.(Val218Met)	Missense	Nondisruptive	Notruncating
178	c.475G>C	p.(Ala159Pro)	Missense	Nondisruptive	Notruncating
179	c.1037C>T	p.(Ser346Leu)	Missense	Nondisruptive	Notruncating
	c.623A>C	p.(Asp208Ala)	Missense	Nondisruptive	Notruncating
181	c.827C>A	p.(Ala276Asp)	Missense	Nondisruptive	Notruncating
	c.711G>T	p.(Met237Ile)	Missense	Nondisruptive	Notruncating
182	c.378C>G	p.(Tyr126*)	Nonsense	Disruptive	Truncating
183	c.818G>A	p.(Arg273His)	Missense	Nondisruptive	Notruncating
184	c.830G>T	p.(Cys277Phe)	Missense	Nondisruptive	Notruncating
186	c.1001G>T	p.(Gly334Val)	Missense	Nondisruptive	Notruncating
189	c.376T>C	p.(Tyr126His)	Missense	Nondisruptive	Notruncating
192	c.797G>T	p.(Gly266Val)	Missense	Nondisruptive	Notruncating
193	c.646G>A	p.(Val216Met)	Missense	Nondisruptive	Notruncating
194	c.583A>T	p.(Ile195Phe)	Missense	Nondisruptive	Notruncating
195	c.733G>A	p.(Gly245Ser)	Missense	Disruptive	Notruncating
198	c.827C>G	p.(Ala276Gly)	Missense	Nondisruptive	Notruncating

#1, Disruptive / Nondisruptive[14,16]; #2, Truncating / Notruncating[17].

Continued Supplementary Table 1 - Description and frequency of *TP53* mutations identified for each lung adenocarcinoma patient (n=221).

ID	c.	p.	Type	Classification#1	Classification#2
200	c.1015G>T	p.(Glu339*)	Nonsense	Disruptive	Truncating
202	c.796G>T	p.(Gly266*)	Nonsense	Disruptive	Truncating
206	c.833C>G	p.(Pro278Arg)	Missense	Nondisruptive	Notruncating
207	c.857A>G	p.(Glu286Gly)	Missense	Nondisruptive	Notruncating
208	c.817C>T	p.(Arg273Cys)	Missense	Nondisruptive	Notruncating
212	c.746G>T	p.(Arg249Met)	Missense	Disruptive	Notruncating
216	c.711G>T	p.(Met237Ile)	Missense	Nondisruptive	Notruncating
218	c.743G>A	p.(Arg248Gln)	Missense	Disruptive	Notruncating
220	c.488A>G	p.(Tyr163Cys)	Missense	Nondisruptive	Notruncating
221	c.920-2A>T	p.(?)	Splice_site	Disruptive	Truncating
222	c.457_484del	p.(Pro153Serfs*8)	Frameshift	Disruptive	Truncating
224	c.747G>T	p.(Arg249Ser)	Missense	Disruptive	Notruncating
227	c.373_374dup	p.(Tyr126Argfs*45)	Frameshift	Disruptive	Truncating
228	c.733G>T	p.(Gly245Cys)	Missense	Disruptive	Notruncating
229	c.713G>A	p.(Cys238Tyr)	Missense	Nondisruptive	Notruncating
231	c.406C>G	p.(Gln136Glu)	Missense	Nondisruptive	Notruncating
232	c.743G>T	p.(Arg248Leu)	Missense	Disruptive	Notruncating
236	c.555_556del	p.(Ser185Argfs*23)	Frameshift	Disruptive	Truncating
237	c.372C>A	p.(Cys124*)	Nonsense	Disruptive	Truncating
238	c.201_213del	p.(Glu68Profs*51)	Frameshift	Disruptive	Truncating
240	c.997del	p.(Arg333Valfs*12)	Frameshift	Disruptive	Truncating
243	c.267del	p.(Ser90Profs*33)	Frameshift	Disruptive	Truncating
	c.313G>C	p.(Gly105Arg)	Missense	Nondisruptive	Notruncating

#1, Disruptive / Nondisruptive[14,16]; #2, Truncating / Notruncating[17].

Continued Supplementary Table 1 - Description and frequency of *TP53* mutations identified for each lung adenocarcinoma patient (n=221).

ID	c.	p.	Type	Classification#1	Classification#2
245	c.591_592delinsTT	p.(Glu198*)	Nonsense	Disruptive	Truncating
246	c.1027G>T	p.(Glu343*)	Nonsense	Disruptive	Truncating
247	c.314G>T	p.(Gly105Val)	Missense	Nondisruptive	Notruncating
	c.524G>T	p.(Arg175Leu)	Missense	Disruptive	Notruncating
248	c.467G>A	p.(Arg156His)	Missense	Nondisruptive	Notruncating
249	c.817C>T	p.(Arg273Cys)	Missense	Nondisruptive	Notruncating
251	c.817C>T	p.(Arg273Cys)	Missense	Nondisruptive	Notruncating
252	c.469G>T	p.(Val157Phe)	Missense	Nondisruptive	Notruncating
253	c.775G>T	p.(Asp259Tyr)	Missense	Nondisruptive	Notruncating
254	c.488A>G	p.(Tyr163Cys)	Missense	Nondisruptive	Notruncating
256	c.469G>T	p.(Val157Phe)	Missense	Nondisruptive	Notruncating
257	c.97-1G>T	p.(?)	Splice_site	Disruptive	Truncating
259	c.517G>A	p.(Val173Met)	Missense	Nondisruptive	Notruncating
260	c.1010G>A	p.(Arg337His)	Missense	Nondisruptive	Notruncating
262	c.613T>C	p.(Tyr205His)	Missense	Nondisruptive	Notruncating
267	c.403T>C	p.(Cys135Arg)	Missense	Nondisruptive	Notruncating
269	c.659A>G	p.(Tyr220Cys)	Missense	Nondisruptive	Notruncating
271	c.493C>T	p.(Gln165*)	Nonsense	Disruptive	Truncating
272	c.477_502del	p.(Met160Hisfs*12)	Frameshift	Disruptive	Truncating
273	c.715A>G	p.(Asn239Asp)	Missense	Disruptive	Notruncating
275	c.626_627del	p.(Arg209Lysfs*6)	Frameshift	Disruptive	Truncating
278	c.396G>C	p.(Lys132Asn)	Missense	Nondisruptive	Notruncating
279	c.733G>A	p.(Gly245Ser)	Missense	Disruptive	Notruncating

#1, Disruptive / Nondisruptive[14,16]; #2, Truncating / Notruncating[17].

Continued Supplementary Table 1 - Description and frequency of *TP53* mutations identified for each lung adenocarcinoma patient (n=221).

ID	c.	p.	Type	Classification#1	Classification#2
281	c.848G>C	p.(Arg283Pro)	Missense	Nondisruptive	Notruncating
284	c.-1_13del	p.(Met1?)	Start_Loss	Disruptive	Truncating
289	c.486_506del	p.(Ile162_His168del)	Deletion/Insertion	Nondisruptive	Notruncating
290	c.637C>T	p.(Arg213*)	Nonsense	Disruptive	Truncating
291	c.718_719delinsGT	p.(Ser240Val)	Missense	Disruptive	Notruncating
292	c.844C>T	p.(Arg282Trp)	Missense	Nondisruptive	Notruncating
293	c.523C>T	p.(Arg175Cys)	Missense	Disruptive	Notruncating
294	c.818G>A	p.(Arg273His)	Missense	Nondisruptive	Notruncating
296	c.471_473delinsTCT	p.(Arg158Leu)	Missense	Nondisruptive	Notruncating
302	c.512A>G	p.(Glu171Gly)	Missense	Disruptive	Notruncating
303	c.991dupC	p.(Gln331Profs*6)	Frameshift	Disruptive	Truncating
	c.524G>A	p.(Arg175His)	Missense	Nondisruptive	Notruncating
	c.1155dupC	p.(Lys386Glnfs*6)	Frameshift	Disruptive	Truncating
	c.427G>A	p.(Val143Met)	Missense	Nondisruptive	Notruncating
306	c.313G>T	p.(Gly105Cys)	Missense	Nondisruptive	Notruncating
	c.733_734delGGinsTT	p.(Gly245Phe)	Missense	Nondisruptive	Notruncating
308	c.1010G>A	p.(Arg337His)	Missense	Nondisruptive	Notruncating
	c.645T>A	p.(Ser215Arg)	Missense	Nondisruptive	Notruncating
310	c.480_481delGGinsTT	p.(Met160_Ala161delinsIleSer)	Deletion/Insertion	Nondisruptive	Notruncating
313	c.856G>A	p.(Glu286Lys)	Missense	Nondisruptive	Notruncating
314	c.614A>T	p.(Tyr205Phe)	Missense	Nondisruptive	Notruncating
315	c.481G>T	p.(Ala161Ser)	Missense	Nondisruptive	Notruncating
	c.993G>T	p.(Gln331His)	Missense	Nondisruptive	Notruncating

#1, Disruptive / Nondisruptive[14,16]; #2, Truncating / Notruncating[17].

Continued Supplementary Table 1 - Description and frequency of *TP53* mutations identified for each lung adenocarcinoma patient (n=221).

ID	c.	p.	Type	Classification#1	Classification#2
316	c.526T>C	p.(Cys176Ser)	Missense	Nondisruptive	Notruncating
319	c.592G>T	p.(Glu198*)	Nonsense	Disruptive	Truncating
320	c.858A>C	p.(Glu286Asp)	Missense	Nondisruptive	Notruncating
321	c.711G>C	p.(Met237Ile)	Missense	Nondisruptive	Notruncating
323	c.469G>T	p.(Val157Phe)	Missense	Nondisruptive	Notruncating
324	c.514G>T	p.(Val172Phe)	Missense	Nondisruptive	Notruncating
325	c.577C>T	p.(His193Tyr)	Missense	Disruptive	Notruncating
327	c.427G>A	p.(Val143Met)	Missense	Nondisruptive	Notruncating
329	c.158G>A	p.(Trp53*)	Nonsense	Disruptive	Truncating
330	c.1001G>T	p.(Gly334Val)	Missense	Nondisruptive	Notruncating
331	c.1010G>A	p.(Arg337His)	Missense	Nondisruptive	Notruncating
333	c.515T>A	p.(Val172Asp)	Missense	Disruptive	Notruncating
339	c.578A>T	p.(His193Leu)	Missense	Disruptive	Notruncating
340	c.413C>T	p.(Ala138Val)	Missense	Nondisruptive	Notruncating
341	c.892G>T	p.(Glu298*)	Nonsense	Disruptive	Truncating
343	c.797G>T	p.(Gly266Val)	Missense	Nondisruptive	Notruncating
344	c.796G>C	p.(Gly266Arg)	Missense	Nondisruptive	Notruncating
348	c.467G>C	p.(Arg156Pro)	Missense	Nondisruptive	Notruncating
349	c.743G>A	p.(Arg248Gln)	Missense	Disruptive	Notruncating
350	c.799C>T	p.(Arg267Trp)	Missense	Nondisruptive	Notruncating
351	c.701A>G	p.(Tyr234Cys)	Missense	Nondisruptive	Notruncating
353	c.524G>A	p.(Arg175His)	Missense	Nondisruptive	Notruncating
360	c.475G>C	p.(Ala159Pro)	Missense	Nondisruptive	Notruncating

#1, Disruptive / Nondisruptive[14,16]; #2, Truncating / Notruncating[17].

Continued Supplementary Table 1 - Description and frequency of *TP53* mutations identified for each lung adenocarcinoma patient (n=221).

ID	c.	p.	Type	Classification#1	Classification#2
361	c.401T>G	p.(Phe134Cys)	Missense	Nondisruptive	Notruncating
362	c.560-1G>T	p.(?)	Splice_site	Disruptive	Truncating
363	c.841G>C	p.(Asp281His)	Missense	Nondisruptive	Notruncating
367	c.329G>T	p.(Arg110Leu)	Missense	Nondisruptive	Notruncating
369	c.1010G>A	p.(Arg337His)	Missense	Nondisruptive	Notruncating
372	c.404G>T	p.(Cys135Phe)	Missense	Nondisruptive	Notruncating
375	c.722C>T	p.(Ser241Phe)	Missense	Disruptive	Notruncating
376	c.625A>T	p.(Arg209*)	Nonsense	Disruptive	Truncating
	c.596G>A	p.(Gly199Glu)	Missense	Nondisruptive	Notruncating
377	c.727_730delATGGinsTTT	p.(Met243Phefs*4)	Frameshift	Disruptive	Truncating
378	c.725G>A	p.(Cys242Tyr)	Missense	Nondisruptive	Notruncating
379	c.375+1G>A	p.(?)	Splice_site	Disruptive	Truncating
382	c.839G>A	p.(Arg280Lys)	Missense	Nondisruptive	Notruncating
385	c.488A>G	p.(Tyr163Cys)	Missense	Nondisruptive	Notruncating
386	c.994-2A>T	p.(?)	Splice_site	Disruptive	Truncating
387	c.774_776delAGA	p.(Glu258del)	Deletion/Insertion	Nondisruptive	Notruncating
388	c.455C>T	p.(Pro152Leu)	Missense	Nondisruptive	Notruncating
390	c.754_762delCTCACCATC	p.(Leu252Ile254del)	Deletion/Insertion	Nondisruptive	Notruncating
391	c.371dupG	p.(Cys124Trpfs*25)	Frameshift	Disruptive	Truncating
395	c.314G>A	p.(Gly105Asp)	Missense	Nondisruptive	Notruncating
397	c.892G>T	p.(Glu298*)	Nonsense	Disruptive	Truncating
400	c.347C>T	p.(Ser116Phe)	Missense	Nondisruptive	Notruncating
403	c.394A>G	p.(Lys132Glu)	Missense	Nondisruptive	Notruncating

#1, Disruptive / Nondisruptive[14,16]; #2, Truncating / Notruncating[17].

Continued Supplementary Table 1 - Description and frequency of *TP53* mutations identified for each lung adenocarcinoma patient (n=221).

ID	c.	p.	Type	Classification#1	Classification#2
404	c.782+1G>A	p.(?)	Splice_site	Disruptive	Truncating
406	c.722C>T	p.(Ser241Phe)	Missense	Disruptive	Notruncating
407	c.273_276delGCCC	p.(Thp91Cysfs*31)	Frameshift	Disruptive	Truncating
408	c.581T>G	p.(Leu194Arg)	Missense	Disruptive	Notruncating
409	c.524G>A	p.(Arg175His)	Missense	Nondisruptive	Notruncating
413	c.733G>T	p.(Gly245Cys)	Missense	Disruptive	Notruncating
415	c.515T>A	p.(Val172Asp)	Missense	Disruptive	Notruncating
416	c.404G>A	p.(Cys135Tyr)	Missense	Nondisruptive	Notruncating
417	c.880G>T	p.(Glu294*)	Nonsense	Disruptive	Truncating
418	c.524G>A	p.(Arg175His)	Missense	Nondisruptive	Notruncating
420	c.920-2A>T	p.(?)	Splice_site	Disruptive	Truncating
421	c.747G>T	p.(Arg249Ser)	Missense	Disruptive	Notruncating
423	c.404G>A	p.(Cys135Tyr)	Missense	Nondisruptive	Notruncating
424	c.818G>T	p.(Arg273Leu)	Missense	Nondisruptive	Notruncating
	c.892G>T	p.(Glu298*)	Nonsense	Disruptive	Truncating
425	c.958A>T	p.(Lys320*)	Nonsense	Disruptive	Truncating
427	c.845G>A	p.(Arg282Gln)	Missense	Nondisruptive	Notruncating
428	c.823T>C	p.(Cys275Arg)	Missense	Nondisruptive	Notruncating
433	c.3G>A	p.(Met1?)	Start_Loss	Disruptive	Truncating
435	c.747G>T	p.(Arg249Ser)	Missense	Disruptive	Notruncating
436	c.814G>T	p.(Val272Leu)	Missense	Nondisruptive	Notruncating
437	c.947_949delinsTCT	p.(Pro316_Gln317delinsLeu)	Deletion/Insertion	Nondisruptive	Notruncating
438	c.391A>T	p.(Asn131Tyr)	Missense	Nondisruptive	Notruncating

#1, Disruptive / Nondisruptive[14,16]; #2, Truncating / Notruncating[17].

Continued Supplementary Table 1 - Description and frequency of *TP53* mutations identified for each lung adenocarcinoma patient (n=221).

ID	c.	p.	Type	Classification#1	Classification#2
441	c.713G>A	p.(Cys238Tyr)	Missense	Nondisruptive	Notruncating
446	c.329G>C	p.(Arg110Pro)	Missense	Nondisruptive	Notruncating
447	c.368del	p.(Thr123Ilefs*47)	Frameshift	Disruptive	Truncating
	c.817C>T	p.(Arg273Cys)	Missense	Nondisruptive	Notruncating
451	c.707_708insT	p.(Met237Hisfs*3)	Frameshift	Disruptive	Truncating
453	c.963del	p.(Lys321Asnfs*24)	Frameshift	Disruptive	Truncating
454	c.538G>T	p.(Glu180*)	Nonsense	Disruptive	Truncating
455	c.548C>T	p.(Ser183Leu)	Missense	Disruptive	Notruncating
	c.747G>T	pArg249Ser)	Missense	Disruptive	Notruncating
621	c.713_714del	p.(Cys238*)	Nonsense	Disruptive	Truncating
	c.743G>A	p.(Arg248Gln)	Missense	Disruptive	Notruncating
	c.572C>T	p.(Pro191Leu)	Missense	Nondisruptive	Notruncating
	c.328C>T	p.(Arg110Cys)	Missense	Nondisruptive	Notruncating
	c.475G>A	p.(Ala159Thr)	Missense	Nondisruptive	Notruncating
775	c.1036G>T	p.(Glu346*)	Nonsense	Disruptive	Truncating
1128	c.329G>T	p.(Arg110Leu)	Missense	Nondisruptive	Notruncating
1153	452C>A	p.(Pro151His)	Missense	Nondisruptive	Notruncating
1262	748C>T	p.(Pro250Ser)	Missense	Disruptive	Notruncating
	457C>T	p.(Pro153Ser)	Missense	Nondisruptive	Notruncating
	413C>T	p.(Ala138Val)	Missense	Nondisruptive	Notruncating
	599A>T	p.(Asn200Ile)	Missense	Nondisruptive	Notruncating
1795	c.1010G>A	p.(Arg337His)	Missense	Nondisruptive	Notruncating
	c.527G>A	p.(Cys176Tyr)	Missense	Nondisruptive	Notruncating

#1, Disruptive / Nondisruptive[14,16]; #2, Truncating / Notruncating[17].

Supplementary Table 2 - Description and frequency of identified mutations in *TP53* gene (n=249).

c.	p.	Type	TP53 Exon	Classification#1	Classification#2	Frequency	Presenting in IARC Database*
c.-1_13del	p.(Met1?)	Start_Loss	2	Disruptive	Truncating	1 (0.40%)	No
c.3G>A	p.(Met1?)	Start_Loss	2	Disruptive	Truncating	1 (0.40%)	Yes
c.84_86delAAA	p.(Glu28_Asn29delinsAsp)	Deletion/Insertion	3	Nondisruptive	Notruncating	1 (0.40%)	No
c.102del	p.(Leu35Cysfs*9)	Frameshift	4	Disruptive	Truncating	1 (0.40%)	Yes
c.128delT	p.(Leu43*fs*1)	Frameshift	4	Disruptive	Truncating	1 (0.40%)	Yes
c.148_149del	p.(Ile50*)	Nonsense	4	Disruptive	Truncating	1 (0.40%)	Yes
c.151G>T	p.(Glu51*)	Nonsense	4	Disruptive	Truncating	1 (0.40%)	Yes
c.158G>A	p.(Trp53*)	Nonsense	4	Disruptive	Truncating	1 (0.40%)	Yes
c.201_213del	p.(Glu68Profs*51)	Frameshift	4	Disruptive	Truncating	1 (0.40%)	Yes
c.267del	p.(Ser90Profs*33)	Frameshift	4	Disruptive	Truncating	1 (0.40%)	Yes
c.273_276delGCCC	p.(Trp91Cysfs*31)	Frameshift	4	Disruptive	Truncating	1 (0.40%)	No
c.313G>C	p.(Gly105Arg)	Missense	4	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.313G>T	p.(Gly105Cys)	Missense	4	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.314G>A	p.(Gly105Asp)	Missense	4	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.314G>T	p.(Gly105Val)	Missense	4	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.328C>T	p.(Arg110Cys)	Missense	4	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.329G>C	p.(Arg110Pro)	Missense	4	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.329G>T	p.(Arg110Leu)	Missense	4	Nondisruptive	Notruncating	3 (1.21%)	Yes
c.347C>T	p.(Ser116Phe)	Missense	4	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.363_375deL	p.(?)	Frameshift	4	Disruptive	Truncating	1 (0.40%)	No
c.368del	p.(Thr123Ilefs*47)	Frameshift	4	Disruptive	Truncating	1 (0.40%)	Yes

#1, Disruptive / Nondisruptive[14,16]; #2, Truncating / Notruncating[17]. *IARC (International Agency for Research on Cancer) TP53 database - <https://p53.iarc.fr/> - The database consist of several TP53 mutations data, such as somatic and germline mutations, polymorphism, function, cell-lines, and mouse model. Therefore, various annotations on the predicted or experimentally functional impact of mutations, clinicopathological features of tumors, and demographic patients' data are available.

Continued Supplementary Table 2 - Description and frequency of identified mutations in *TP53* gene (n=249).

c.	p.	Type	TP53 Exon	Classification#1	Classification#2	Frequency	Presenting in IARC Database*
c.371dupG	p.(Cys124Trpfs*25)	Frameshift	4	Disruptive	Truncating	1 (0.40%)	No
c.372C>A	p.(Cys124*)	Nonsense	4	Disruptive	Truncating	1 (0.40%)	Yes
c.373_374dup	p.(Tyr126Argfs*45)	Frameshift	5	Disruptive	Truncating	1 (0.40%)	No
c.376T>C	p.(Tyr126His)	Missense	5	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.378C>G	p.(Tyr126*)	Nonsense	5	Disruptive	Truncating	1 (0.40%)	Yes
c.388C>G	p.(Leu130Val)	Missense	5	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.391A>T	p.(Asn131Tyr)	Missense	5	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.394A<T	p.(Lys132*)	Nonsense	5	Disruptive	Truncating	1 (0.40%)	Yes
c.394A>G	p.(Lys132Glu)	Missense	5	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.396_397delGA	p.(Lys132Asnfs*16)	Frameshift	5	Disruptive	Truncating	1 (0.40%)	No
c.396G>C	p.(Lys132Asn)	Missense	5	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.401T>G	p.(Phe134Cys)	Missense	5	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.403T>C	p.(Cys135Arg)	Missense	5	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.404G>A	p.(Cys135Tyr)	Missense	5	Nondisruptive	Notruncating	2 (0.81%)	Yes
c.404G>T	p.(Cys135Phe)	Missense	5	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.406C>G	p.(Gln136Glu)	Missense	5	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.413C>T	p.(Ala138Val)	Missense	5	Nondisruptive	Notruncating	2 (0.81%)	Yes
c.415A>T	p.(Lys139*)	Nonsense	5	Disruptive	Truncating	1 (0.40%)	Yes
c.427G>A	p.(Val143Met)	Missense	5	Nondisruptive	Notruncating	2 (0.81%)	Yes
c.446C>G	p.(Ser149Cys)	Missense	5	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.452C>A	p.(Pro151His)	Missense	5	Nondisruptive	Notruncating	1 (0.40%)	Yes

#1, Disruptive / Nondisruptive[14,16]; #2, Truncating / Notruncating[17]. *IARC (International Agency for Research on Cancer) TP53 database - <https://p53.iarc.fr/> - The database consist of several TP53 mutations data, such as somatic and germline mutations, polymorphism, function, cell-lines, and mouse model. Therefore, various annotations on the predicted or experimentally functional impact of mutations, clinicopathological features of tumors, and demographic patients' data are available.

Continued Supplementary Table 2 - Description and frequency of identified mutations in *TP53* gene (n=249).

c.	p.	Type	TP53 Exon	Classification#1	Classification#2	Frequency	Presenting in IARC Database*
c.455C>T	p.(Pro152Leu)	Missense	5	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.457_484del	p.(Pro153Serfs*8)	Frameshift	5	Disruptive	Truncating	1 (0.40%)	No
c.457C>T	p.(Pro153Ser)	Missense	5	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.466C>G	p.(Arg156Gly)	Missense	5	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.467G>A	p.(Arg156His)	Missense	5	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.467G>C	p.(Arg156Pro)	Missense	5	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.469G>T	p.(Val157Phe)	Missense	5	Nondisruptive	Notruncating	4 (1.61%)	Yes
c.472C>G	p.(Arg158Gly)	Missense	5	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.473G>T	p.(Arg158Leu)	Missense	5	Nondisruptive	Notruncating	4 (1.61%)	Yes
c.475G>A	p.(Ala159Thr)	Missense	5	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.475G>C	p.(Ala159Pro)	Missense	5	Nondisruptive	Notruncating	3 (1.21%)	Yes
c.477_502del	p.(Met160Hisfs*12)	Frameshift	5	Disruptive	Truncating	1 (0.40%)	No
c.480_481delGGinsTT	p.(Met160_Ala161delinsIleSer)	Deletion/Insertion	5	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.481G>T	p.(Ala161Ser)	Missense	5	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.486_506del	p.(Ile162_His168del)	Deletion/Insertion	5	Nondisruptive	Notruncating	1 (0.40%)	No
c.488A>G	p.(Tyr163Cys)	Missense	5	Nondisruptive	Notruncating	3 (1.21%)	Yes
c.488delA	p.(Tyr163Serfs*7)	Frameshift	5	Disruptive	Truncating	1 (0.40%)	No
c.490A>T	p.(Lys164*)	Nonsense	5	Disruptive	Truncating	1 (0.40%)	Yes
c.493C>T	p.(Gln165*)	Nonsense	5	Disruptive	Truncating	1 (0.40%)	Yes
c.499C>T	p.(Gln167*)	Nonsense	5	Disruptive	Truncating	1 (0.40%)	Yes
c.503A>G	p.(His168Arg)	Missense	5	Nondisruptive	Notruncating	1 (0.40%)	Yes

#1, Disruptive / Nondisruptive[14,16]; #2, Truncating / Notruncating[17]. *IARC (International Agency for Research on Cancer) TP53 database - <https://p53.iarc.fr/> - The database consist of several TP53 mutations data, such as somatic and germline mutations, polymorphism, function, cell-lines, and mouse model. Therefore, various annotations on the predicted or experimentally functional impact of mutations, clinicopathological features of tumors, and demographic patients' data are available.

Continued Supplementary Table 2 - Description and frequency of identified mutations in *TP53* gene (n=249).

c.	p.	Type	TP53 Exon	Classification#1	Classification#2	Frequency	Presenting in IARC Database*
c.512A>G	p.(Glu171Gly)	Missense	5	Disruptive	Notruncating	1 (0.40%)	Yes
c.514G>T	p.(Val172Phe)	Missense	5	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.515T>A	p.(Val172Asp)	Missense	5	Disruptive	Notruncating	2 (0.81%)	Yes
c.517G>A	p.(Val173Met)	Missense	5	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.523C>T	p.(Arg175Cys)	Missense	5	Disruptive	Notruncating	1 (0.40%)	Yes
c.524G>A	p.(Arg175His)	Missense	5	Nondisruptive	Notruncating	4 (1.61%)	Yes
c.524G>T	p.(Arg175Leu)	Missense	5	Disruptive	Notruncating	1 (0.40%)	Yes
c.526T>C	p.(Cys176Ser)	Missense	5	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.527G>A	p.(Cys176Tyr)	Missense	5	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.535C>T	p.(His179Tyr)	Missense	5	Disruptive	Notruncating	1 (0.40%)	Yes
c.536A>G	p.(His179Arg)	Missense	5	Nondisruptive	Notruncating	2 (0.81%)	Yes
c.538G>T	p.(Glu180*)	Nonsense	5	Disruptive	Truncating	1 (0.40%)	Yes
c.548C>T	p.(Ser183Leu)	Missense	5	Disruptive	Notruncating	1 (0.40%)	Yes
c.555_556del	p.(Ser185Argfs*23)	Frameshift	5	Disruptive	Truncating	1 (0.40%)	Yes
c.569delC	p.(Pro190Leufs*57)	Frameshift	6	Disruptive	Truncating	1 (0.40%)	No
c.572C>T	p.(Pro191Leu)	Missense	6	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.577C>T	p.(His193Tyr)	Missense	6	Disruptive	Notruncating	1 (0.40%)	Yes
c.578A>T	p.(His193Leu)	Missense	6	Disruptive	Notruncating	1 (0.40%)	Yes
c.581T>G	p.(Leu194Arg)	Missense	6	Disruptive	Notruncating	1 (0.40%)	Yes
c.583A>T	p.(Ile195Phe)	Missense	6	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.584T>C	p.(Ile195Thr)	Missense	6	Disruptive	Notruncating	1 (0.40%)	Yes

#1, Disruptive / Nondisruptive[14,16]; #2, Truncating / Notruncating[17]. *IARC (International Agency for Research on Cancer) TP53 database - <https://p53.iarc.fr/> - The database consist of several TP53 mutations data, such as somatic and germline mutations, polymorphism, function, cell-lines, and mouse model. Therefore, various annotations on the predicted or experimentally functional impact of mutations, clinicopathological features of tumors, and demographic patients' data are available.

Continued Supplementary Table 2 - Description and frequency of identified mutations in *TP53* gene (n=249).

c.	p.	Type	TP53 Exon	Classification#1	Classification#2	Frequency	Presenting in IARC Database*
c.586C>T	p.(Arg196*)	Nonsense	6	Disruptive	Truncating	1 (0.40%)	Yes
c.591_592delinsTT	p.(Glu198*)	Nonsense	6	Disruptive	Truncating	3 (1.21%)	Yes
c.596G>A	p.(Gly199Glu)	Missense	6	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.599A>T	p.(Asn200Ile)	Missense	6	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.613T>C	p.(Tyr205His)	Missense	6	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.614A>T	p.(Tyr205Phe)	Missense	6	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.623A>C	p.(Asp208Ala)	Missense	6	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.625A>T	p.(Arg209*)	Nonsense	6	Disruptive	Truncating	1 (0.40%)	Yes
c.626_627del	p.(Arg209Lysfs*6)	Frameshift	6	Disruptive	Truncating	1 (0.40%)	Yes
c.631A>G	p.(Thr211Ala)	Missense	6	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.637C>T	p.(Arg213*)	Nonsense	6	Disruptive	Truncating	1 (0.40%)	Yes
c.645T>A	p.(Ser215Arg)	Missense	6	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.646G>A	p.(Val216Met)	Missense	6	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.652G>A	p.(Val218Met)	Missense	6	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.659A>G	p.(Tyr220Cys)	Missense	6	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.701A>G	p.(Tyr234Cys)	Missense	7	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.707_708insT	p.(Met237Hisfs*3)	Frameshift	7	Disruptive	Truncating	1 (0.40%)	No
c.707A>G	p.(Tyr236Cys)	Missense	7	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.711G>C	p.(Met237Ile)	Missense	7	Nondisruptive	Notruncating	3 (1.21%)	Yes
c.713_714del	p.(Cys238*)	Nonsense	7	Disruptive	Truncating	1 (0.40%)	Yes
c.713G>A	p.(Cys238Tyr)	Missense	7	Nondisruptive	Notruncating	2 (0.81%)	Yes

#1, Disruptive / Nondisruptive[14,16]; #2, Truncating / Notruncating[17]. *IARC (International Agency for Research on Cancer) TP53 database - <https://p53.iarc.fr/> - The database consist of several TP53 mutations data, such as somatic and germline mutations, polymorphism, function, cell-lines, and mouse model. Therefore, various annotations on the predicted or experimentally functional impact of mutations, clinicopathological features of tumors, and demographic patients' data are available.

Continued Supplementary Table 2 - Description and frequency of identified mutations in *TP53* gene (n=249).

c.	p.	Type	TP53 Exon	Classification#1	Classification#2	Frequency	Presenting in IARC Database*
c.715A>G	p.(Asn239Asp)	Missense	7	Disruptive	Notruncating	1 (0.40%)	Yes
c.718_719delinsGT	p.(Ser240Val)	Missense	7	Disruptive	Notruncating	1 (0.40%)	Yes
c.722C>T	p.(Ser241Phe)	Missense	7	Disruptive	Notruncating	2 (0.81%)	Yes
c.725G>A	p.(Cys242Tyr)	Missense	7	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.727_730delATGGinsTTT	p.(Met243Phefs*4)	Frameshift	7	Disruptive	Truncating	1 (0.40%)	No
c.730G>T	p.(Gly244Cys)	Missense	7	Disruptive	Notruncating	1 (0.40%)	Yes
c.733_734delGGinsTT	p.(Gly245Phe)	Missense	7	Nondisruptive	Notruncating	1 (0.40%)	No
c.733G>A	p.(Gly245Ser)	Missense	7	Disruptive	Notruncating	2 (0.81%)	Yes
c.733G>T	p.(Gly245Cys)	Missense	7	Disruptive	Notruncating	3 (1.21%)	Yes
c.734G>T	p.(Gly245Val)	Missense	7	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.742C>T	p.(Arg248Trp)	Missense	7	Disruptive	Notruncating	2 (0.81%)	Yes
c.743G>A	p.(Arg248Gln)	Missense	7	Disruptive	Notruncating	3 (1.21%)	Yes
c.743G>T	p.(Arg248Leu)	Missense	7	Disruptive	Notruncating	1 (0.40%)	Yes
c.746G>T	p.(Arg249Met)	Missense	7	Disruptive	Notruncating	2 (0.81%)	Yes
c.747G>T	p.(Arg249Ser)	Missense	7	Disruptive	Notruncating	4 (1.61%)	Yes
c.747G>T	p.(Arg249Ser)	Missense	7	Disruptive	Notruncating	1 (0.40%)	Yes
c.748_750delCCC	p.(Pro250del)	Deletion/Insertion	7	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.748C>T	p.(Pro250Ser)	Missense	7	Disruptive	Notruncating	1 (0.40%)	Yes
c.751A>T	p.(Ile251Phe)	Missense	7	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.754_762delCTCACCATC	p.(Leu252Ile254del)	Deletion/Insertion	7	Nondisruptive	Notruncating	1 (0.40%)	No
c.772_774del	p.(Glu258del)	Deletion/Insertion	7	Nondisruptive	Notruncating	1 (0.40%)	Yes

#1, Disruptive / Nondisruptive[14,16]; #2, Truncating / Notruncating[17]. *IARC (International Agency for Research on Cancer) TP53 database - <https://p53.iarc.fr/> - The database consist of several TP53 mutations data, such as somatic and germline mutations, polymorphism, function, cell-lines, and mouse model. Therefore, various annotations on the predicted or experimentally functional impact of mutations, clinicopathological features of tumors, and demographic patients' data are available.

Continued Supplementary Table 2 - Description and frequency of identified mutations in *TP53* gene (n=249).

c.	p.	Type	TP53 Exon	Classification#1	Classification#2	Frequency	Presenting in IARC Database*
c.775G>T	p.(Asp259Tyr)	Missense	7	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.796G>C	p.(Gly266Arg)	Missense	8	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.796G>T	p.(Gly266*)	Nonsense	8	Disruptive	Truncating	1 (0.40%)	Yes
c.797G>A	p.(Gly266Glu)	Missense	8	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.797G>T	p.(Gly266Val)	Missense	8	Nondisruptive	Notruncating	2 (0.81%)	Yes
c.799C>T	p.(Arg267Trp)	Missense	8	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.809T>C	p.(Phe270Ser)	Missense	8	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.811G>A	p.(Glu271Lys)	Missense	8	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.814G>A	p.(Val272Met)	Missense	8	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.814G>T	p.(Val272Leu)	Missense	8	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.817C>T	p.(Arg273Cys)	Missense	8	Nondisruptive	Notruncating	5 (2.02%)	Yes
c.818G>A	p.(Arg273His)	Missense	8	Nondisruptive	Notruncating	3 (1.21%)	Yes
c.818G>T	p.(Arg273Leu)	Missense	8	Nondisruptive	Notruncating	6 (2.42%)	Yes
c.819_841del	p.(Val274Profs*24)	Frameshift	8	Disruptive	Truncating	1 (0.40%)	No
c.820G>T	p.(Val274Phe)	Missense	8	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.823T>C	p.(Cys275Arg)	Missense	8	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.825T>G	p.(Cys275Trp)	Missense	8	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.827C>A	p.(Ala276Asp)	Missense	8	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.827C>G	p.(Ala276Gly)	Missense	8	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.830G>T	p.(Cys277Phe)	Missense	8	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.833C>G	p.(Pro278Arg)	Missense	8	Nondisruptive	Notruncating	1 (0.40%)	Yes

#1, Disruptive / Nondisruptive[14,16]; #2, Truncating / Notruncating[17]. *IARC (International Agency for Research on Cancer) TP53 database - <https://p53.iarc.fr/> - The database consist of several TP53 mutations data, such as somatic and germline mutations, polymorphism, function, cell-lines, and mouse model. Therefore, various annotations on the predicted or experimentally functional impact of mutations, clinicopathological features of tumors, and demographic patients' data are available.

Continued Supplementary Table 2 - Description and frequency of identified mutations in *TP53* gene (n=249).

c.	p.	Type	TP53 Exon	Classification#1	Classification#2	Frequency	Presenting in IARC Database*
c.839G>A	p.(Arg280Lys)	Missense	8	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.841G>C	p.(Asp281His)	Missense	8	Nondisruptive	Notruncating	2 (0.81%)	Yes
c.844C>T	p.(Arg282Trp)	Missense	8	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.845G>A	p.(Arg282Gln)	Missense	8	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.848G>C	p.(Arg283Pro)	Missense	8	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.856G>A	p.(Glu286Lys)	Missense	8	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.857A>G	p.(Glu286Gly)	Missense	8	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.857A>T	p.(Glu286Val)	Missense	8	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.858A>C	p.(Glu286Asp)	Missense	8	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.880G>T	p.(Glu294*)	Nonsense	8	Disruptive	Truncating	1 (0.40%)	Yes
c.892G>T	p.(Glu298*)	Nonsense	8	Disruptive	Truncating	3 (1.21%)	Yes
c.947_949delinsTCT	p.(Pro316_Gln317delinsLeu)	Deletion/Insertion	9	Nondisruptive	Notruncating	1 (0.40%)	No
c.958A>T	p.(Lys320*)	Nonsense	9	Disruptive	Truncating	1 (0.40%)	Yes
c.963del	p.(Lys321Asnfs*24)	Frameshift	9	Disruptive	Truncating	1 (0.40%)	Yes
c.991 dupC	p.(Gln331Profs*6)	Frameshift	9	Disruptive	Truncating	2 (0.81%)	No
c.991C>T	p.(Gln331*)	Nonsense	9	Disruptive	Truncating	2 (0.81%)	Yes
c.993G>T	p.(Gln331His)	Missense	9	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.1001G>T	p.(Gly334Val)	Missense	10	Nondisruptive	Notruncating	2 (0.81%)	Yes
c.1006G>T	p.(Glu336*)	Nonsense	10	Disruptive	Truncating	1 (0.40%)	Yes
c.1010G>A	p.(Arg337His)	Missense	10	Nondisruptive	Notruncating	11 (4.44%)	Yes
c.1010G>T	p.(Arg337Leu)	Missense	10	Nondisruptive	Notruncating	1 (0.40%)	Yes

#1, Disruptive / Nondisruptive[14,16]; #2, Truncating / Notruncating[17]. *IARC (International Agency for Research on Cancer) TP53 database - <https://p53.iarc.fr/> - The database consist of several TP53 mutations data, such as somatic and germline mutations, polymorphism, function, cell-lines, and mouse model. Therefore, various annotations on the predicted or experimentally functional impact of mutations, clinicopathological features of tumors, and demographic patients' data are available.

Continued Supplementary Table 2 - Description and frequency of identified mutations in *TP53* gene (n=249).

c.	p.	Type	TP53 Exon	Classification#1	Classification#2	Frequency	Presenting in IARC Database*
c.1015G>T	p.(Glu339*)	Nonsense	10	Disruptive	Truncating	1 (0.40%)	Yes
c.1027G>T	p.(Glu343*)	Nonsense	10	Disruptive	Truncating	1 (0.40%)	Yes
c.1036G>T	p.(Glu346*)	Nonsense	10	Disruptive	Truncating	1 (0.40%)	Yes
c.1037C>T	p.(Ser346Leu)	Missense	10	Nondisruptive	Notruncating	1 (0.40%)	No
c.997del	p.(Arg333Valfs*12)	Frameshift	10	Disruptive	Truncating	1 (0.40%)	Yes
c.1132T>C	p.(Ser378Pro)	Missense	11	Nondisruptive	Notruncating	1 (0.40%)	Yes
c.1155dupC	p.(Lys386Glnfs*6)	Frameshift	11	Disruptive	Truncating	2 (0.81%)	No
c.97-1G>T	p.(?)	Splice_site	Intron 3 (between exons 3-4)	Disruptive	Truncating	1 (0.40%)	Yes
c.375+1G>A	p.(?)	Splice_site	Intron 4 (between exons 4-5)	Disruptive	Truncating	1 (0.40%)	Yes
c.560-1G>T	p.(?)	Splice_site	Intron 5 (between exons 5-6)	Disruptive	Truncating	1 (0.40%)	Yes
c.782_782+18del	p.(?)	Splice_site	Intron 7 (between exons 7-8)	Disruptive	Truncating	1 (0.40%)	No
c.782+1G>A	p.(?)	Splice_site	Intron 7 (between exons 7-8)	Disruptive	Truncating	1 (0.40%)	Yes
c.920-2A>T	p.(?)	Splice_site	Intron 8 (between exons 8-9)	Disruptive	Truncating	2 (0.81%)	Yes
c.994-2A>T	p.(?)	Splice_site	Intron 9 (between exons 9-10)	Disruptive	Truncating	2 (0.81%)	Yes

#1, Disruptive / Nondisruptive[14,16]; #2, Truncating / Notruncating[17]. *IARC (International Agency for Research on Cancer) TP53 database - <https://p53.iarc.fr/> - The database consist of several TP53 mutations data, such as somatic and germline mutations, polymorphism, function, cell-lines, and mouse model. Therefore, various annotations on the predicted or experimentally functional impact of mutations, clinicopathological features of tumors, and demographic patients' data are available.

Supplementary Table 3 - Association of adenocarcinoma patients' features and *TP53* mutational status.

		TP53 STATUS (N=366)													
		Wild-type (n =145)		Nondisruptive (n=125)		Disruptive (n=96)				Truncating (n=63)		Notruncating (n=158)			
FEATURES	Parameters	n (%)		n (%)		n (%)		p value	n (%)		n (%)		p value		
AGE (YEAR)*	Median (range)	65	(27 – 86)	64	(36 – 88)	62.5	(26 – 84)	0.047	61	(26 – 81)	64	(36 – 88)	0.003		
	<=64	66	(34.4)	66	(34.4)	60	(31.3)	0.036	46	(24.0)	80	(41.6)	0.001		
	>64	79	(45.4)	59	(33.9)	36	(20.7)		17	(9.8)	78	(44.8)			
SEX	Female	73	(42.4)	49	(28.5)	50	(29.1)	0.095	36	(20.9)	63	(36.6)	0.041		
	Male	72	(37.1)	76	(39.2)	46	(23.7)		27	(13.9)	95	(49.0)			
SELF-REPORTED SKIN COLOR	White	94	(43.1)	70	(32.1)	54	(24.8)	0.253	39	(17.9)	85	(39.0)	0.136		
	Non-White	51	(34.4)	55	(37.2)	42	(28.4)		24	(16.2)	73	(49.3)			
SMOKING STATUS	Never	56	(54.4)	28	(27.2)	19	(18.4)	0.004	14	(13.6)	33	(32.0)	0.005		
	Quitter	41	(29.9)	52	(38.0)	44	(32.1)		22	(18.3)	52	(43.4)			
	Current	46	(38.3)	42	(35.0)	32	(26.7)		26	(19.0)	70	(51.1)			
	Missing	2		3		1			1		3				
LOSS OF WEIGHT**	No	56	(49.1)	32	(28.1)	26	(22.8)	0.385	15	(13.2)	43	(37.7)	0.257		
	Yes, <=10%	38	(35.8)	38	(35.8)	30	(28.3)		24	(22.6)	44	(41.6)			
	Yes, >10%	31	(41.3)	23	(30.7)	21	(28.0)		13	(17.3)	31	(41.4)			
	Missing	20		32		19			11		40				
DISEASE STAGE AT DIAGNOSIS***	I/II	27	(54.0)	17	(34.0)	6	(12.0)	0.072	4	(8.0)	19	(38.0)	0.117		
	III	21	(44.6)	13	(27.7)	13	(27.7)		9	(19.1)	17	(36.2)			
	IV	93	(36.5)	89	(34.9)	73	(28.6)		46	(18.0)	116	(45.5)			
	Missing	4		6		4			4		6				

n, number of patients; CNS, central nervous system; *p*-value: significance of Fisher exact test/ χ^2 test for categorical variables and Kruskal-Wallis test for continuous variables (age); *age was categorized into two groups considering the median age of the entire series as the cutoff; **Loss of weight <10% and >10% of total body weight; PS ECOG, performance status ECOG (Eastern Cooperative Oncology Group); *** according to AJCC 7th edition; **** Not Otherwise Specified consist of samples that standard could be assessed. Significant associations are indicated in bold. #, previously reported [20].

Continued Supplementary Table 3 - Association of adenocarcinoma patients' features and *TP53* mutational status.

		TP53 STATUS (N=366)											
FEATURES	Parameters	Wild-type (n =145)		Nondisruptive (n=125)		Disruptive (n=96)		p value	Truncating (n=63)		Notruncating (n=158)		p value
		n (%)		n (%)		n (%)			n (%)		n (%)		
PS ECOG	0	31	(52.5)	19	(32.2)	9	(15.3)	0.036	7	(11.9)	21	(35.6)	0.027
	1	67	(46.5)	40	(27.8)	37	(25.7)		20	(13.9)	57	(39.6)	
	2	23	(31.5)	29	(39.7)	21	(28.8)		14	(19.2)	36	(49.3)	
	3/4	13	(28.2)	16	(34.8)	17	(37.0)		14	(30.4)	19	(41.4)	
	Missing	11		21		12			8		25		
METASTASIS AT DIAGNOSIS	No	48	(49.5)	30	(30.9)	19	(19.6)	0.002	13	(13.4)	36	(37.1)	0.001
	Yes, CNS	20	(23.3)	33	(38.4)	33	(38.4)		24	(27.9)	42	(48.8)	
	Yes, Others	72	(44.7)	51	(31.7)	38	(23.6)		21	(13.0)	68	(42.2)	
	Missing	5		11		6			5		12		
ADENOCARCINOMA STANDARD	Acinar	26	(44.1)	20	(33.9)	13	(22.0)	0.002	8	(13.6)	25	(42.4)	0.003
	Solid	21	(38.2)	16	(29.1)	18	(32.7)		11	(20.0)	23	(41.8)	
	Lepidic	8	(66.7)	4	(33.3)	0	(0.0)		0	(0.0)	4	(33.3)	
	Mix	28	(54.9)	11	(21.6)	12	(23.5)		9	(17.6)	14	(27.5)	
	Nos****	48	(28.9)	71	(42.8)	47	(28.3)		30	(18.1)	88	(53.0)	
	Others	12	(70.6)	3	(17.6)	2	(11.8)		2	(11.8)	3	(17.6)	
	Missing	2		0		4			3		1		

n, number of patients; *CNS*, central nervous system; *p*-value: significance of Fisher exact test/ χ^2 test for categorical variables and Kruskal-Wallis test for continuous variables (*age*); **age* was categorized into two groups considering the median age of the entire series as the cutoff; **Loss of weight <10% and >10% of total body weight; *PS ECOG*, performance status ECOG (Eastern Cooperative Oncology Group); *** according to AJCC 7th edition; **** Not Otherwise Specified consist of samples that standard could be assessed. Significant associations are indicated in bold. #, previously reported [20].

Continued Supplementary Table 3 - Association of adenocarcinoma patients' features and TP53 mutational status.

		TP53 STATUS (N=366)											
		Wild-type (n =145)		Nondisruptive (n=125)		Disruptive (n=96)			Truncating (n=63)		Notruncating (n=158)		
FEATURES	Parameters	n (%)		n (%)		n (%)		p value	n (%)		n (%)		p value
EGFR STATUS	Wild-type	113	(40.8)	91	(32.9)	73	(26.4)	0.612	47	(17.0)	117	(42.2)	0.750
	Mutated	30	(36.1)	32	(38.6)	21	(25.3)		15	(18.1)	38	(45.8)	
	Missing	2		2		2			1		3		
KRAS STATUS#	Wild-type	99	(38.1)	97	(37.3)	64	(24.6)	0.122	42	(16.1)	120	(46.0)	0.240
	Mutated	43	(44.3)	25	(25.8)	29	(29.9)		19	(19.6)	35	(36.1)	
	Missing	3		3		3			2		3		
ASIAN ANCESTRY	Low	46	(43.8)	38	(36.2)	21	(20.0)	0.220	14	(13.3)	45	(42.9)	0.373
	Intermedium	45	(43.3)	32	(30.8)	27	(26.0)		17	(16.3)	42	(40.4)	
	High	34	(33.0)	35	(34.0)	34	(33.0)		22	(21.4)	47	(45.6)	
	Missing	20		20		14			10		24		
AFRICAN ANCESTRY	Low	59	(55.7)	31	(29.2)	16	(15.1)	<0.0001	13	(12.3)	34	(32.1)	0.001
	Intermedium	39	(37.9)	38	(36.9)	26	(25.2)		17	(16.5)	47	(45.6)	
	High	27	(26.2)	36	(35.0)	40	(38.8)		23	(22.3)	53	(51.5)	
	Missing	20		20		14			10		24		

n, number of patients; CNS, central nervous system; *p*-value: significance of Fisher exact test/ χ^2 test for categorical variables and Kruskal-Wallis test for continuous variables (age); *age was categorized into two groups considering the median age of the entire series as the cutoff; **Loss of weight <10% and >10% of total body weight; PS ECOG, performance status ECOG (Eastern Cooperative Oncology Group); *** according to AJCC 7th edition; **** Not Otherwise Specified consist of samples that standard could be assessed. Significant associations are indicated in bold. #, previously reported [20].

Continued Supplementary Table 3 - Association of adenocarcinoma patients' features and TP53 mutational status.

		TP53 STATUS (N=366)											
FEATURES	Parameters	Wild-type (n =145)		Nondisruptive (n=125)		Disruptive (n=96)		p value	Truncating (n=63)		Notruncating (n=158)		p value
		n (%)		n (%)		n (%)			n (%)		n (%)		
EUROPEAN ANCESTRY	Low	59	(55.7)	31	(29.2)	16	(15.1)	<0.0001	26	(24.8)	49	(46.7)	0.001
	Intermedium	39	(37.9)	38	(36.9)	26	(25.2)		18	(17.5)	47	(45.6)	
	High	27	(26.2)	36	(35.0)	40	(38.8)		9	(8.7)	38	(36.5)	
	Missing	20		20		14			10		24		
NATIVE AMERICAN ANCESTRY	Low	50	(46.3)	40	(37.0)	18	(16.7)	0.022	9	(8.3)	49	(45.4)	0.007
	Intermedium	45	(42.9)	31	(29.5)	29	(27.6)		18	(17.1)	42	(40.0)	
	High	30	(30.3)	34	(34.3)	35	(35.4)		26	(26.3)	43	(43.4)	
	Missing	20		20		14			10		24		
VITAL STATUS	Alive – no disease	6	(37.5)	6	(37.5)	4	(25.0)	0.184	2	(12.5)	8	(50.0)	0.217
	Alive – active disease	61	(47.7)	43	(33.6)	24	(18.8)		15	(11.7)	52	(40.6)	
	Death by cancer	73	(34.6)	72	(34.1)	66	(31.3)		44	(20.9)	94	(44.5)	
	Death by others	3	(37.5)	3	(37.5)	2	(25.0)		2	(25.0)	3	(37.5)	
	Missing	2		1		0			0		1		

n, number of patients; CNS, central nervous system; *p*-value: significance of Fisher exact test/ χ^2 test for categorical variables and Kruskal-Wallis test for continuous variables (age); *age was categorized into two groups considering the median age of the entire series as the cutoff; **Loss of weight <10% and >10% of total body weight; PS ECOG, performance status ECOG (Eastern Cooperative Oncology Group); *** according to AJCC 7th edition; **** Not Otherwise Specified consist of samples that standard could be assessed. Significant associations are indicated in bold. #, previously reported [20].

Supplementary Table 4 – Association of *TP53* mutations and patients' features from cBioportal online dataset.

FEATURES		TP53 STATUS (N=547)				p value
		Wild-type (n =248)		Mutated (n=299)		
		n (%)		n (%)		
Parameters						
AGE (YEAR)	Median (range)	67.5	(39 – 87)	63.0	(33 – 88)	0.001
	<=64	84	(40.0)	126	(60.0)	0.002
	>64	136	(54.6)	113	(45.4)	
	Missing	28		60		
SEX	Female	142	(45.2)	127	(54.8)	0.950
	Male	106	(45.5)	172	(54.5)	
	Missing	0		0		
SELF-REPORTED SKIN COLOR	White	211	(47.5)	233	(52.5)	0.073
	Black/African	18	(31.6)	39	(68.4)	
	Other	18	(43.9)	23	(56.1)	
	Missing	1		4		

n, number of patients; *p*-value: significance of Fisher exact test/ χ^2 test for categorical variables and U-Mann-Whitney test for continuous variables (age); *age was categorized into two groups considering the median age of the entire series as the cutoff; Significant associations are indicated in bold.

5.2 Frequência da mutação p.Gly12Cys no gene *KRAS* em pacientes brasileiros.

Este artigo avaliou a frequência de pacientes carreadores da mutação no gene *KRAS* em pacientes do Hospital de Amor de Barretos e Laboratórios Bacchi. Ademais, foi avaliado o perfil de ancestralidade genética dos pacientes, e sua associação com os dados clínicos patológicos e sobrevida dos pacientes.

Frequency of *KRAS* p.Gly12Cys Mutation in Brazilian Patients With Lung Cancer

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INTRODUCTION

Lung cancer is the deadliest cancer worldwide, and in Brazil.^{1,2} In the past decade, targeted therapies have revolutionized the clinical management of lung cancer, particularly in non-small-cell lung cancer (NSCLC) subtype.³⁻⁸ The most successful examples of targeted therapies are the EGFR and ALK inhibitors, used for *EGFR*-mutated and *ALK*-translocated tumors, respectively.^{9,10}

KRAS is one of the most frequently mutated genes in NSCLC. The frequency of *KRAS* mutations varies among distinct populations, accounting for approximately 25% in Whites and < 10% in East Asians.¹¹ *KRAS* driver mutations are mostly located in codons 12 and 13, and the most frequent one is the p.Gly12Cys (c.34G>T) mutation.¹²⁻¹⁸ In lung cancer, *KRAS* mutations are associated with smokers and with a more aggressive phenotype.^{12,19-22} Efforts have been made in the past decade for rendering *KRAS* mutations susceptible to targeting.²³ However, until lately, *KRAS*-mutated tumors were, unfortunately, undruggable.¹⁰

Recently, the agents AMG-510 (sotorasib, Amgen, Thousand Oaks, CA) and MRTX849 (adagrasib, Mirati Therapeutics, San Diego, CA) were developed to target the *KRAS* p.Gly12Cys mutation.^{24,25} These specific inhibitors locked *KRAS* p.Gly12Cys mutation in an inactive state, hampering the oncogenic signals and allowed the normal function of remained wild-type *KRAS*.²⁴⁻²⁶ In a phase I study, 32.2% (19 out of 59) of sotorasib-treated patients presented with objective response, and 88.1% (52 out of 59) presented with the disease control.²⁶ In a phase I and II study, 94% (17 out of 18) adagrasib-treated lung patients presented with disease control, and objective response was not yet available (KRYSTAL-1 study; ClinicalTrials.gov identifier: NCT03785249).²⁵

The frequency of *KRAS* p.Gly12Cys in admixture NSCLC populations remains scarce. Herein, we report the frequency of the *KRAS* p.Gly12Cys mutation in a series of 844 Brazilian NSCLC cases, followed by the data gathered from Brazil's previously reported studies.

METHODS

This retrospective study included 844 patients diagnosed with NSCLC. Seven hundred fifty-four patients

were diagnosed at Barretos Cancer Hospital (BCH), and 90 patients were diagnosed at Bacchi Laboratory. Tobacco exposure, performance status, and overall survival data were provided for a subset of patients (BCH). This study was approved by the local IRB (Project no. 630/2012), and all procedures were performed following the Helsinki Declaration.

KRAS mutational status was evaluated from FFPE tumor tissue using different methodologies. The cases diagnosed at Barretos Cancer Hospital from 2014 to 2017 (n = 319) were genotyped by polymerase chain reaction followed by direct Sanger sequencing, and from 2018 to 2020 (n = 435) was assessed by next-generation sequencing, using the TruSight Tumor 15 (Illumina Waltham, MA) as reported by our group.^{12,27,28} The cases diagnosed at Bacchi Laboratory were analyzed by qPCR TaqMan-MGB allelic discrimination assay (n = 67) and by FoundationOne (n = 23) between 2018 and 2020.^{29,30} Genetic ancestry was analyzed in a subset of patients from Barretos Cancer Hospital (n = 660 out of 844), as previously described.¹²

For statistical analysis, the percentage was used to describe categorical variables, and medians were used to describe continuous variables. Fisher's exact test and χ^2 test were used for the association between *KRAS* mutations and the clinicopathologic data. The log-rank test and the Kaplan-Meier curves were used to analyze patients' overall survival. The Cox regression method was used to investigate the association of clinicopathologic data to the outcome (death). All tests were made in the software IBM SPSS Statistics version 22 with a limit of statistical significance of 0.05.

RESULTS

We evaluated the frequency of *KRAS* mutations in a series of 844 NSCLC (Table 1). The median age of the cohort was 64 years, 55.4% (n = 468 out of 844) were male, 88.2% (n = 744 out of 844) were adenocarcinoma, and 2.2% (n = 19 out of 844) were squamous-cell carcinoma. Concerning tobacco consumption, 63.5% (n = 536 out of 844) were current or quitter smoking, 65.3% (n = 552 out of 754) were diagnosed in an advanced stage of the disease, and

ASSOCIATED CONTENT

Appendix

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

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TABLE 1. Association of Patients' Characteristics and *KRAS* Mutational Status

Characteristics	Parameters	<i>KRAS</i> Status (N = 844)		P ^a
		Wild-Type (n = 630) No. (%)	Mutated (n = 214) No. (%)	
Age, years	Median (range)	64 (21-94)	64 (31-87)	.150 ^b
	≤ 64	319 (73.8)	113 (26.2)	.689
	> 64	295 (75.3)	97 (24.7)	
	Missing	16	4	
Sex	Female	286 (73.6)	100 (26.4)	.524
	Male	354 (75.6)	114 (24.4)	
Smoking status	Never	155 (92.3)	13 (7.7)	< .0001
	Quitter	174 (71.6)	69 (28.4)	
	Current	201 (68.6)	92 (31.4)	
	Missing	100	40	
Disease stage at diagnosis ^c	I or II	66 (71.0)	27 (29.0)	.337
	III	80 (80.0)	20 (20.0)	
	IV	412 (74.6)	140 (25.4)	
	Missing	72	27	
Histology	Adenocarcinoma	549 (73.8)	195 (26.2)	.095
	Squamous-cell carcinoma	18 (94.7)	1 (5.3)	
	Other ^d	63 (77.8)	18 (22.2)	
ECOG PS	0	74 (74.7)	25 (25.3)	.004
	1	248 (78.5)	68 (21.5)	
	2	99 (78.6)	27 (21.4)	
	3 or 4	49 (59.9)	33 (40.2)	
	Missing	160	61	
Asian ancestry	Low	171 (76.0)	54 (24.0)	.725
	Intermedium	169 (77.5)	49 (22.5)	
	High	161 (74.2)	56 (25.8)	
	Missing	129	55	
African ancestry	Low	160 (72.4)	61 (27.6)	.180
	Intermedium	166 (75.5)	54 (24.5)	
	High	175 (79.9)	44 (20.1)	
	Missing	129	55	
European ancestry	Low	171 (77.4)	50 (22.6)	.406
	Intermedium	170 (77.6)	49 (22.4)	
	High	160 (72.7)	60 (27.3)	
	Missing	129	55	
Native American ancestry	Low	190 (81.5)	43 (18.5)	.006
	Intermedium	145 (68.7)	66 (31.3)	
	High	166 (76.9)	50 (23.1)	
	Missing	129	55	
Vital status	Alive with disease	165 (78.6)	45 (21.4)	.370
	Alive with no disease	12 (75.0)	4 (25.0)	
	Death by disease	367 (75.2)	121 (24.8)	
	Death by others causes	7 (58.3)	5 (41.7)	
	Missing	79	39	

Abbreviations: AJCC, American Joint Committee on Cancer; ECOG PS, Eastern Cooperative Oncology Group performance status; NOS, not otherwise specified.

^aFisher's exact test or χ^2 test.

^bMann-Whitney test.

^cAccording to AJCC 7th edition.

^dIncluding NOS.

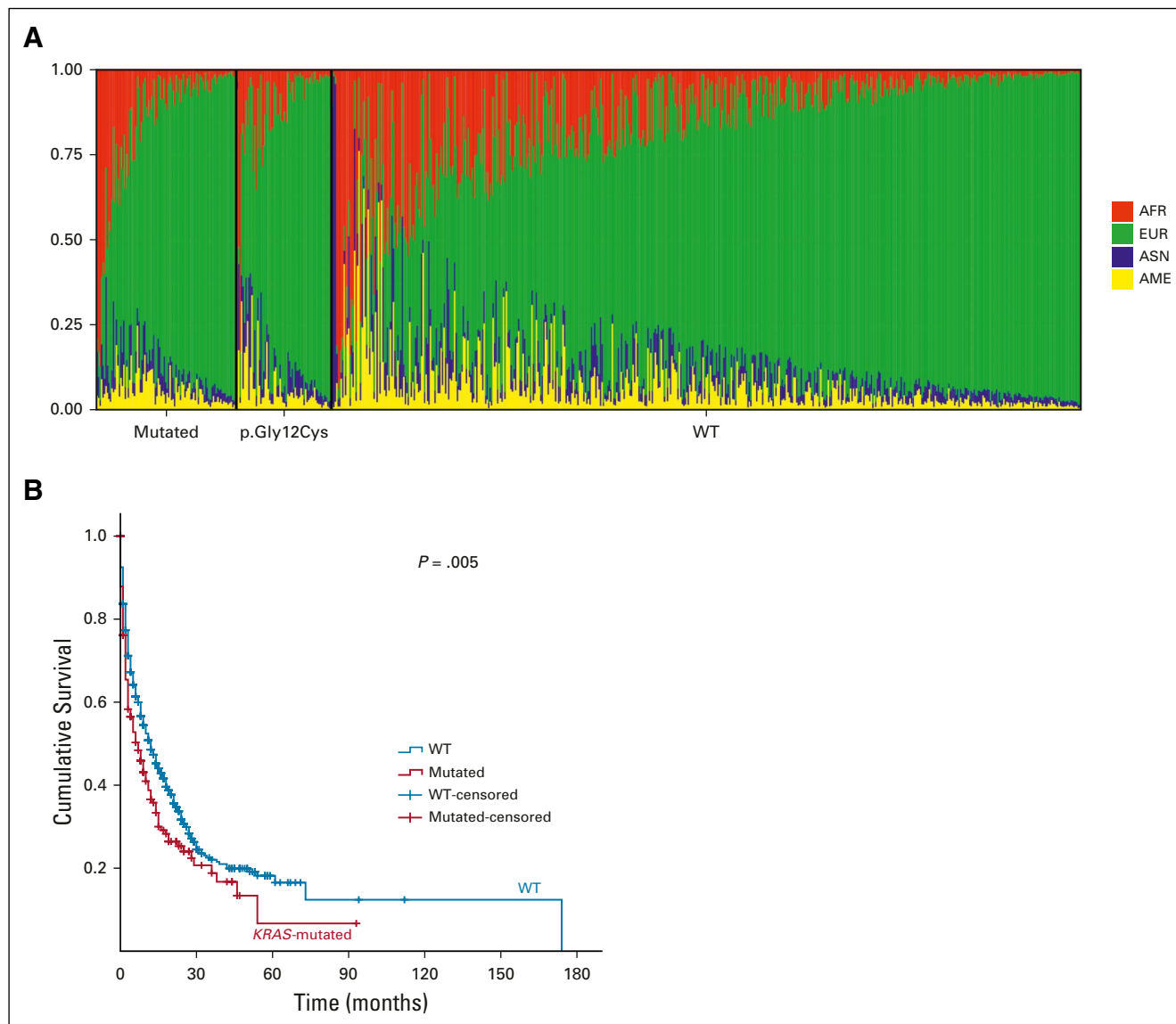


FIG 1. (A) Ancestry background of patients, divided in mutated patients and wild-type patients (n = 660). (B) Kaplan-Meier comparing wild-type patients with mutated patients. AFR, African; AME, Native American; ASN, Asian; EUR, European; WT, wild-type.

9.7% (n = 82 out of 844) were diagnosed with worse Eastern Cooperative Oncology Group performance status (ECOG PS; Table 1).

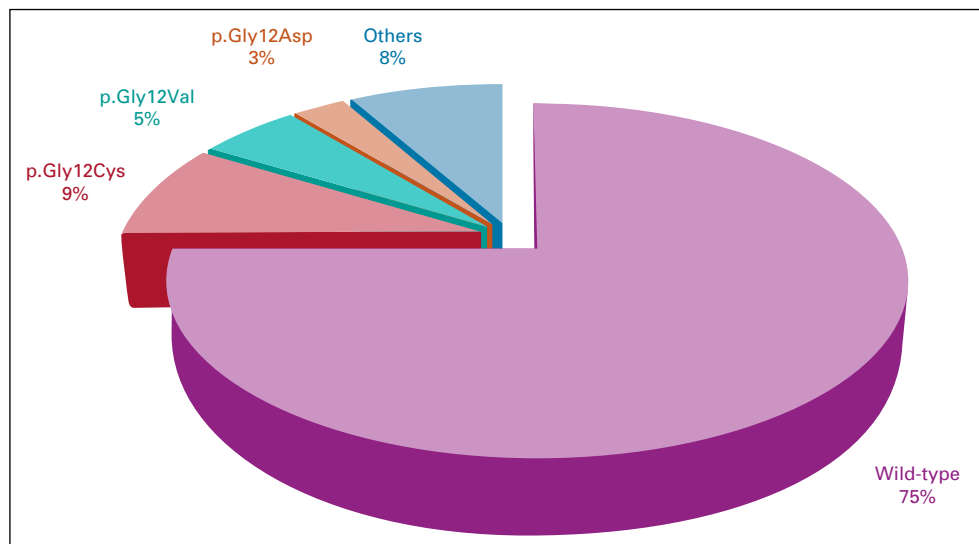
KRAS was mutated in 214 cases (25.3%; Table 1). A detailed description of *KRAS* mutation variants is described at Appendix Table A1. Briefly, in the adenocarcinoma subtype, 26.2% (n = 195 out of 744) were *KRAS*-mutated, with p.Gly12Cys being the most frequent mutation identified in 9.4% (n = 70 out of 744), followed by p.Gly12Val in 6.2% (n = 46 out of 744). Among squamous-cell carcinomas, 5.3% (n = 1 out of 19) were *KRAS*-mutated (p.Gly12Asp). Concerning other histologies, 22.2% (n = 18 out of 81) were *KRAS*-mutated, with p.Gly12Cys being the most frequent mutation identified in 7.4% (n = 6 out of 81; Appendix Table A1).

The genetic ancestry evaluation in a subset of patients (n = 660 out of 844) showed the following proportion of

TABLE 2. Frequency of *KRAS* Mutations in Brazilian Patients

Author	Year	No.	<i>KRAS</i> -Mutated (%)	p.Gly12Cys (%)
Bacchi et al ¹⁷	2012	206	30 (15)	15 (7)
De melo et al ¹⁶	2015	125	33 (26)	15 (12)
Andreis et al ¹⁵	2019	619	189 (31)	70 (11)
Leal et al ¹²	2019	444	90 (20)	32 (7)
Freitas et al ¹⁸	2020	495	133 (27)	46 (9)
Mascarenhas et al ³⁷	2020	513	124 (24)	31 (6)
This study	2021	844	214 (25)	76 (9)
Total	—	3,247	813 (25)	285 (9)

FIG 2. Frequency of *KRAS* mutations in Brazilian patients with non–small-cell lung cancer (n = 3,247).



ancestry background: 72.2% for European, 14.0% for African, 6.4% for Asian, and 7.5% for Native American (Fig 1A).

KRAS mutation status was further associated with clinicopathologic and ancestry features (Table 1). Significant associations were found between the presence of *KRAS* mutations and smoking status, ECOG PS at diagnosis, and Native American ancestry (Table 1). Patients harboring *KRAS* mutation had worse overall survival than wild-type patients (Fig 1B). Besides, smoking and higher ECOG PS at diagnosis were significantly associated with higher risk of death by multivariate Cox regression analysis ($P < .0001$ and $P < .0001$, respectively).

We further gathered *KRAS* mutational status reported in the NSCLC Brazilian population (Table 2; Fig 2). Among the 3,247 cases, the *KRAS* mutational frequency was 25.0% (n = 813 out of 3,247)—ranging from 15% to 31% among studies (Table 2). The *KRAS* p.Gly12Cys mutation frequency was 35.0% (n = 285 out of 813) of the *KRAS*-mutated cases, corresponding to 9% (285 out of 3,247) of all Brazilian NSCLC cases—ranging from 6.0% to 12.0% (Table 2; Fig 2).

DISCUSSION

The *KRAS* p.Gly12Cys mutation became a new target for personalized therapy with the sotorasib and adagrasib.^{23,25,26,31} Our study analyzed the frequency of p.Gly12Cys mutation in the Brazilian NSCLC population. We observed that 25% of the 3,247 cases were *KRAS*-mutated, and the most common variant was the p.Gly12Cys, present in 285 (9%) of the cases. Currently, expanded access is available for Brazilian patients and also for patients around the world, since both are non-US Food and Drug Administration-approved drugs. Once US Food and Drug Administration approves any of these drugs—sotorasib and

adagrasib—compassionate drug use may be the option for obtaining access for Brazilian patients.

In our study, the presence of *KRAS* mutations was associated with smoking status (current or quitter) and worse overall survival. These data are in agreement with the literature.^{12,19-22} A recent review reported that *KRAS* mutations are present in 18%-32% of lung adenocarcinoma, 12.8% of large cell carcinoma, 10% of adenosquamous carcinomas, and 1.6%-7.1% of squamous-cell carcinomas in White patients.³² Moreover, African-American patients with NSCLC are more frequently identified with *KRAS* mutations than White patients.³² The frequency of *KRAS* mutations in Western populations with lung adenocarcinoma is about 26% and about 6% in the squamous-cell carcinoma population.³³ In Asian patients, the frequency of *KRAS* mutations is 11.2% of patients with NSCLC.³³ According to The Cancer Genome Atlas, *KRAS* mutations are found in 33% of lung adenocarcinoma.³⁴ A study involving 5,738 NSCLC cases reported 14% of *KRAS*-mutated cases in Latin American except for Brazil (Argentina, Mexico, Colombia, Peru, Costa Rica, and Panama).³⁵

The role of genetic ancestry in *KRAS* mutational status in NSCLC is poorly explored. A recent metadata analysis showed that *KRAS* mutations were more present in White and Black NSCLC patient groups than in Asian.³⁶ In a previous study, our group reported that *KRAS* mutations were associated with low Asian genetic ancestry background.¹² In the current study, using a panel of genetic ancestry markers, these findings were not confirmed in a multivariate analysis. Therefore, further studies using an admixture of populations are needed to clarify this important issue.

In conclusion, we showed that approximately 10% of Brazilian patients with NSCLC harbor the *KRAS* p.Gly12Cys variant and are therefore potentially responsive to the new anti-*KRAS* agents.

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APPENDIX

TABLE A1. Frequency of *KRAS* Mutations Identified According to Histology of the Tumor (n = 214)

Codon	Adenocarcinoma, No. (%)	Squamous-Cell Carcinoma, No. (%)	Other Histologies, No. (%)	Total No. (%)
12				186 (86.9)
p.Gly12Cys	70 (35.9)	0 (0.0)	6 (33.3)	76 (35.5)
p.Gly12Val	46 (23.6)	0 (0.0)	4 (22.2)	50 (23.4)
p.Gly12Asp	31 (15.9)	1 (100.0)	3 (16.7)	35 (16.4)
p.Gly12Ala	11 (5.6)	0 (0.0)	3 (16.7)	14 (6.5)
p.Gly12Ser	6 (3.1)	0 (0.0)	0 (0.0)	6 (2.8)
p.Gly12Arg	2 (1.0)	0 (0.0)	0 (0.0)	2 (0.9)
p.Gly12Phe	3 (1.5)	0 (0.0)	0 (0.0)	3 (1.4)
13				18 (8.4)
p.Gly13Asp	7 (3.6)	0 (0.0)	0 (0.0)	7 (3.3)
p.Gly13Cys	6 (3.1)	0 (0.0)	2 (11.0)	8 (3.7)
p.Gly13dup	1 (0.5)	0 (0.0)	0 (0.0)	1 (0.5)
p.Gly13Tyr	2 (1.0)	0 (0.0)	0 (0.0)	2 (0.9)
Other				10 (4.7)
p.Ser17Thr	1 (0.5)	0 (0.0)	0 (0.0)	1 (0.5)
p.Gly10Ala	1 (0.5)	0 (0.0)	0 (0.0)	1 (0.5)
p.Leu19Phe	2 (1.0)	0 (0.0)	0 (0.0)	2 (0.9)
p.Gln61His	2 (1.0)	0 (0.0)	0 (0.0)	2 (0.9)
p.Gln61Leu	3 (1.6)	0 (0.0)	0 (0.0)	3 (1.4)
Amplification	1 (0.5)	0 (0.0)	0 (0.0)	1 (0.5)

5.3 Frequência de inserções no éxon 20 do gene *ERBB2* em pacientes brasileiros.

Este artigo avaliou a frequência de pacientes carreadores de inserções no éxon 20 do gene *ERBB2* em pacientes brasileiros diagnosticados com câncer de pulmão de não pequenas células no Hospital de Amor de Barretos.

BRIEF REPORT

ERBB2 exon 20 insertions are rare in Brazilian non-small cell lung cancer

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Abstract

ERBB2 exon 20 insertions may impact the clinical management of lung cancer patients. However, the frequency of *ERBB2* exon 20 insertions in lung cancer patients in Brazil is scarce. Here, we analyzed 722 Brazilian non-small cell lung cancer (NSCLC) patients from Barretos Cancer Hospital that were indicated to require routine lung cancer molecular testing. *ERBB2* exon 20 insertions were evaluated by a targeted panel using next-generation sequencing (NGS). Clinicopathological and molecular data were collected from patient medical records. Among the 722 NSCLC patients, 85.2% had lung adenocarcinomas, 53.9% were male, 66.8% were quitter or current smokers, and 63.2% were diagnosed at an advanced stage of the disease. We identified 0.8% (6/722) of patients who harbored the insertion p.(Tyr772_Ala775dup) at exon 20 of the *ERBB2* gene. All *ERBB2* mutated patients were diagnosed with lung adenocarcinoma, were never smokers, and wild-type for *EGFR*, *KRAS*, and *ALK* hotspot alterations. Less than 1% of Brazilian NSCLC patients harbor *ERBB2* exon 20 insertions, yet they could benefit in future from the new drugs in development.

KEYWORDS

Brazil, *ERBB2*, exon 20 insertions, non-small cell lung cancer

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INTRODUCTION

Lung cancer remains the deadliest cancer worldwide.¹ The International Agency for Research on Cancer estimated about 2.2 million new cases and 1.8 million deaths to the year 2020 around the world.^{1,2} In Brazil, lung cancer is one of the most frequent diagnosed cancers, and the leading cause of cancer-related deaths.^{1,2}

Targeted therapies towards specific molecular alterations in non-small cell lung cancer (NSCLC) tumors, such as *EGFR* mutations and *ALK* translocations, have improved the clinical management and prognosis for patients.³ The human epidermal growth factor receptor 2 gene (*ERBB2*) is a proto-oncogene that has emerged as a candidate for targeted therapies in lung adenocarcinoma patients.⁴ *ERBB2* gene is amplified in about 2% of NSCLC tumors, and *ERBB2* mutations have been reported in 2%–4% of NSCLC tumors.⁴ Most *ERBB2* mutations are duplications or insertions of 12 nucleotides at the exon 20.⁴ This insertion adds four aminoacids (TyrValMetAla) in the kinase domain at codon 775, leading putatively to increased gene activation.^{4–6} Additionally, *ERBB2* exon 20 insertions are mutually exclusive with *EGFR* and *KRAS* mutations as either *ALK* translocations.^{4–6} A recent comprehensive review highlighted the importance of *ERBB2* mutations in the clinical management of NSCLC patients, and several drugs targeting *ERBB2* insertion in exon 20, such as trastuzumab deruxtecan, poziotinib and pyrotinib, have been under clinical trials.⁶

The frequency of *ERBB2* exon 20 insertions in lung cancer patients in Brazil is scarce. Therefore, we aimed to evaluate the frequency of *ERBB2* exon 20 insertions and their clinicopathological and molecular features in a series of Brazilian NSCLC patients.

METHODS

We evaluated a retrospective series of 722 Brazilian NSCLC patients, coming from almost all Brazilian regions (Supplementary Figure 1), diagnosed at Barretos Cancer Hospital (Barretos, São Paulo, Brazil). All patients were indicated to routine lung cancer molecular testing (*EGFR*, *KRAS*, and *ERBB2*) and *ALK* translocations at the Department of Molecular Diagnosis from the institution between the years 2018 and 2021. The clinicopathological and molecular data were collected from the medical records of patients. The present study was approved by the Barretos Cancer Hospital IRB (project no. 630/2012) and waived written informed consent due to the retrospective nature of the study. All procedures were performed following the Declaration of Helsinki.

DNA was isolated from formalin-fixed paraffin-embedded (FFPE) tumor using the commercial kit QIAamp DNA Micro Kit (Qiagen). DNA concentration and purity were evaluated by Nanodrop 2000 (Thermo Scientific) and by Qubit 2.0 Fluorometer (Thermo Fisher Scientific) with Qubit dsDNA HS assay kit (Thermo Fisher Scientific).

The mutational status of the exon 20 from *ERBB2* gene (NM_004448) was assessed by next-generation sequencing using the targeted panel TruSight Tumor 15 (Illumina) on the MiSeq instrument, according to the manufacturer's instructions. For the read alignment and variant calling, we used the BaseSpace BWA Enrichment version 2.1 (Illumina) and the Sophia DDM software version 4.2 (Sophia Genetics SA). Only *ERBB2* insertions in exon 20 with depth higher than 500x and allele frequency higher than 5% were selected.

Detection of *ALK* rearrangements were routinely performed in FFPE sections using Ventana *ALK* (D5F3) CDx Assay (Roche) according to the manufacturer's instructions on automated equipment. Slides were evaluated by a specialist pathologist. *ALK* rearrangement was defined as positive by the presence of strong granular cytoplasmic staining in tumor cells (any percentage).

For statistical analysis, we used the frequency and percentage to describe categorical variables and median to describe continuous variables, using the software IBM SPSS Statistics Version 22 (IBM Corp).

RESULTS

The clinicopathological and molecular features of the 722 Brazilian NSCLC patients is summarized in Table 1. Among NSCLC patients, 85.2% ($n = 615/722$) had lung adenocarcinomas, 1.9% ($n = 14/722$) had squamous cell carcinoma, and 12.9% ($n = 93/722$) were from other NSCLC histology. The median age was 64.0 years old, and 53.9% were male ($n = 389/722$). Concerning tobacco use, 66.8% ($n = 482/722$) were quitters or current smokers, 53.6% ($n = 387/722$) were diagnosed with a performance status of 0/1, 46.6% ($n = 336/722$) presented with loss of weight 6 months prior the diagnosis, and 63.2% ($n = 456/722$) were diagnosed with advanced stage of the disease.

Molecularly, 26.2% ($n = 189/722$) were *KRAS*-mutated, 22.6% ($n = 163/722$) were *EGFR*-mutated, and 5.3% ($n = 38/722$) were *ALK*-translocated (Table 1). *ERBB2* exon 20 insertions were identified in 0.8% ($n = 6/722$) of all patients.

We identified six lung adenocarcinoma patients harboring the *ERBB2* inframe insertion p.(Tyr772_Ala775dup) in exon 20 (Figure 1a/b and Table A.1). The features of mutant *ERBB2* patients are described in Table 2. Most patients were female, never smokers, and diagnosed at an advanced stage of disease with metastasis of lung/pleura, bone, and lymph node. All six patients were diagnosed with lung adenocarcinoma and were wild-type for *EGFR*, *KRAS* and *ALK*-translocations (Table 2; Figure 2).

DISCUSSION

Molecular *ERBB2* therapies, namely targeting exon 20 insertion, are being explored in NSCLC patients.⁴ Herein, we

TABLE 1 Clinicopathological and molecular features of NSCLC patients

Variable	Category	NSCLC patients (<i>n</i> = 722)	
		N	(%)
Age (years) ^a	Median (range)	64.0 (26–94)	
	≤64	373	51.7%
	>64	349	48.3%
Sex	Female	333	46.1%
	Male	389	53.9%
Tobacco use	Never smoker	177	24.5%
	Quitter smoker	249	34.5%
	Current smoker	233	32.3%
	Missing	63	8.7%
Loss of weight ^b	No	216	29.9%
	Yes ≤10% of weight	194	26.9%
	Yes >10% of weight	142	19.7%
	Missing	170	23.5%
ECOG PS	0	111	15.4%
	1	276	38.2%
	2	140	19.4%
	3/4	74	10.2%
	Missing	121	16.8%
Histology	Adenocarcinoma	615	85.2%
	Squamous cell carcinoma	14	1.9%
	Others ^c	93	12.9%
Stage at diagnosis ^d	I/II	28	3.9%
	III	154	21.3%
	IV	456	63.2%
	Missing	84	11.6%
Metastasis at diagnosis	No	182	25.2%
	Yes, central nervous system	146	20.2%
	Yes, others	273	37.8%
	Missing	121	16.8%
EGFR status	Wild-type	559	77.4%
	Mutant	163	22.6%
KRAS status	Wild-type	533	73.8%
	Mutant	189	26.2%
ERBB2 exon 20 insertions	Wild-type	716	99.2%
	Mutated	6	0.8%
ALK status	Wild-type	621	86.0%
	Mutant	38	5.3%
	Missing/inconclusive	63	8.7%
Vital status	Alive	336	46.5%
	Death	352	48.8%
	Missing	34	4.7%

n, number of patients.

^aAge was categorized into two groups considering the median of the entire series as the cutoff.^bLast 6 months before the diagnosis.^cAdenosquamous, NOS (not otherwise specified), large cell, sarcomatoid carcinoma, and neuroendocrine carcinoma.^dAccording to AJCC seventh edition.

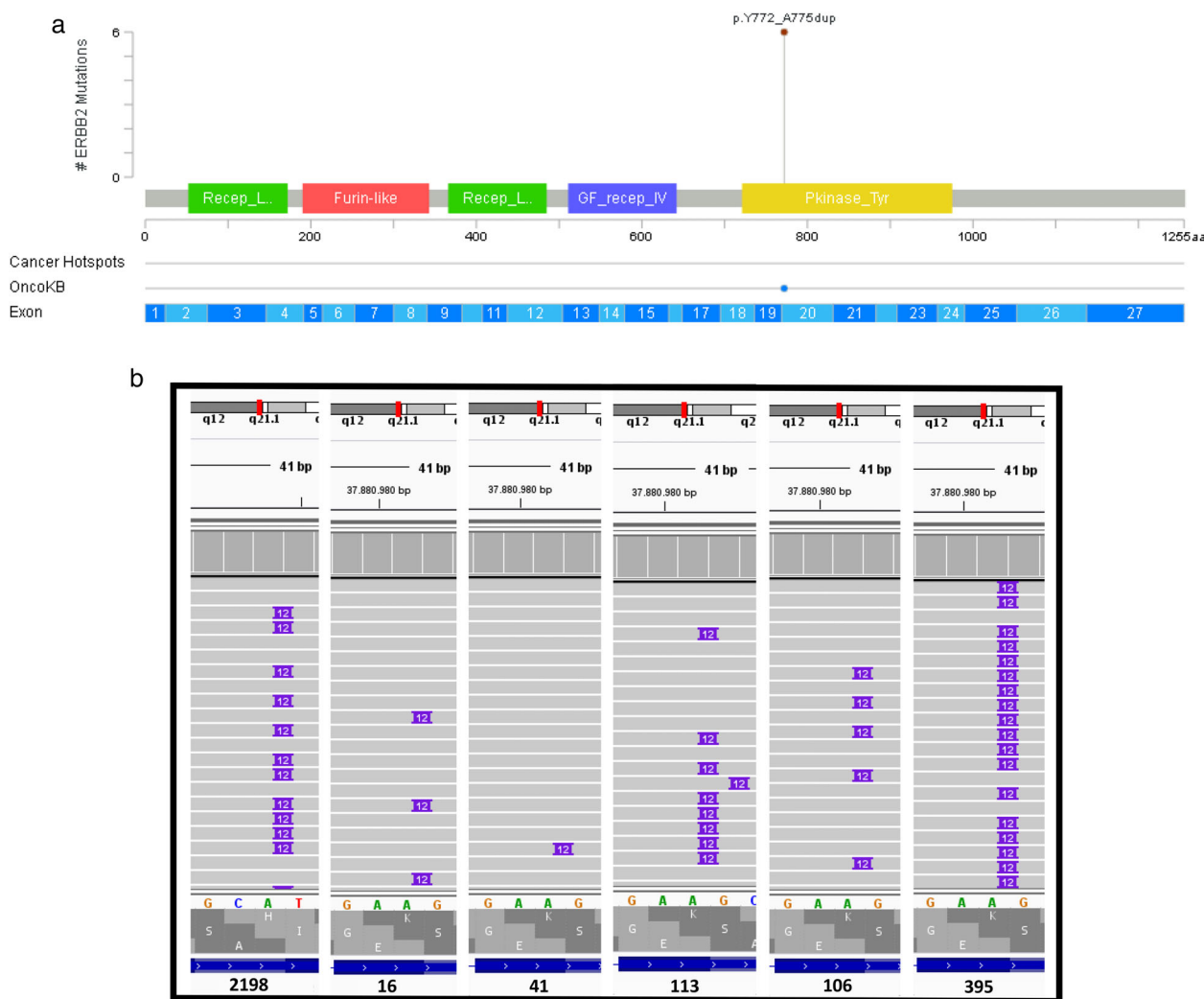


FIGURE 1 (a) *ERBB2* exon 20 insertions identified in non-small cell lung cancer (NSCLC) patients ($n = 6$); Created in cbiportal.gov. (b) *ERBB2* exon 20 insertions identified observed in IGV software

report the frequency of *ERBB2* exon 20 insertions in a series of 722 Brazilian NSCLC patients.

The frequency of NSCLC patients harboring *ERBB2* mutations ranges from 2%–5% in the literature.^{6–8} The Cancer Genome Atlas (TCGA) reported a frequency of 2.6% ($n = 6/230$) for *ERBB2*-mutated patients in lung adenocarcinomas.⁷ Concerning only *ERBB2* exon 20 insertions, TCGA reported a frequency of 1.3% ($n = 3/230$).⁷ A recent comprehensive review on the topic reported that *ERBB2* exon 20 insertions are present in about 1.5% of NSCLC patients and account for 90% of *ERBB2* mutations.⁶ The study by Li et al. reported an *ERBB2* mutational frequency of 3% ($n = 4/148$) in lung adenocarcinomas patients – all identified mutations were insertions at exon 20.⁸ Recently, Carrot-Zhang et al. analyzed *ERBB2* in Latin Americans (Mexico and Colombia patients), but no *ERBB2* exon 20 insertion was reported.⁹ Moreover, a Brazilian study using the Foundation One or Foundation ACT, described 5% of NSCLC patients harboring *ERBB2* mutations ($n = 26/513$), with 1.4% (7/513) exhibiting the exon 20 insertions

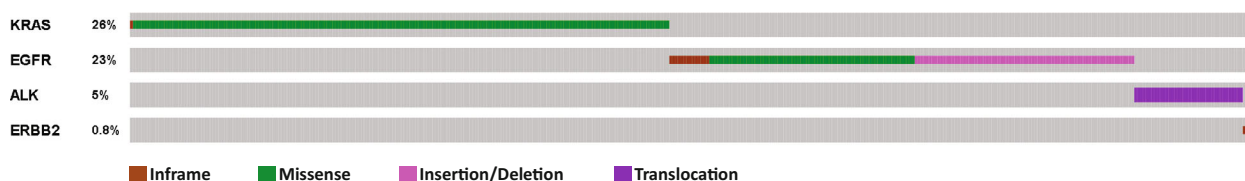
(p. A775_G776INSYVMA), but no additional information was reported.¹⁰

Our study constituted a larger assessment of *ERBB2* exon 20 insertions in Brazilian patients. Only six out of 722 (0.8%) were mutated, being mainly female and never smokers, in agreement with studies from other geographic regions.^{4,6,8} Considering the frequency reported in our study and by Mascarenhas et al.,¹⁰ we may infer that about 1% of NSCLC patients harbor *ERBB2* exon 20 insertions in Brazil. As expected, *ERBB2* mutations were mutually exclusive with *EGFR*, *KRAS* and *ALK* alterations. In our study, all *ERBB2* exon 20 insertions were the p.(Tyr772_Ala775dup) inframe insertion. According to the review by Friedlaender et al., the most common *ERBB2* exon 20 insertions in NSCLC patients are p.(Tyr772dupTyrValMetAla) and the p.(Ala775_Gly776insTyrValMetAla).⁶ Due to the low number of *ERBB2* exon 20 insertion mutated patients in our study, no statistically significant association was performed.

Notably, we found six patients in our series harboring *ERBB2* exon 20 insertion mutations that could benefit from

TABLE 2 Clinicopathological and molecular features from *ERBB2*-mutant patients ($n = 6$)

Variable	<i>ERBB2</i> -mutant patients p.(Tyr772_Ala775dup)					
	ID-113	ID-16	ID-395	ID-41	ID-2198	ID-106
Age (year)	75.0	75.0	66.0	52.0	61.0	58.0
Sex	Female	Male	Female	Female	Male	Female
Tobacco Use	Never smoker	Never smoker	Never smoker	Never smoker	Missing	Never smoker
Loss of weight ^a	Yes, <= 10%	No	Missing	Yes, <= 10%	No	No
ECOG PS	2	Missing	3	1	Missing	1
Histology	Adenocarcinoma	Adenocarcinoma	Adenocarcinoma	Adenocarcinoma	Adenocarcinoma	Adenocarcinoma
stage at diagnosis ^b	IV	IV	IV	IV	II	IV
Metastasis at diagnosis	Lung / pleura	Lung / pleura, Bone, lymph node	Bone	Lung / pleura	No	Liver, lymph node
<i>EGFR</i> status	Wild-type	Wild-type	Wild-type	Wild-type	Wild-type	Wild-type
<i>KRAS</i> status	Wild-type	Wild-type	Wild-type	Wild-type	Wild-type	Wild-type
<i>ALK</i> status	Wild-type	Wild-type	Wild-type	Wild-type	Missing	Wild-type
Vital status	Alive-active disease	Alive-active disease	Death-cancer	Death-cancer	Alive-active disease	Death-cancer
Overall survival (months)	27.8	27.2	3.5	28.6	2.8	2.0

^aLast 6 months before the diagnosis.^bAccording to AJCC seventh edition.FIGURE 2 Representative molecular alterations in non-small cell lung cancer (NSCLC) patients ($n = 722$). Only mutant patients are shown ($n = 396$)

treatment with tyrosine kinase inhibitors (TKIs), such as poziotinib, pyrotinib, and trastuzumab deruxtecan. A phase II basket trial (ZENITH20) evaluated 90 patients harboring *ERBB2* exon 20 insertion mutations treated with poziotinib which showed an objective response rate (ORR) of 27.8% (95% CI: 18.9–38.2), with 25 of 90 patients achieving partial response, a disease control rate of 70% (95% CI: 59.4–79.2), and a median progression-free survival (PFS) of 5.5 months (95% CI: 3.9–5.8). Patients pretreated with three or more prior treatment lines had greater responses (ORR, 37.1%).¹¹ Another phase II trial study evaluated 60 advanced (IIIB–IV) lung adenocarcinoma patients harboring *ERBB2* mutations and previously treated with platinum-based chemotherapy.¹² In this study, 49 patients were identified with *ERBB2* exon 20 insertions (12-bp insertion, $n = 44$; 9-bp insertion, $n = 5$) and there were 11 patients with missense mutations.¹² From the 60 patients, 18 showed an ORR (30%, 95% CI: 18.8%–43.2%), all with partial response, and a median PFS and overall survival of 6.9 months (95% CI: 5.5–8.3). In patients with different mutation types, the ORR was higher in 44 patients harboring 12- and 9-bp exon 20 insertions (27.3% and 60.0%, respectively).¹² Finally, the

open-label phase 2 DESTINY-Lung01 study evaluated trastuzumab deruxtecan in 91 nonsquamous metastatic NSCLC patients harboring *ERBB2* mutations that relapsed during standard treatment or had refractory to standard treatment (platinum-based chemotherapy, anti-PD-1 or anti-PD-L1 treatment), and reported an ORR in 55% of the patients.⁴

Importantly, *ERBB2* can be deregulated by other mutations, namely missense in other regions, and harbor gene amplification mechanisms,^{6,8} which were not explored in the present study, and could lead to a higher percentage of *ERBB2* genomic alterations in Brazilian lung cancer patients.

In conclusion, we report that less than 1% of Brazilian NSCLC patients harbor the *ERBB2* exon 20 insertions. Nevertheless, they could putatively benefit from *ERBB2* targeted therapies.

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CONFLICT OF INTEREST

The authors have nothing to disclose and confirm they have not received any grants or support related to this study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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5.4 Implementação de nova metodologia de detecção de fusões dos genes *NTRK1,2*, e *3* e sua frequência em pacientes diagnosticados com câncer de pulmão não pequenas células e validação.

Este artigo descreveu um nova metodologia para avaliar a presença de fusões nos genes *NTRK1,2* e *3*, e avaliou a sua frequência em pacientes brasileiros diagnosticados com câncer de pulmão de não pequenas células no Hospital de Amor de Barretos.



OPEN Detection of *NTRK* fusions by RNA-based nCounter is a feasible diagnostic methodology in a real-world scenario for non-small cell lung cancer assessment

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NTRK1, 2, and 3 fusions are important therapeutic targets for NSCLC patients, but their prevalence in South American admixed populations needs to be better explored. *NTRK* fusion detection in small biopsies is a challenge, and distinct methodologies are used, such as RNA-based next-generation sequencing (NGS), immunohistochemistry, and RNA-based nCounter. This study aimed to evaluate the frequency and concordance of positive samples for *NTRK* fusions using a custom nCounter assay in a real-world scenario of a single institution in Brazil. Out of 147 NSCLC patients, 12 (8.2%) cases depicted pan-*NTRK* positivity by IHC. Due to the absence of biological material, RNA-based NGS and/or nCounter could be performed in six of the 12 IHC-positive cases (50%). We found one case exhibiting an *NTRK1* fusion and another an *NTRK3* gene fusion by both RNA-based NGS and nCounter techniques. Both *NTRK* fusions were detected in patients diagnosed with lung adenocarcinoma, with no history of tobacco consumption. Moreover, no concomitant *EGFR*, *KRAS*, and *ALK* gene alterations were detected in *NTRK*-positive patients. The concordance rate between IHC and RNA-based NGS was 33.4%, and between immunohistochemistry and nCounter was 40%. Our findings indicate that *NTRK* fusions in Brazilian NSCLC patients are relatively rare (1.3%), and RNA-based nCounter methodology is a suitable approach for *NTRK* fusion identification in small biopsies.

Lung cancer remains the most deadly cancer worldwide and in Brazil^{1,2}. Non-small cell lung cancer (NSCLC) is the most common histologic type of lung cancer, representing about 85% of cases. NSCLC is a heterogeneous disease, and its molecular profiling has shown the presence of molecular alterations in several oncogenes that could be therapeutically targeted, which have revolutionized the treatment of patients with NSCLC over the last years^{3,4}.

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The *NTRK1* (Neurotrophic Receptor Tyrosine Kinase 1), *NTRK2* (Neurotrophic Receptor Tyrosine Kinase 2), and *NTRK3* (Neurotrophic Receptor Tyrosine Kinase 3) genes are members of the TRK (tropomyosin-receptor kinase) family, playing crucial roles in cell growth, proliferation, neuronal differentiation, survival, and metabolism in central nervous system cells⁵. The *NTRK* fusion arises as a result of genomic rearrangements (intra-chromosomal or inter-chromosomal) that juxtapose the 3' region of the *NTRK* gene with the 5' sequencing of the partner gene, leading to the aberrant expression of the gene and constitutive activation of the kinase domain⁶. Nevertheless, screening for *NTRK* fusions may be complex due to the diversity of both partners and breaking points locals. Larotectinib and Entrectinib are Food and Drug Administration (FDA)-approved targeted therapies that inhibit TRK fusion proteins and benefit patients with solid tumors harboring *NTRK* rearrangements⁷.

The frequency of *NTRK* fusions varies according to the tumor type, reported in 2–17% of thyroid cancers, 5–15% of salivary gland tumors, and ~1% of NSCLC^{8–11}. Because of the low frequency and incompletely characterized partners in tumors like NSCLC, assays allowing the detection of several fusions or a two-step screening by immunohistochemistry (IHC) followed by confirmation by RNA-based next-generation sequencing (NGS) have been recommended^{12–15}. However, due to the large number of driver alterations and the scarcity of tumor tissue usually available in NSCLC patients, multiplexed assays may improve *NTRK* fusion detection¹⁶. The nCounter assay is a robust semi-automatized platform, particularly for degraded biological material, such as formalin-fixed paraffin-embedded (FFPE) tissue, that offers a cost-effective solution with high specificity and sensitivity for detecting *NTRK* and other therapy-targeted fusions, with a reduced rate of false positive and false negative when using a custom panel with multiplex capabilities^{16–19}.

Here, we aimed to evaluate the frequency of *NTRK* fusions in a real-world scenario of a routine molecular profile of NSCLC and assess the feasibility of a nCounter custom assay for rearrangement alterations in a Brazilian single center.

Results

Characterization of patients' clinicopathological and molecular features

The clinicopathological and molecular features of the consecutive cohort of 147 formalin-fixed paraffin-embedded (FFPE) lung tumors, which were evaluated for pan-TRK, are summarized in Table 1 and Fig. 1. Molecularly, 24.5% (n = 36/147) of patients harbored *KRAS* (*Kirsten Rat Sarcoma Virus*) mutations, 16.3% (n = 24/147) *EGFR* (*Epidermal Growth Factor Receptor*) mutations, and 4.8% (n = 7/147) *ALK* (*Anaplastic Lymphoma Kinase*) fusions.

We observed 8.2% (n = 12/147) of cases with pan-TRK positive immunostaining (Fig. 2). The most frequent histology of IHC-positive patients was adenocarcinoma in 66.7% (n = 8/12) of patients, the median age of patients at diagnosis was 61.0 years, 58.3% (n = 7/12) were male, and 83.3% (n = 10/12) were former or current smokers (Table 2). Clinically, 58.3% (n = 7/12) of patients were diagnosed in an advanced stage of disease, 25.0% (n = 4/12) presented weight loss 6 months prior to diagnosis, and most patients presented a good performance status (Table 2). Molecularly, one patient exhibited an *EGFR* mutation p.(Leu858Arg), three patients contained the *KRAS* mutation, the p.(Gly12Cys) present in two, and a p.(Gly12Val) in one patient.

Detection of *NTRK* fusions by RNA-based NGS and RNA-based nCounter assays

Next, we tested the 12 IHC-positive cases for *NTRK* fusions using two molecular methods: NGS panel Archer FusionPlex solid tumor and our custom fusion panel nCounter Elements XT (Fig. 1). Due to the absence of biological material in the FFPE biopsies, we were able to perform the NGS test on 50.0% (n = 6/12) of the positive pan-TRK (Table 2).

Out of the six samples tested by NGS, two samples were positive for the presence of *NTRK* fusions (*EML4-Echinoderm microtubule-associated protein-like 4*)-*NTRK3* and (*PRKAR1A-Protein Kinase CAMP-Dependent Type I Regulatory Subunit Alpha*)-*NTRK1*, two were negative, and two were inconclusive (Table 2 and Fig. 3). Simultaneously, we performed our custom nCounter Elements XT fusion panel in 41.7% (n = 5/12) of the positive pan-TRK samples (Table 2). From five tested samples, two were positive for the presence of *NTRK* fusions (*NTRK1* and *NTRK3*) detected by 3'–5' imbalance, and three samples were negative (Fig. 4). To corroborate the 3'–5' imbalance results, we included the two positive *NTRK* fusion non-lung cancer samples in Fig. 4. Since our assay does not use specific breakpoint probes for *NTRK* genes, the fusion partners are not reported.

We further evaluated the concordance rate between the results obtained from NGS, IHC, and nCounter assays (Table 2). Three of the six samples tested using the NGS assay were also analyzed by the nCounter assay, with a concordance rate of 100% (n = 3/3; two positive and one negative samples). When comparing the results obtained from the NGS assay with the pan-TRK IHC assay, we observed a concordance rate of 33.4% (n = 2/6; two positive samples). Similarly, when comparing the nCounter assay with the pan-TRK IHC assay, we observed a concordance rate of 40% (n = 2/5; two positive samples). Additionally, when comparing only positive pan-TRK samples with IHC stain intensity defined as 2+ or 3+ with the NGS assay and nCounter assay (Table 3), we observed a concordance rate of 40.0% (n = 2/5) and 66.7% (n = 2/3), respectively.

Overall, the frequency of *NTRK* fusions in NSCLC patients is 1.36% (n = 2/147).

Characterization of *NTRK*-positive patients (nCounter and NGS)

Molecularly, none of the patients had other genetic alterations in the *EGFR*, *KRAS*, and *ALK* genes (Table 2). Both male and female patients had no history of tobacco consumption, were diagnosed with lung adenocarcinoma, and presented no weight loss prior to 6 months of diagnosis (Table 2). The female patient was diagnosed at 38 with a stage IVA disease, which had metastasized to the lung and pleura, and received carboplatin with pemetrexed and pembrolizumab as first-line treatment, followed by carboplatin with paclitaxel as second-line treatment after disease progression. The other patient was male, diagnosed at 71 with a disease staged as IIIB, and was submitted

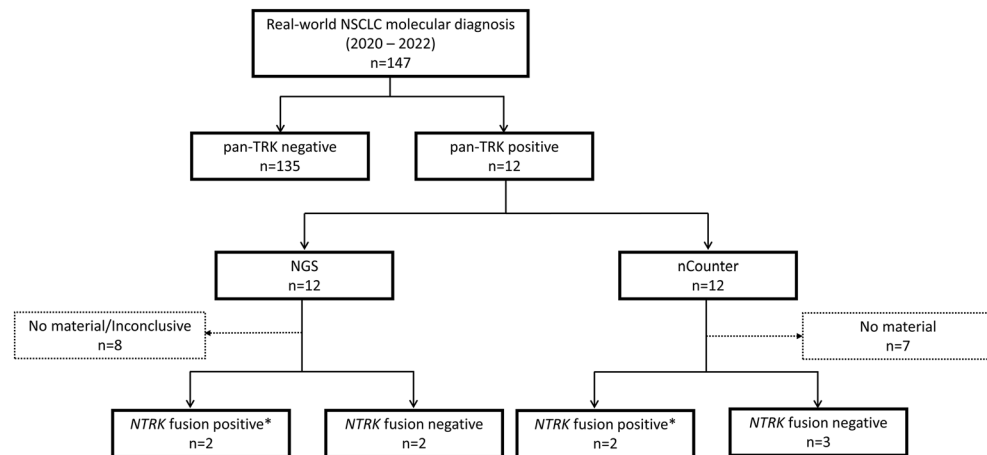
Variable	Parameter	n	%
	Mean (min–max)	64.0 (32.0–94.0)	
Age (year)	≤ 64	77	52.4
	> 64	70	47.6
Sex	Male	83	56.5
	Female	64	43.5
Smoking	Never	37	25.2
	Quitter	49	33.3
	Current	57	38.8
	No information	4	2.7
Loss of weight ^a	No	77	52.4
	≤ 10%	23	15.6
	> 10%	29	19.8
	No information	18	12.2
ECOG PS at diagnosis	0	27	18.4
	1	79	53.7
	2	22	15.0
	3	10	6.8
	No information	9	6.1
Histology	Adenocarcinoma	109	74.1
	Squamous cell	7	4.8
	NSCLC ^b	31	21.1
Stage at diagnosis ^c	I/II	28	19.0
	III	26	17.7
	IV	83	56.5
	No information	10	6.8
Metastasis at diagnosis	No	54	36.7
	Yes, CNS	24	16.3
	Yes, Others	59	40.2
	No information	10	6.8
pan-TRK IHC	Negative	135	91.8
	Positive	12	8.2
EGFR mutations	Wild-type	119	81.0
	Mutated	24	16.3
	No information	4	2.7
KRAS mutations	Wild-type	99	67.3
	Mutated	36	24.5
	No information	12	8.2
ALK fusions	Wild-type	130	88.4
	Mutated	7	4.8
	No information	10	6.8
Vital status	Alive	92	62.6
	Deceased	54	36.7
	No information	1	0.7

Table 1. Clinicopathological and molecular features of NSCLC consecutively evaluated for pan-TRK (n = 147). n, number of patients; ECOG PS (Eastern Cooperative Oncology Group Performance Status); NSCLC, Non-small cell lung cancer; ^aprior to 6 months to diagnosis; ^bincluding the following histologies: NSCLC NOS (not otherwise specified), neuroendocrine large cell carcinoma, adenosquamous carcinoma, ^caccording to AJCC 8th edition; ^dpan-TRK IHC (immunohistochemistry).

to surgery (lobectomy) with adjuvant chemotherapy (cisplatin with pemetrexed) as curative treatment. None of the patients received anti-NTRK inhibitors, such as Larotrectinib or Entrectinib.

Discussion

In the present study, we evaluated the feasibility of assessing *NTRK* fusions in a real-world scenario of routine molecular profiling of consecutive 147 NSCLC, using a custom fusion panel of nCounter assay from a single Brazilian Center.



* *NTRK* fusions detected in the same samples by both techniques

Figure 1. Flow chart of the study design. We selected 147 FFPE cases diagnosed with NSCLC at Barretos Cancer Hospital that were routinely evaluated for molecular diagnosis.

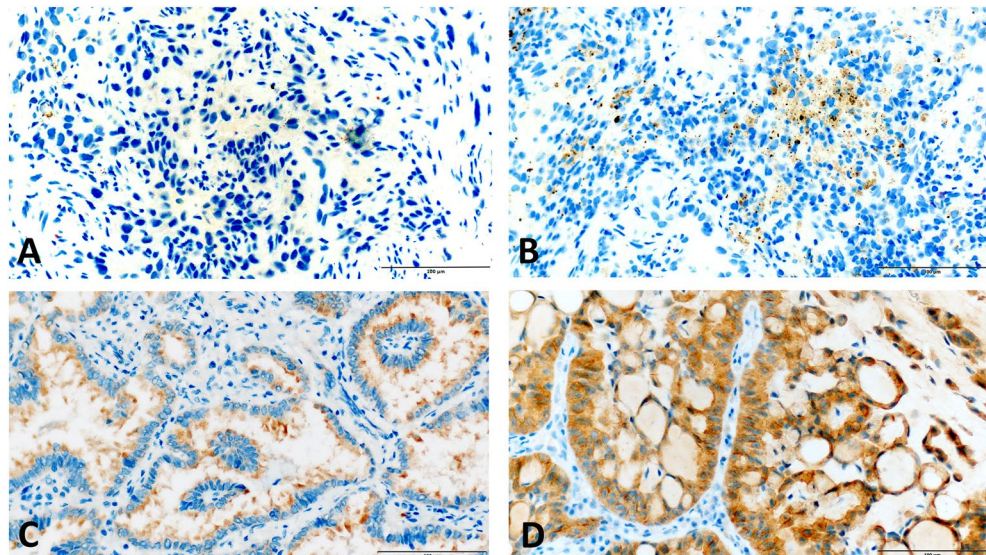


Figure 2. Microscopy figure of the pan-TRK immunohistochemistry. (A) pan-TRK-negative immunohistochemistry (B) pan-TRK-positive cytoplasmic 1+ (C) pan-TRK-positive cytoplasmic 2+ (D) pan-TRK-positive cytoplasmic 3+. Brown color indicates pan-TRK positivity by DAB staining.

We observed the presence of *NTRK* fusions (*NTRK1* and *NTRK3*) in 1.36% ($n = 2/147$) of patients. Previous studies reported that the frequency of *NTRK* fusions ranges from 0.1 to 3.3% in NSCLC patients worldwide, with fusions in *NTRK1* and *NTRK3* being more common than *NTRK2*^{7,10,15,20–27}. In Hispanic/Latin patients with lung cancer, a recent meta-analysis reported *NTRK* fusions in 1% of patients²⁰. A real-world study reported 3.5% ($n = 10/289$) of samples with pan-TRK expression²⁷. The authors, due to insufficient material, were able to confirm the presence of *NTRK* fusion (*EML4-NTRK3*) in only one patient by NGS, rendering an *NTRK* fusion frequency of 0.35% ($n = 1/289$)²⁷. *NTRK* fusions are reported predominantly in patients with no smoking history and diagnosed with metastatic disease^{7,27}. Likewise, our patients with *NTRK* fusion were never-smokers and diagnosed with advanced disease (IVA and IIIB). Molecularly, the presence of *NTRK* fusions in our series was mutually exclusive with other driver mutations and fusions, as previously described^{17,27}.

Additionally, we evaluated the concordance rate between pan-TRK immunohistochemistry, RNA-based NGS, and our custom nCounter assay. Since the majority of the cases were routine small biopsies, and a panel of IHC markers initially diagnosed the cases, then were further evaluated for molecular alterations, namely *EGFR*, *KRAS*, *ALK*, and PD-L1, no more biological material with tumor content was available for molecular validation in half of the pan-TRK-positive cases. We observed that 33.4% ($n = 2/6$) of tested samples using NGS were positive for *NTRK* fusion, and 40.0% ($n = 2/5$) of tested samples using nCounter were positive for *NTRK* fusion. We observed

Patients with positive NTRK (pan-TRK)																
	Age (year)	Sex	Tobacco Use	ECOG PS	Histology	Stage at diagnosis ^a	Metastasis at diagnosis	Sample specimen	Vital status	OS (months) ^b	EGFR mutation	KRAS mutation	ALK fusion	IHC pan-TRK	NGS ^c	nCounter ^d
	ID-2068	71	Male	Never	0	LUAD	IIIB	No	Surgical	Alive	22.0	WT	WT	Cytoplasmic 3+	EML4-NTRK3	NTRK3
	ID-2649	54	Male	Former	2	SQC	IIIB	No	Biopsy	Alive	5.8	Missing	Inconclusive	Cytoplasmic 2+	Inconclusive	NTA
	ID-2143	67	Male	Former	1	LUAD	IIIC	No	Biopsy	Deceased	4.8	WT	WT	Cytoplasmic 1+	NTA	NTA
	ID-2386	54	Female	Former	2	LUAD	IVB	Bone, liver, CNS	Biopsy	Deceased	1.0	WT	WT	Cytoplasmic 2+	NTA	NTA
	ID-2393	60	Male	Former	1	NSCLC	IVB	Bone, liver	Biopsy	Alive	6.3	WT	WT	Cytoplasmic 2+	NTA	NTA
	ID-2443	74	Male	Former	2	NSCLC	IVA	Kidney	Biopsy	Alive	1.6	WT	WT	Cytoplasmic 2+ and membranous 1+	NTA	Negative
	ID-2406	58	Female	Current	1	LUAD	IVB	Lung/pleura, adrenal, subcutaneous	Biopsy	Deceased	3.7	WT	p(Gly12Cys)	Cytoplasmic 1+	NTA	NTA
	ID-381	62	Female	Former	0	LUAD	IIIA	No	Biopsy	Alive	45.9	WT	WT	Cytoplasmic 2+	Inconclusive	NTA
	ID-2183	67	Male	Current	1	NSCLC	IVB	Bone, liver	Biopsy	Deceased	11.7	WT	WT	Cytoplasmic 2+ and membranous 1+	Negative	NTA
	ID-2642	94	Male	Former	2	LUAD	IA3	No	Biopsy	Alive	2.8	WT	p(Gly12Val)	Cytoplasmic 1+	Negative	Negative
	ID-2644	59	Female	Current	1	LUAD	IVA	Lung/pleura	Biopsy	Deceased	7.5	p(Leu858Arg)	WT	Cytoplasmic 1+	NTA	Negative
	ID-426	38	Female	Never	1	LUAD	IVA	Lung/pleura	Biopsy	Deceased	19.3	WT	WT	Cytoplasmic 2+	PRKARIA-NTRK1	NTRK1

Table 2. Clinicopathological and molecular features of positive pan-TRK NSCLC cases (n = 12). Positive cases are in bold. ^a According to AJCC 8th edition. ^b Since the date of diagnosis. ^c NGS (Next-generation sequencing)–Archer FusionPlex solid tumor panel. ^d nCounter Elements XT panel. ECOG PS (Eastern Cooperative Oncology Group Performance Status), LUAD (Lung adenocarcinoma), NSCLC (Non-small cell lung cancer), SQC (Squamous Cell Carcinoma), OS (Overall Survival), IHC (Immunohistochemistry), NTA (Not Tissue Available).

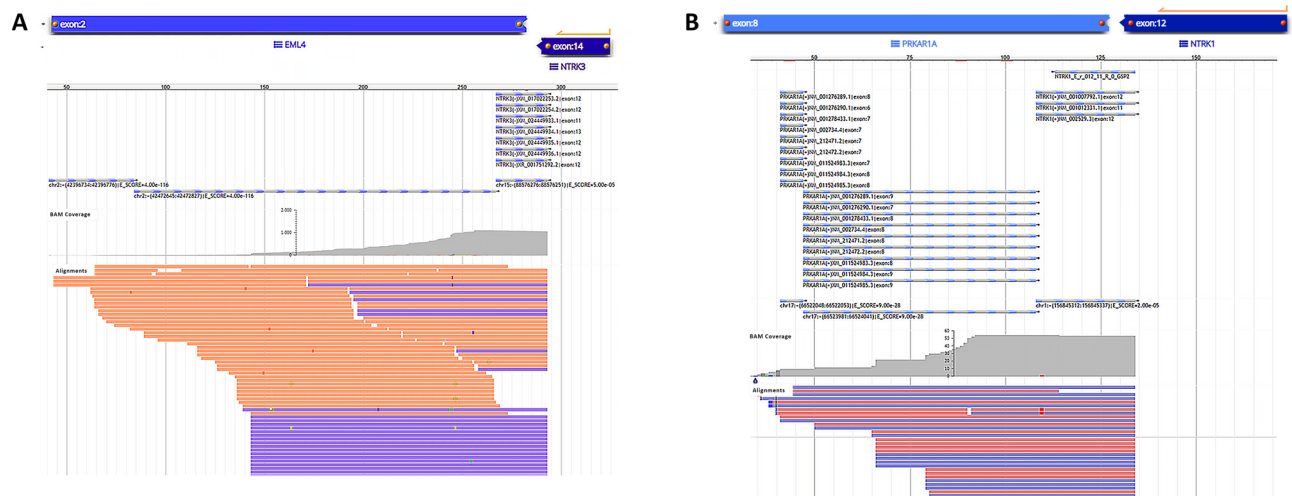


Figure 3. NGS analysis showing sequenced reads using Archer VR FusionPlex VR (JBrowse 1.11.6) of *NTRK* genes fusion. (A) Visualization of *EML4* and *NTRK3* genes. (B) Visualization of *PRKARIA* and *NTRK1* genes.

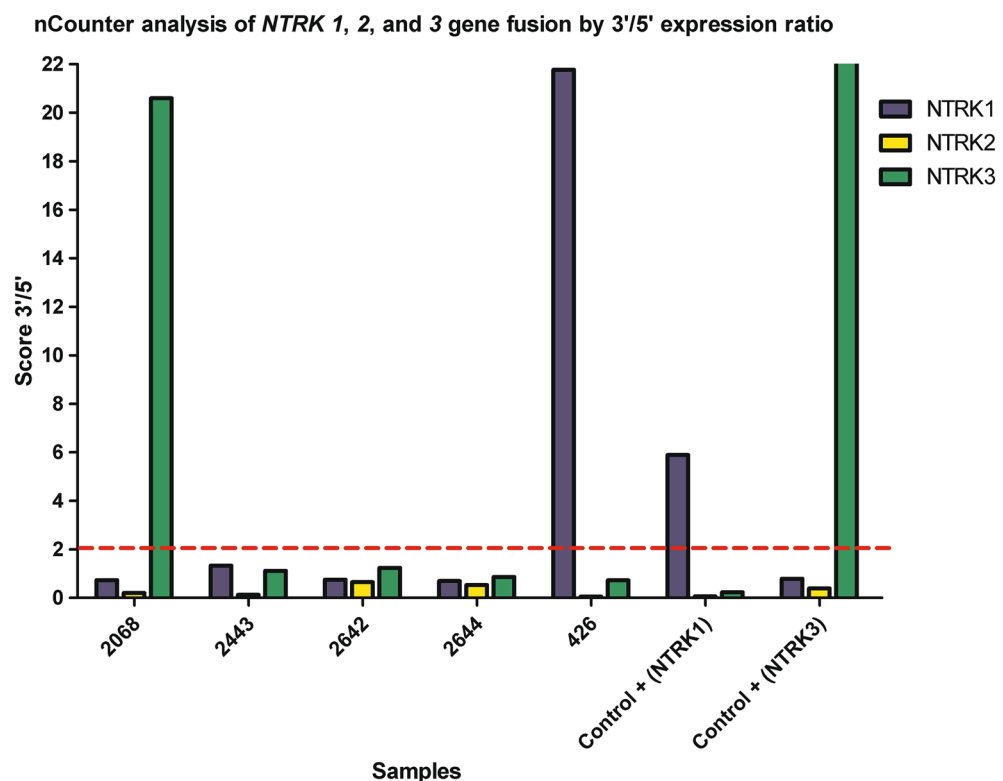


Figure 4. Representative graph of *NTRK* gene fusions obtained from the analyzed samples and two positive *NTRK* fusion controls (cutoff=2). The y-axis represents the packing ratio between the 3' and 5' regions for the *NTRK* genes. The x-axis represents the RNA samples analyzed in the study.

a concordance rate of 100% between the RNA-based NGS assay and our custom nCounter assay for *NTRK* fusion detection. Similarly, previous studies reported discordances between immunohistochemistry assays and more robust techniques (RNA-based NGS and nCounter) for *NTRK* fusion detection^{15,18,28}. This may be due to methodology limitations since the pan-TRK immunohistochemistry assay detects wild-type and aberrant TRK proteins. In contrast, the RNA-based NGS and nCounter assays detect only the fusions²⁹.

Importantly, detecting NSCLC patients harboring *NTRK* fusions is critical since the patients may benefit from targeted therapies, such as Larotrectinib and Entrectinib^{7,11}. However, none of our patients were treated with Larotrectinib or Entrectinib. Also, *NTRK* fusions are associated with resistance to EGFR-TKIs (Tyrosine Kinase Inhibitors) in NSCLC patients⁷. Thus, *NTRK* fusions have emerged as a pivotal biomarker for NSCLC patients.

	Elements XT nCounter panel genes and Housekeeper (HK)						
	Probes for specific genes						
	ALK-fusion ^a	ROS1-fusion ^a	RET-fusion ^a	METΔex14 ^b	NTRK1-fusion	NTRK2-fusion	NTRK3-fusion
Probes 5' and 3' for imbalance fusion detection	ALK_ex1	ROS1_ex1_(5'-UTR)	RET_ex1-2		NTRK1_ex1	NTRK2_ex1	NTRK3_ex20b
	ALK_ex5	ROS1_ex18-19	RET_ex2-3		NTRK1_ex2-3-4	NTRK2_ex3	NTRK3_ex20
	ALK_ex8-9	ROS1_ex24	RET_ex6-7		NTRK1_ex5	NTRK2_ex4	NTRK3_ex19
	ALK_ex18	ROS1_ex29-30	RET_ex11		NTRK1_ex7	NTRK2_ex5-6	NTRK3_ex17
	ALK_ex22-23	ROS1_ex37	RET_ex14-15		NTRK1_ex14	NTRK2_ex8	NTRK3_ex9
	ALK_ex26-27	ROS1_ex40	RET_ex15-16-17		NTRK1_ex15	NTRK2_ex18	NTRK3_ex7
	ALK_ex29	ROS1_ex41/42	RET_ex18		NTRK1_ex17	NTRK2_ex19	NTRK3_ex4-5
	ALK_ex29_(3'-UTR)	ROS1_ex43_(3'-UTR)	RET_ex19_(3'-UTR)		NTRK1_ex17b	NTRK2_ex20	NTRK3_ex3
						NTRK2_ex21	
Probes for specific genes	EML4_ex13-ALK_ex20	CD74_ex6-ROS1_ex32	KIFB5_ex16-RET_ex12	MET_ex13-14			
	EML4_ex20-ALK_ex20	SDC4_ex2-ROS1_ex32	KIFB5_ex22-RET_ex12	MET_ex13-15			
	EML4_ex6-ALK_ex20	SLC34A2_ex13-ROS1_ex32	KIFB5_ex23-RET_ex12				
	EML4_ex18-ALK_ex20	SLC34A2_ex4-ROS1_ex32	CCDC6_ex1-RET_ex12				
	KIF5B_ex24-ALK_ex20	EZR_ex10-ROS1_ex34					
	KIF5B_ex17-ALK_ex20	SDC4_ex4-ROS1_ex34					
	TFG_ex5-ALK_ex20	GOPC_ex8-ROS1_ex35					
		GOPC_ex4-ROS1_ex36					
Housekeeper genes (HK)		LRIG3_ex16-ROS1_ex34					
Housekeeper genes (HK)		SYMPK	HPRT1	GAPDH	GUSB	OAZ1	POLR2A

Table 3. Probes of the custom NSCLC gene fusion panel of Barretos Cancer Hospital in the Elements XT nCounter. ^aPreviously published by Novaes et al. ¹⁶. ^bPreviously published by Aguado et al. ³⁵.

Since *NTRK* fusions occur in a wide range of partners, with most of them in a low frequency, assays that identify the specific breakpoint are not ideal¹¹. Our results showed high efficacy in avoiding false positive cases for *NTRK* fusions when using our custom nCounter methodology, with complete concordance with the RNA-based NGS approach. Furthermore, the nCounter technology is highly robust, with multiplex capabilities, high sensitivity, easy to execute, faster, and more cost-effective compared to NGS assays, and shows a high success rate in samples with poor quality, such as FFPE samples^{19,30}. Nevertheless, one area for improvement is the absence of knowledge of the fusion partner, in addition to the high cost of the equipment. Overall, these results suggest that our custom nCounter methodology could serve as a standard approach for routine biomarker testing gene fusions (*NTRK1,2,3*, *ALK*, *RET* (*Rearranged During Transfection*), *ROS1* (*c-ros Oncogene 1*), and *METΔex14* (*Mesenchymal Epithelial Transition exon 14 skipping*) in NSCLC patients.

These findings indicate that a custom RNA-based nCounter methodology is feasible for routine *NTRK* fusion detection and that the frequency of these alterations in Brazilian NSCLC patients is rare (1.3%).

Methods

From 2020 to 2022, we evaluated 147 FFPE consecutive cases diagnosed with NSCLC at Barretos Cancer Hospital that were routinely evaluated for their molecular profile, which included the mutation status of *EGFR*, *KRAS*, *BRAF* (*V-raf Murine Sarcoma Viral Oncogene Homolog B*), and *HER2* (*Human epidermal growth factor receptor 2*) by NGS, using the TruSight Tumor 15 panel (Illumina, USA)^{31,32}, immunohistochemistry (IHC) of *ALK* and *PD-L1* (Programmed death ligand 1)³³, and evaluation of *NTRK1/2/3* fusions. The *NTRK* fusions triage was initially done by pan-TRK IHC, followed by molecular NGS validation (Fig. 1). The clinicopathological and molecular data were collected from the patient's medical records. The institutional review board-Barretos Cancer Hospital IRB-approved the study protocol (CAAE 05744712.3.0000.5437) and waived written informed consent due to the study's retrospective nature.

NTRK1/2/3 fusion detection by Immunohistochemistry

Automated immunohistochemical for TRK A, B, and C (pan-TRK) expression was performed for all cases on an automated staining system (BenchMark Ventana Ultra™) as previously described³⁴. The UltraView DAB IHC detection Kit was briefly used to visualize antibody reactions. The slides were counterstained with hematoxylin, and controls were used to verify appropriate staining. To perform the reticulum staining, we used the Reticulum/Nuclear Fast Red Stain Kit (Artisan) on Artisan PRO, Dako Agilent Platform. Two pathologists reviewed the slides. We quantified the percentage of stained tumor cells in the subcellular compartments: cytoplasmic, membranous, and nuclear, as previously reported¹⁴. Additionally, the staining intensity for each compartment was defined on a 0 to 3 scale as follows: strong staining (3+), which was visible with the use of a 20× or 40× objective; moderate staining (2+), which required the use of a 10× or 20× objective; weak staining (1+), which involved the

use of a 40× objective; and negative staining (0), which was defined as complete absence of expression (Fig. 4). As previously reported, a positive cutoff of at least 1% of tumor cells was defined¹⁴.

RNA isolation

RNA isolation was performed from FFPE tumor samples, sectioned on slides with a thickness of 10 μm. One slide was stained with hematoxylin and eosin (H&E) and evaluated by a pathologist for identification, sample adequacy assessment, and selection of the tumor tissue area (minimum of 60% tumor area). RNA was isolated using the RNeasy FFPE Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Measurement of RNA quantity was done with TapeStation 4150 (Agilent Technologies).

Fusion detection by Archer FusionPlex solid tumor

Analysis of *NTRK* fusion was performed using the Archer FusionPlex Custom Solid Panel with Anchored Multiplex PCR (ArcherDX, Boulder, CO, USA) as previously described³⁴. Briefly, the target-enriched cDNA library was prepared with the Archer FusionPlex solid tumor (ArcherDX, Boulder, CO, USA) using an amount of 100 ng of RNA as per the manufacturer's description. In short, the reverse transcription of RNA was followed by real-time quantitative PCR (Polymerase Chain Reaction) to determine the sample quality. Then, End-repair, adenylation, and universal half-functional adapter ligation of double-stranded cDNA fragments were followed by two rounds of PCR with universal primers and gene-specific primers, covering 53 target genes that rendered the library fully functional for clonal amplification and sequencing using the MiSeq (Illumina, USA). With the Archer Analysis software version 6.0 (ArcherDX, Boulder, CO, USA), the produced libraries were analyzed for relevant fusions.

Detection of *NTRK* fusions by nCounter Technology

Detection of *NTRK1,2,3* rearrangement was performed using the nCounter Elements XT (NanoString Technologies, Seattle, WA, USA) custom fusion panel developed at Molecular Diagnostic Laboratory, Barretos Cancer Hospital. The panel was previously designed to detect *ALK*, *RET*, and *ROS1*¹⁶ and was now updated to detect *MET*Δex14³⁵ and *NTRK1/2/3* fusions. The specific probes are detailed in Table 3.

Briefly, 100 ng RNA was hybridized with specific probes for 21 h at 67 °C. Hybridized complexes were purified using the PrepStation (NanoString Technologies, Seattle, WA, USA) and then hybridized in the cartridge. Finally, the cartridge was scanned by the Digital Analyzer (NanoString Technologies, Seattle, WA, USA) for counting transcripts. Normalization of transcripts was performed by the nSolver Analysis Software v4.0 (NanoString Technologies, Seattle, WA, USA) using the ratio of geometric mean for each sample and arithmetic mean for all samples for positive assays controls and reference gene (housekeeper). Samples with counts lower than 300 counts for the *GAPDH* gene were considered inconclusive.

Detection of *NTRK1,2,3* rearrangement was based on 3'/5' probes imbalance, and no specific probes for breakpoints were used due to the large number of partners and breakpoints for the *NTRK* gene. The calculation of the imbalance probes was defined by the ratio between the geometric mean of 3' probes and the average of 5' probes, considering thresholds for positive *NTRK1/2/3* rearrangement equal to 2. Two cases were included as controls: an infant-type hemispheric glioma³⁴ and an infantile fibrosarcoma harboring *NTRK1* and *NTRK3* fusions, initially detected by RNA-based NGS (Archer FusionPlex solid tumor). All analyses were performed in R environment v3.4.1.

Statistical analysis

We described categorical variables using percentages and continuous variables using the medians for statistical analysis. To assess the concordance rate between all the techniques, we calculated the percentage of samples with concordant and discordant results between the techniques. Frequencies and medians were performed using IBM SPSS Statistics Version 25 (IBM, Armonk, Nova York, USA). Graphs were created using GraphPad Prism v5.01 (GraphPad Software Inc., Boston, Massachusetts USA).

Statement of ethics

The institutional review board-Barretos Cancer Hospital IRB-approved the study protocol (CAAE 05744712.3.0000.5437) and waived written informed consent due to the study's retrospective nature. All procedures were performed following the Helsinki Declaration.

Data availability

The data supporting this study's findings are available from Dr. Rui Manuel Reis. However, restrictions apply to the availability of patients' clinical data, which were used under ethics committee approval for the current study. Data are, however, available from the authors upon reasonable request and with permission of Dr. Rui Manuel Reis (corresponding author).

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Author contributions

R.O.C., L.F.L., and R.M.R. wrote the main manuscript text; R.M.R. designed the study. F.E.P., G.N.B., and M.B. performed the molecular experiments. M.T.R. and G.R.T. organized and/or reviewed the histological samples. B.G.Z., J.M.D., F.A.F.S., C.E.B., A.A.J., M.X.R., E.L.M., T.S.A., and R.E.N.N.O. collected, organized, and reviewed the clinical data. E.S.A., F.E.P., G.N.B., M.B., R.O.C., M.T.R., and R.M.R. contributed to data analysis and interpretation. All authors have reviewed the manuscript.

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Competing interests

The authors declare no competing interests.

Additional information

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5.5 Frequência de fusões gênicas e *METΔex14* em pacientes diagnosticados com câncer de pulmão no Hospital de Câncer de Barretos.

Este artigo descreve a frequência das principais fusões e da alteração *METΔex14* em pacientes diagnosticados com câncer de pulmão e que foram molecularmente avaliados por técnicas multiples.

ALK, RET, ROS1, NTRK1-2-3 fusions and METΔex14 in Brazilian Lung Cancer:

Clinicopathologic impact and prognostic values

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Abstract

Introduction: Targeted therapies have revolutionized lung cancer treatment, significantly improving overall survival. *ALK/RET/ROS1/NTRK* gene fusions and *METexΔ14* are effective therapeutic targets. However, the frequency of these alterations and their clinicopathological implications in admixed populations, such as in Brazil, are scarce. **Aims:** To evaluate the role of *ALK*, *RET*, *ROS1*, *NTRK1-2-3* gene fusions and the genomic alteration *METΔex1* using real-world data from Brazilian patients diagnosed with lung cancer. **Materials and Methods:** We evaluated 416 patients indicated for *ALK/RET/ROS1/NTRK1-3* fusions and *METexΔ14* detection using custom multiplex nCounter RNA-based techniques. A targeted NGS panel assessed *EGFR*, *KRAS*, *BRAF*, and *ERBB2* status. PD-L1 expression was evaluated by immunohistochemistry. Clinical data were collected from medical records, and frequencies, associations, and survival analyses were performed. **Results:** Of the 416 patients, 73.8% had lung adenocarcinoma, 56.7% were male, 73.3% were current or former smokers, and 64.4% were diagnosed with stage IV. The most frequent alteration was *ALK* fusions in 9.1% of patients, followed by *METexΔ14* in 4.1%, *RET* fusions in 1.6%, *ROS1* fusions in 0.7%, and *NTRK* fusion in 0.3% of cases. Fusions and *METexΔ14* were associated with never-smoker patients and low and high PD-L1 expression, respectively. Among patients with actionable fusions, a subset received targeted therapies and showed better median overall survival than those who did received other therapies. **Conclusion:** Overall, *ALK/RET/ROS1/NTRK1-3* fusions and *METexΔ14* are present in almost 16% of Brazilian lung cancer patients. These driver alterations were associated with better outcomes in targeted treated patients.

Introduction

Lung Cancer is the deadliest cancer worldwide[1]. However, non-small cell lung cancer (NSCLC) treatment has been revolutionized due to target therapies, with EGFR and ALK inhibitors used for patients harboring *EGFR* mutations and *ALK* fusions as the most successful examples [2–4]. Besides the *ALK* gene, fusions in other driver genes, such as *RET*, *ROS1* and *NTRK1-2-3* genes and *METΔex14* (*MET* exon 14 skipping) are also identified in NSCLC tumors, which can also be targeted by tyrosine kinase inhibitors (TKIs) [3,5].

Epidemiologic, *ALK* fusion frequency varies from 1% to 5% in NSCLC patients, *RET* fusions from 1% to 3%, *ROS1* fusions from 1% to 2%, *NTRK1-2-3* fusions in less than 1%, and *METΔex14* in about 3% [3,5–7]. A recent study based in a real-world repository reported fusions in *ALK*, *RET*, *ROS1*, and *NTRK* genes in about 5% of NSCLC patients, and *METΔex14* in 3% [7]. Thus, due to their clinical and diagnostic impact, assessing the gene fusion status involving *ALK*, *RET*, *ROS1*, *NTRK1-2-3*, and *METΔex14*, is mandatory for personalized oncology [3,7]. Patients harboring fusions involving the *ALK* or *RET* gene who are treated with TKIs show longer survival than patients treated with chemotherapy [3,8]. For *METΔex14* and fusions in the genes *ROS1* and *NTRK1-2-3*, data directly comparing TKIs with chemotherapy are not available; However, indirect comparisons with historical chemotherapy data showed that a personalized treatment approach results in better patient outcomes [8]. Moreover, excluding the presence of *ALK* and *RET* fusions is essential before starting immunotherapy due to the lack of efficacy when fusions are presented [3,8].

Despite data showing the role of gene fusions for patients with NSCLC, in Brazil, the data are scarce yet. Here, we aimed to evaluate the frequency and the clinical implications of gene fusions involving the genes *ALK*, *RET*, *ROS1*, *NTRK1-2-3*, and the genomic alteration *METΔex14* using real-world data from Brazilian patients diagnosed with lung cancer from a single institution.

Materials and Methods

Patients and Samples

We retrospectively included 451 Lung Cancer patients from Barretos Cancer Hospital who were routinely evaluated for fusions (*ALK*, *RET*, *ROS1*, and *NTRK1/2/3*) and *MetΔex14* using nCounter, Idylla, or Next-Generarion Sequencing (NGS) by the Department of Molecular Diagnosis. About 8% (n=35/451) were excluded from the study due to inconclusive results for fusions (**Figure 1**). From included patients (n=416), 85.8% (n=357/416) were evaluated by nCounter, 16.3% (n=68/416) by Idylla, and 1.9% (n=8/416) by NGS. Additionally, 2.9% (n=12/416) were evaluated by nCounter and Idylla, 1.2% (n=5/416) by nCounter and NGS, and two patients were evaluated by all techniques (nCounter, Idylla and NGS). The clinicopathological and molecular data were collected from the patient’s medical records. The institutional review board-Barretos Cancer Hospital IRB-approved the study protocol (CAAE 05744712.3.0000.5437) and waived written informed consent due to the study’s retrospective nature.

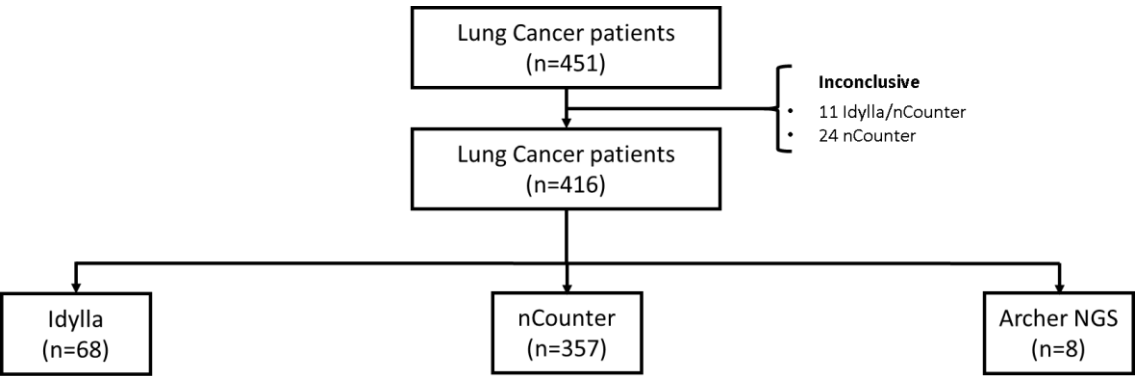


Figure 1 - Workflow for fusions and *METΔex14* evaluation. Only patients with fusion evaluation using multiplex techniques were included. Patients with inconclusive results were excluded.

DNA/RNA isolation

DNA and RNA isolation was performed from FFPE (Formalin-Fixed Paraffin-embedded) tumor samples sectioned on slides with a thickness of 10µm. One slide was stained with hematoxylin and eosin (H&E) and evaluated by a pathologist for identification, sample adequacy assessment, and selection of tumor area. DNA was isolated using the commercial kit QIAamp DNA Micro Kit (Qiagen, Hilden, Germany) according to the manufacturer’s specifications. DNA concentration and purity were

evaluated by NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA) and by Qubit 2.0 Fluorometer (Thermo Fisher Scientific) with Qubit dsDNA HS assay kit (Thermo Fisher Scientific). RNA was isolated using the RNeasy FFPE Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA concentration and purity were evaluated by NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA).

Fusion and *METΔex14* detection by nCounter Technology

ALK, *RET*, *ROS1*, *NTRK1-2-3* fusions, and *METΔex14* were performed using the nCounter Elements XT (NanoString Technologies, Seattle, WA, USA) custom fusion panel, as described previously [9,10]. Briefly, 100 ng RNA was hybridized with specific probes, purified using the PrepStation (NanoString Technologies, Seattle, WA, USA), and then hybridized in the cartridge. The Digital Analyzer (NanoString Technologies, Seattle, WA, USA) scanned the cartridge for counting transcripts. Normalization of transcripts was performed by the nSolver Analysis Software v4.0 (NanoString Technologies, Seattle, WA, USA). Samples with geometric mean lower than 100 and *GAPDH* counting lower than 500 were considered inconclusive. When the geometric mean was lower than 100 and *GAPDH* counting was higher than 500, only *METΔex14* was inconclusive.

Fusions were identified for imbalance – considering the ratio between the 5' and 3' probes- and for specific partners for *ALK*, *RET*, and *ROS1* genes, while for *NTRK1-2-3* genes, fusions were identified only by imbalance, as described[10]. *METΔex14* was determined by the ratio of wild-type and mutated probes[10].

Fusion and *METΔex14* detection by NGS

Analysis of fusions and *METΔex14* was performed using the Archer FusionPlex Custom Solid Panel with Anchored Multiplex PCR (ArcherDX, Boulder, CO, USA) as described [9]. Briefly, 100 ng of RNA was used to prepare the target-enriched cDNA library with the Archer FusionPlex solid tumor (ArcherDX, Boulder, CO, USA), as per the manufacturer's description. Sample quality was determined by real-time quantitative PCR (Polymerase Chain Reaction). Then, End-repair, adenylation, and universal half-functional

adapter ligation of double-stranded cDNA fragments were followed by two rounds of PCR with universal primers and gene-specific primers, covering 53 target genes that rendered the library fully functional for clonal amplification and sequencing using the MiSeq (Illumina, San Diego, CA, USA). The presence of fusions and *MET* Δ *ex14* were analyzed with the Archer Analysis software version 6.0 (ArcherDX, Boulder, CO, USA).

Fusion and *MET* Δ *ex14* detection by Idylla

Analysis of fusions and *MET* Δ *ex14* was performed using the Idylla Gene Fusion Assay kit (Biocartis, Mechelen, Belgium). Briefly, cartridge was manually loaded with an unextracted FFPE tissue sections (one slide) into a lysis chamber and loaded onto Idylla-Biocartis equipment, according to manufacturer's instruction.

Mutational Profile

The mutational analysis of hotspot regions for *EGFR*, *KRAS*, *ERBB2*, and *BRAF* genes was assessed by NGS using the TruSight Tumor 15 (Illumina, San Diego, CA, USA) on the MiSeq instrument, according to manufacturer's instruction as described [11]. The read alignment and variant calling were performed with Sophia DDM[®] software (Sophia Genetics SA, Saint Sulpice, Switzerland).

A subset of patients (when NGS was inconclusive) was also analyzed by real-time PCR using the COBAS platform (ROCHE, Basileia, Switzerland) as described [12]. Cobas DNA Sample Preparation Kit (ROCHE, Basileia, Switzerland) was used for manual sample preparation followed by the Cobas z 480 analyzer for automated amplification and detection following Cobas EGFR Mutation Test v2 kit (ROCHE, Basileia, Switzerland) for *EGFR* gene and Cobas 4800 BRAF V600 Mutation Test kit for *BRAF* p.(Val600Glu) mutation.

PD-L1 expression by Immunohistochemistry

A subset of patients (n=322) was evaluated for PD-L1 expression by immunohistochemistry (IHC) with the 22C3 clone (PharmaDx antibody) as described

[12,13]. PD-L1 expression was reported as Tumor Proportional Score (TPS), with results categorized as no-expression (< 1%), Low-expression (1-49%), and high-expression ($\geq$ 50%) [14]. In addition to TPS, PD-L1 was also dichotomized as no expressed (TPS <1%) and expressed (TPS $\geq$ 1%).

Genetic Ancestry analysis

The genetic ancestry background was performed in a subset of patients (n = 328) using a set of 46 ancestry-informative markers, INDELs, selected based on their high information content for European (EUR), African (AFR), Asian (ASN), and Native American (AME) population groups as described [11,14,15].

Statistical Analysis

Categorical variables were reported as absolute numbers and frequencies, and continuous variables were reported using the mean value. As previously described, individual ancestry proportions were defined as categorical variables and divided into tertiles (low, intermedium, and high) [14]. Chi-square and Fisher's exact test were used to associate the presence of fusions and *MET* Δ *ex14* with molecular and clinicopathological data. The multinomial logistic regression test was used to investigate the independent association of clinicopathological and molecular data with the presence of fusions and *MET* Δ *ex14* status. Kaplan-Meier method and Log-rank test was used to estimate patient's survival. Overall survival (OS) was defined as the time interval between the histological diagnosis date (biopsy or surgery) and date of death or last follow-up. The Cox regression method was used to investigate the association of clinicopathological data to the outcome. All statistical analyses were performed using IBM SPSS Statistics, version 25.0 (IBM Corp, Armonk, NY, USA) with a limit of statistical significance of 0.05.

Results

The clinicopathological features for this lung cancer series (n=416) are summarized in **Table 1**.

Table 1 – Summary of clinical and molecular features of the patients (n=416)

Characteristic	Parameter	n	%
Age at diagnosis (years)	Median (Min-Max)	64.0 (20.0 – 94.0)	
	≤ 64	218	52.4
	> 64	198	47.6
Sex	Female	180	43.3
	Male	236	56.7
Self-declared skin color	White	283	68.0
	Non-white	93	22.4
	Missing	40	9.6
Tobacco status	Never smoker	93	22.4
	Quitter smoker	128	30.8
	Current smoker	177	42.5
	Missing	18	4.3
Loss of weight at diagnosis	Yes	130	31.3
	No	189	45.4
	Missing	97	23.3
ECOG PS	0-1	249	59.9
	2-4	109	26.2
	Missing	58	13.9
Histology	Adenocarcinoma	307	73.8
	Squamous	30	7.2
	Adenosquamous	4	1.0
	NSCLC SOE	66	15.9
	Others*	9	2.2
Stage at diagnosis**	I/II	67	16.1
	III	73	17.5
	IV	268	64.4
	Missing	8	1.9
Metastasis at diagnosis	No	142	34.1
	Yes, CNS	90	21.6
	Yes, Others	177	42.5
	Missing	7	1.7
Vital status	Alive	146	35.1
	Deceased	244	58.7
	Loss of Follow-up	26	6.3
ALK-fusions	Negative	378	90.9
	Positive	38	9.1
RET-fusions	Negative	410	98.6
	Positive	6	1.4
ROS1-fusions	Negative	413	99.3
	Positive	3	0.7
NTRK-fusions	Negative	414	99.5
	Positive	2	0.5
METΔex14	Not detected	389	93.5
	Detected	17	4.1
	Inconclusive	10	2.4
EGFR status	Wild-type	392	94.2
	Mutant	13	3.1
	Inconclusive	11	2.6
KRAS status	Wild-type	339	81.5
	Mutant	43	10.3
	Inconclusive	34	8.2
BRAF status	Wild-type	375	90.1
	Mutant	9	2.2
	Missing	32	7.7
ERBB2 status	Wild-type	369	88.7
	Mutant	13	3.1
	Missing	34	8.2
PD-L1 expression level	No (<1%)	156	37.5
	Low (1%-49%)	80	19.2
	High (≥50%)	80	19.2
	Inconclusive	6	1.4
	Missing	94	22.6

ECOG PS, Performance Status Eastern Cooperative Oncology Group; CNS, Central Nervous System. *Others (Large cell carcinomas, small cell carcinomas, Neuroendocrine large cell carcinomas, atypical carcinoid). **According to AJCC 8th edition.

The median age at diagnosis was 64.0 years; the majority were male (n=236/416; 56.7%); quitter or current smoker (n=305/416; 73.3%); with adenocarcinoma histology (n=307/416; 73.8%); and diagnosed with advanced disease (n=268/416; 64.4%). Clinically, most patients received initial palliative treatment (n=262/416; 63.0%), and 38 patients

received TKIs in any line of palliative treatment, with the ALK-inhibitor, Alectinib, being the most common. (Table 2 and Table 3).

Table 2 – First line treatment description (n=416)

Characteristic	Parameter	n	%
Intention of first treatment	Curative	128	30.8
	Palliative	262	63.0
	Missing	26	6.3
Progression after first treatment	No	91	21.9
	Yes	275	66.1
	Loss of follow up	45	10.8
	Missing	5	1.2
Chemotherapy – First Treatment	No	142	34.1
	Yes, curative	81	19.5
	Yes, palliative	167	40.1
	Missing	26	6.3
Radiotherapy – First treatment	No	219	52.6
	Yes, curative	65	15.6
	Yes, palliative	106	25.5
	Missing	26	6.3
Surgery – First Treatment	No	310	74.5
	Yes, curative	64	15.4
	Yes, palliative	16	3.8
	Missing	26	6.3
TKI – First treatment	No	369	88.7
	Yes, palliative	21	5.0
	Missing	26	6.3
Immunotherapy – First Treatment	No	350	84.1
	Yes, curative	5	1.2
	Yes, palliative	30	7.2
	Unknown	5	1.2
TKIs – First Treatment	Missing	26	6.3
	None	350	84.1
	Erlotinib	1	0.2
	Gefitinib	6	1.4
	Alectinib	6	1.4
	Capmatinib	1	0.2
	Entrectinib	1	0.2
	Lazertinib	1	0.2
	Lorlatinib	1	0.2
	Missing	26	6.3

TKIs, Tyrosine Kinase Inhibitors

Table 3 – Overview of Treatments Administered to Patients Receiving Second-Line Therapy Following Progression on Initial Treatment (n=167).

Characteristic	Parameter	n	%
Chemotherapy – Second Treatment	No	72	43.1
	Yes	95	56.9
Radiotherapy – Second treatment	No	97	58.1
	Yes	70	41.9
Surgery – Second Treatment	No	163	97.6
	Yes	4	2.4
TKI – Second treatment	No	149	89.2
	Yes	18	10.8
Immunotherapy – Second Treatment	No	153	91.6
	Yes	14	8.4
TKIs – Second Treatment	None	149	89.2
	Crizotinib	3	1.8
	Gefitinib	4	2.4
	Alectinib	6	3.6
	Brigatinib	1	0.6
	Lorlatinib	1	0.6
	Tepotinib	1	0.6
	Clinical Research*	2	1.2

*TKIs, Tyrosine Kinase Inhibitors. *Patient is a participant of a blind study.*

Genetic Ancestry Analysis

The genetic ancestry could be assessed for 78.8% (n=328/416) of cases. The proportion of each ancestry population evaluated was as follows: 70.6% for European, 15.2% for African, 7.9% for Native American, and 6.3% for Asian (Figure 2).

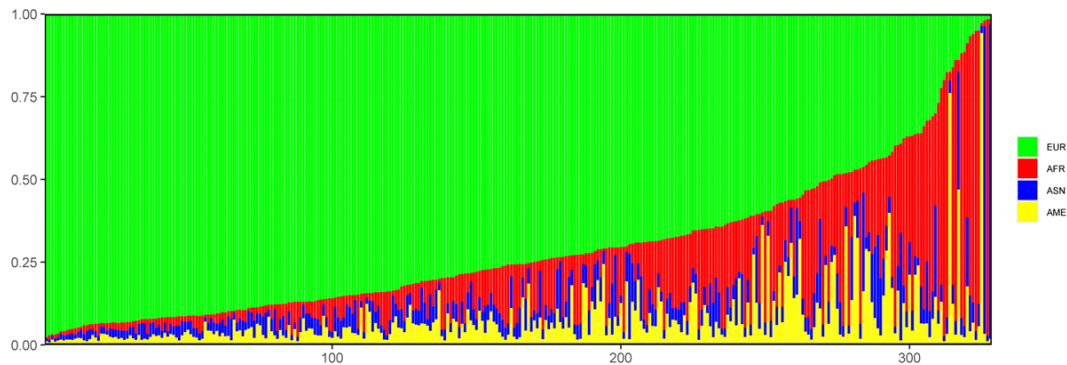


Figure 2- Graphical representation of ancestry proportions for lung cancer patients of the cohort (n=328). EUR, European, AFR, African, ASN, Asian, and AME, Native American.

Molecular Profile

Molecularly, 9.1% (n=38/416) of patients had *ALK*-fusions, 1.4% (n=6/416) had *RET*-fusions, 0.7% (n=3/416) had *ROS1*-fusions, 0.3% (n=1/416) had *NTRK1*, 0.3% (n=1/416) had *NTRK3*, and 4.1% (n=17/416) had *METΔex14* (Figure 3). No *NTRK2*-fusions were identified.

Among the *ALK* fusions positive cases, 68% (n = 26/38) involved the *EML4* gene as the partner, while in 32% (n = 12/38), the partner gene was unknown. For the *RET* gene, 66% (n=4/6) of patients had fusion with an unknown partner, while one patient (n=1/6) had the *CCDC6* gene and another patient (n=1/6) had the *KIF5B* as the gene partner. Additionally, *NTRK1* fusion involved the *PRKAR1A* as a partner, and *NTRK3* fusion also involved the *EML4* gene. We observed that fusions events and *METΔex14* were mutually exclusive to each other and other driver genes (Figure 3).

Considering mutations, 10.3% (n=43/416) had *KRAS* mutations, 3.1% (n=13/416) had *EGFR* mutations, 3.1% (n=13/416) had *ERBB2* mutations, and 2.2% (n=9/416) had *BRAF* mutations (Figure 3, Table 1).

Additionally, 37.5% (n=156/416) of patients' sample presented PD-L1 expression <1%, 19.2% (n=80/416) presented 1%-49%, and 19.2% (n=80/416) presented $\geq 50\%$ (Table 1).

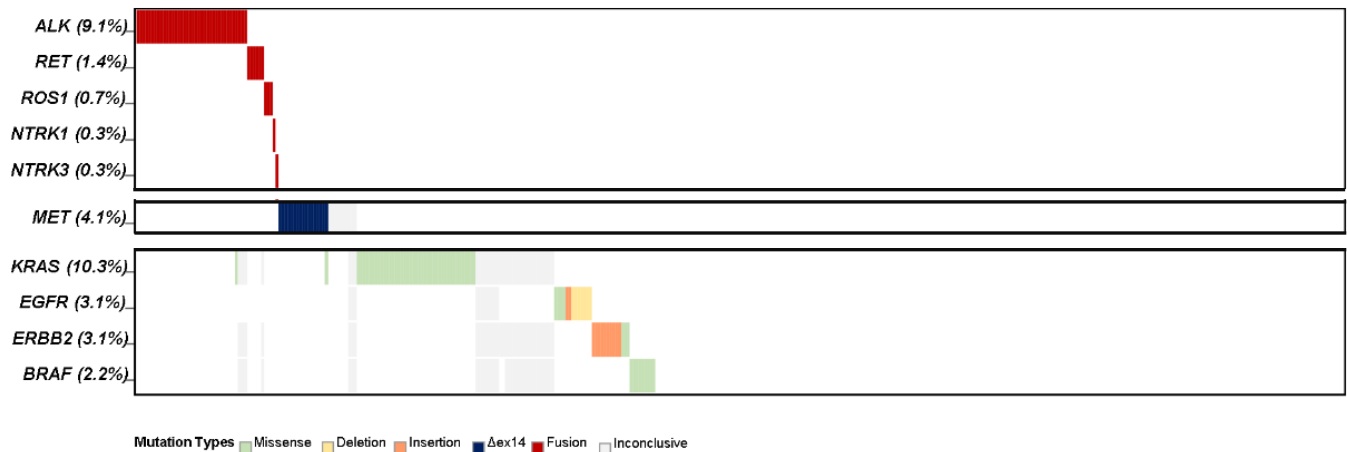


Figure 3- Oncoprint with fusions and other molecular alterations identified (n=416).

Concordance ratio between molecular techniques

We further evaluated the concordance rate between the results obtained from NGS, Idylla, and nCounter assays. We observed 100% concordance between all three techniques for detecting fusions in the genes *RET*, *ROS1*, *NTRK1-2-3*, and also for *MET*Δex14. We observed 100% concordance for *ALK*-fusions between NGS vs nCounter (n=5) and Idylla vs NGS (n=2). Considering samples analyzed by nCounter and Idylla, negative samples had 100% concordance in both techniques, but only 33% (n=1/3) of concordance was observed for positive samples (Table 4).

Table 4 – Concordance between techniques for *ALK* fusion.

ALK evaluation		nCounter		NGS		Idylla		IHC	
		Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive
nCounter	Negative			5	0	9	0	239	3*
	Positive			0	0	2	1	9	26
NGS	Negative	5	0			2	0	5	0
	Positive	0	0			0	0	0	0
Idylla	Negative	9	0	2	0			0	0
	Positive	2	1	0	0			3	0
IHC	Negative	239	3*	5	0	0	0		
	Positive	9	26	0	0	3	0		

*1 focal positive

Association of gene fusions and *METΔex14* status with clinicopathological features

Next, we performed statistical analysis to evaluate the association of fusions and *METΔex14* status with the clinicopathological and molecular data. Due to the low number of events of *RET* (n=6), *ROS1* (n=3), *NTRK1* (n=1), and *NTRK3* (n=1), the associations were performed only for *ALK* and *MET* (Tables 5 and 6).

Table 5 – Associations between ALK fusions and clinicopathological and molecular features.

Characteristics		ALK-fusions (n=416)				p-value
		Negative (n=389)		Positive (n=38)		
		n	%	n	%	
Age at diagnosis (year)	Median (Min-Max)	64.0 (20.0 – 94.0)		56.5 (28.0 – 77.0)		<0.0001*
	≤ 64	191	87.6	27	12.4	0.017
	> 64	187	94.4	11	5.6	
Sex	Female	162	90.0	18	10.0	0.610
	Male	216	91.5	20	8.5	
Self-declared skin color	White	258	91.2	25	8.8	0.835
	Non-white	84	90.3	9	9.7	
	Missing	46		4		
Tobacco status	Never smoker	70	75.3	23	24.7	<0.0001
	Quitter smoker	118	92.2	10	2.8	
	Current smoker	172	97.2	5	7.8	
	Missing	18		0		
Loss of weight at diagnosis	No	118	90.8	12	9.2	0.842
	Yes	173	91.5	16	8.5	
	Missing	87		10		
Performance Status ECOG	0-1	224	90.0	25	10.0	0.697
	2-4	100	91.7	9	8.3	
	Missing	54		4		
Histology	Adenocarcinoma	270	87.9	37	12.1	0.003
	NSCLC NOS	66	100.0	0	0	
	Others**	42	97.7	1 ²	2.3	
Stage at diagnosis***	I/II	63	94.0	4	6.0	0.503
	III	67	91.8	6	8.2	
	IV	240	89.6	28	10.4	
	Missing	8		0		
Metastasis at diagnosis	No	132	93.0	10	7.0	0.415
	Yes, Others	160	90.4	17	9.6	
	Yes, CNS	79	87.8	11	12.2	
	Missing	7		0		
Vital status	Alive	122	84.7	22	15.3	0.007
	Deceased	229	93.5	16	6.5	
	Loss of Follow-up	27		0		
EGFR Status	Wild-type	354	90.3	38	9.7	0.385
	Mutant	13	100	0	0	
	Missing/Inconclusive	21		0		
KRAS Status	Wild-type	305	90.0	34	10.0	0.155
	Mutant	42	97.7	1	2.3	
	Missing/Inconclusive	31		3		
BRAF Status	Wild-type	340	90.7	35	9.3	0.613
	Mutant	9	100	0	0	
	Missing/Inconclusive	29		3		
ERBB2 Status	Wild-type	335	90.5	35	9.5	0.397
	Mutant	12	100	0	0	
	Missing/Inconclusive	31		3		
PD-L1 expression	No expression	149	95.5	7	4.5	0.006
	Low expression 1-49%	67	83.8	13	16.3	
	High expression =>50%	75	93.8	5	6.3	
	Inconclusive/Missing	87		13		
Asian Ancestry	Low	100	94.3	6	5.7	0.158
	Intermedium	106	91.4	10	8.6	
	High	92	86.8	14	13.2	
	Missing	80		8		
African Ancestry	Low	101	95.3	5	4.7	0.156
	Intermedium	100	88.5	13	11.5	
	High	97	89.0	12	11.0	
	Missing	80		8		
European Ancestry	Low	97	89.8	11	10.2	0.032
	Intermedium	96	86.5	15	13.5	
	High	105	96.3	4	3.7	
	Missing	80		8		
Native American Ancestry	Low	100	93.5	7	6.5	0.196
	Intermedium	100	87.0	15	13.0	
	High	98	92.5	8	7.5	
	Missing	80		8		

ECOG PS, Performance Status Eastern Cooperative Oncology Group; CNS, Central Nervous System. *U-Mann-Whitney, **Others (Large cell carcinomas, small cell carcinomas, Neuroendocrine large cells carcinomas, atypical carcinoid), ***According to AJCC 8th edition.

Table 6 – Associations between *METΔex14* and clinicopathological and molecular features.

Characteristics		METΔex14 (n=406)				p-value
		Negative (n=389)		Positive (n=38)		
		n	%	n	%	
Age at diagnosis (year)	Median (Min-Max)	64.0 (20.0 – 94.0)		73.0 (51.0 – 82.0)		0.027
	≤ 64	209	96.8	7	3.2	0.332
	> 64	180	94.7	10	5.3	
Sex	Female	166	95.4	8	4.6	0.804
	Male	223	96.1	9	3.9	
Self-declared skin color	White	264	94.6	15	5.4	0.264
	Non-white	87	97.8	2	2.2	
	Missing	38		0		
Tobacco status	Never smoker	83	91.2	8	8.8	0.009
	Quitter smoker	118	95.2	6	4.8	
	Current smoker	172	98.9	2	1.1	
	Missing	16		1		
Loss of weight at diagnosis	No	124	95.4	6	4.6	1
	Yes	173	95.1	9	4.9	
	Missing	92		2		
Performance Status ECOG	0-1	233	96.3	9	3.7	0.768
	2-4	102	95.3	5	4.7	
	Missing	54		3		
Histology	Adenocarcinoma	287	95.0	15	5.0	0.634
	NSCLC NOS	64	98.5	1	1.5	
	Others**	38	97.4	1	2.6	
Stage at diagnosis***	I/II	64	97.0	2	3.0	0.936
	III	70	95.9	3	4.1	
	IV	249	95.4	12	4.6	
	Missing	6		0		
Metastasis at diagnosis	No	136	96.5	5	3.5	0.771
	Yes, Others	164	95.9	7	4.1	
	Yes, CNS	84	94.4	5	5.6	
	Missing	50		0		
Vital status	Alive	135	95.7	6	4.3	1
	Deceased	229	95.4	11	4.6	
	Loss of Follow-up	25		0		
EGFR Status	Wild-type	354	90.3	38	9.7	0.385
	Mutant	13	100	0	0	
	Missing/Inconclusive	21		0		
KRAS Status	Wild-type	305	90.0	34	10.0	0.155
	Mutant	42	97.7	1	2.3	
	Missing/Inconclusive	31		3		
BRAF Status	Wild-type	340	90.7	35	9.3	0.613
	Mutant	9	100	0	0	
	Missing/Inconclusive	29		3		
ERBB2 Status	Wild-type	335	90.5	35	9.5	0.397
	Mutant	12	100	0	0	
	Missing/Inconclusive	31		3		
PD-L1 expression	No expression	149	95.5	7	4.5	0.006
	Low expression 1-49%	67	83.8	13	16.3	
	High expression =>50%	75	93.8	5	6.3	
	Inconclusive/Missing	87		13		
Asian Ancestry	Low	100	94.3	6	5.7	0.158
	Intermedium	106	91.4	10	8.6	
	High	92	86.8	14	13.2	
	Missing	80		8		
African Ancestry	Low	101	95.3	5	4.7	0.156
	Intermedium	100	88.5	13	11.5	
	High	97	89.0	12	11.0	
	Missing	80		8		
European Ancestry	Low	97	89.8	11	10.2	0.032
	Intermedium	96	86.5	15	13.5	
	High	105	96.3	4	3.7	
	Missing	80		8		
Native American Ancestry	Low	100	93.5	7	6.5	0.196
	Intermedium	100	87.0	15	13.0	
	High	98	92.5	8	7.5	
	Missing	80		8		

ECOG PS, Performance Status Eastern Cooperative Oncology Group; CNS, Central Nervous System. *U-Mann-Whitney, **Others (Large cell carcinomas, small cell carcinomas, Neuroendocrine large cells carcinomas, atypical carcinoid), ***According to AJCC 8th edition.

ALK-fusions were associated with younger age at diagnosis ($p<0.0001$), patients who never smoked ($p<0.0001$), adenocarcinoma histology ($p=0.003$), lower European genetic ancestry, and low PD-L1 expression ($p=0.006$; Table 5). *MET* Δ ex14 were associated with older age at diagnosis ($p=0.027$), patients who never smoked ($p=0.009$), and high PD-L1 expression ($p<0.0001$; Table 6). The Clinicopathological features for patients harboring *RET*, *ROS1*, and *NTRK* fusions are described in Table 7.

Table 7 – Features of patients harboring fusions in *RET*, *ROS1*, and *NTRKs* genes.

Characteristic	Parameter	<i>RET</i> (n=6)		<i>ROS1</i> (n=3)		<i>NTRKs</i> (n=2)	
		n	%	n	%	n	%
	Median (Min-Max)	61.0 (34 – 73)		67.0 (55 – 73)		54.5 (38 – 71)	
Age at diagnosis (years)	≤ 64	3	50	1	33.3	1	50.0
	> 64	3	50	2	66.7	1	50.0
Sex	Female	2	33.3	2	67.7	1	50.0
	Male	4	66.7	1	33.3	1	50.0
Self-declared skin color	White	6	100	2	67.7	2	100
	Non-white	0	0	1	33.3	0	0
	Missing	0	0	0	0	0	0
Tobacco status	Never smoker	3	50.0	1	33.3	2	100
	Quitter smoker	1	16.7	1	33.3	0	0
	Current smoker	1	16.7	1	33.3	0	0
	Missing	1	16.7	0	0	0	0
Loss of weight at diagnosis	Yes	2	33.3	2	67.7	2	100
	No	3	50.0	1	33.3	0	0
	Missing	1	16.7	0	0	0	0
Performance Status ECOG	0-1	3	50.0	3	100	2	100
	2-4	2	33.3	0	0	0	0
	Missing	1	16.7	0	0	0	0
Histology	Adenocarcinoma	5	83.3	2	67.7	2	0
	Squamous	0	0	0	0	0	0
	Adenosquamous	0	0	0	0	0	0
	NSCLC SOE	1	16.7	1	33.3	0	0
	Others	0	0	0	0	0	0
Stage at diagnosis	I/II	0	0	0	0	0	0
	III	1	16.7	1	33.3	1	50.0
	IV	5	83.3	2	67.7	1	50.0
	Missing	0	0	0	0	0	0
Metastasis at diagnosis	No	1	16.7	1	33.3	1	50.0
	Yes, CNS	2	33.3	1	33.3	0	0
	Yes, Others	3	50.0	1	33.3	1	50.0
	Missing	0	0	0	0	0	0
PD-L1 expression	<1%	0	0	0	0	2	100
	1 – 49%	2	33.3	2	67.7	0	0
	≥50%	2	33.3	1	33.3	0	0
	Missing	2	33.3	0	0	0	0
Vital status	Alive	3	50.0	1	33.3	1	50.0
	Deceased	3	50.0	2	67.7	1	50.0
	Loss of Follow-up	0	0	0	0	0	0

ECOG PS, Performance Status Eastern Cooperative Oncology Group; CNS, Central Nervous System. *Others (Large cell carcinomas, small cell carcinomas, Neuroendocrine large cells carcinomas, atypical carcinoid). **According to AJCC 8th edition.

Then, we grouped all fusions in *ALK*, *RET*, *ROS1*, *NTRK1*, and *NTRK3* for further associations. Thus, fusions were associated with younger patients at diagnosis ($p<0.0001$), patients who never smoked ($p<0.0001$), and adenocarcinoma histology ($p=0.004$; Table 8).

Table 8 – Associations between gene fusions and clinicopathological and molecular features.

		ALK-RET-ROS1-NTRK1,2,3 (n=416)				
Characteristics		Negative (n=378)		Positive (n=49)		p-value
		n	%	n	%	
	Median (Min-Max)	64.0 (20.0 – 94.0)		57.0 (28.0 – 77.0)		<0.0001
Age at diagnosis (year)	≤ 64	186	85.3	32	14.7	0.067
	> 64	181	91.4	17	8.6	
Sex	Female	157	87.2	23	12.8	0.646
	Male	210	89.0	26	11.0	
Self-declared skin color	White	248	87.6	35	12.4	0.718
	Non-white	83	89.2	10	10.8	
	Missing	36		4		
Tobacco status	Never smoker	64	68.8	29	31.2	<0.0001
	Quitter smoker	116	90.6	12	9.4	
	Current smoker	170	96.0	7	4.0	
	Missing	17		1		
Loss of weight at diagnosis	No	112	86.2	18	13.8	0.385
	Yes	169	89.4	20	10.6	
	Missing	86		11		
Performance Status ECOG	0-1	216	86.7	33	13.3	0.486
	2-4	98	89.9	11	10.1	
	Missing	53		5		
Histology	Adenocarcinoma	261	85.0	46	15.0	0.004
	NSCLC NOS	64	97.0	2	3.0	
	Others**	42	97.7	1	2.3	
Stage at diagnosis***	I/II	63	94.0	4	6.0	0.247
	III	64	87.7	9	12.3	
	IV	232	86.6	36	13.4	
	Missing	8		0		
Metastasis at diagnosis	No	129	90.8	13	9.2	0.337
	Yes, Others	155	87.6	22	12.4	
	Yes, CNS	76	84.4	14	15.6	
	Missing	7		0		
Vital status	Alive	117	81.3	27	18.8	0.007
	Deceased	223	91.0	22	9.0	
	Loss of Follow-up	27		0		
EGFR Status	Wild-type	343	87.5	49	12.5	0.241
	Mutant	13	100	0	0	
	Missing/Inconclusive	11		0		
KRAS Status	Wild-type	295	87.0	44	13.0	0.043
	Mutant	42	97.7	1	2.3	
	Missing/Inconclusive	30		4		
BRAF Status	Wild-type	330	88.0	45	12.0	0.398
	Mutant	9	100	0	0	
	Missing/Inconclusive	28		4		
ERBB2 Status	Wild-type	325	87.8	45	12.2	0.374
	Mutant	12	100	0	0	
	Missing/Inconclusive	30		4		
PD-L1 expression	No expression	147	94.2	9	5.8	0.001
	Low expression 1-49%	63	78.8	17	21.3	
	High expression =>50%	72	90.0	8	10.0	
	Inconclusive/Missing	85		15		
Asian Ancestry	Low	99	93.4	7	6.6	0.093
	Intermedium	102	87.9	14	12.1	
	High	89	84.0	17	16.0	
	Missing	77		11		
African Ancestry	Low	99	93.4	7	6.6	0.118
	Intermedium	99	87.6	14	12.4	
	High	92	94.4	17	15.6	
	Missing	77		11		
European Ancestry	Low	93	86.1	15	13.9	0.016
	Intermedium	93	83.8	18	16.2	
	High	104	95.4	5	4.6	
	Missing	77		11		
Native American Ancestry	Low	96	89.7	11	10.3	0.221
	Intermedium	97	84.3	18	15.7	
	High	97	91.5	9	8.5	
	Missing	77		11		

ECOG PS, Performance Status Eastern Cooperative Oncology Group; CNS, Central Nervous System. *U-Mann-Whitney, **Others (Large cell carcinomas, small cell carcinomas, Neuroendocrine large cells carcinomas, atypical carcinoid), ***According to AJCC 8th edition.

The Logistic Regression analysis (Table 9) showed fusions independently associated with patients who never smoked ($p=0.007$) and PD-L1 Low-expression ($p=0.002$). Furthermore, the presence *METΔex14* was independently associated with patients who never smoked ($p=0.002$) and PD-L1 high-expression ($p=0.001$).

Table 9 – Multivariate Logistic regression analysis for Fusions and *METΔex14* clinical associations.

Characteristics		Fusions (<i>ALK-RET-ROS1-NTRK1,3</i>)				<i>METΔex14</i>			
		OR	95% CI		p-value	OR	95% CI		p-value
			Lower	Upper			Lower	Upper	
Tobacco History	Current	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
	Quitter	8.98	1.92	41.94	0.05	3.45	0.59	20.11	0.169
	Never	28.20	6.26	126.99	<0.0001	13.99	2.53	77.30	0.002
PD-L1 expression	No (<1%)	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
	Low (1-49%)	4.22	1.67	10.69	0.002	1.68	0.10	27.82	0.715
	High (≥50%)	2.24	0.78	6.42	0.130	32.73	3.96	270.26	0.001

OR, Odds Ratio; CI, Confidence Interval.

Impact of Gene Fusions and *METΔex14* Overall Survival

The median 5-year OS for patients was 15.0 months (95% CI: 11.6 – 18.4 months), and the median follow-up was 10.9 months. First, we compared patients harboring fusions in *ALK*, *RET*, *ROS1*, *NTRK1*, and *NTRK3*, patients harboring *METΔex14*, and others. Grouped gene fusions showed better 5-year OS (median not reached) than other patients (14.3 months, CI 95%: 10.9 – 17.7 months), while *METΔex14* had worse overall survival (8.0 months, 2.3 – 13.6 months; $p=0.049$; Figure 4A).

Then, considering patients harboring any fusion, we observed those patients who received TKI for fusions ($n=21/49$) in any line of treatment (median not reached) had better OS than patients harboring any fusion who did not receive TKI treatments ($n=28/49$; 7.6 months, 95% CI: 0 – 18.8 months; $p<0.0001$; Figure 4B).

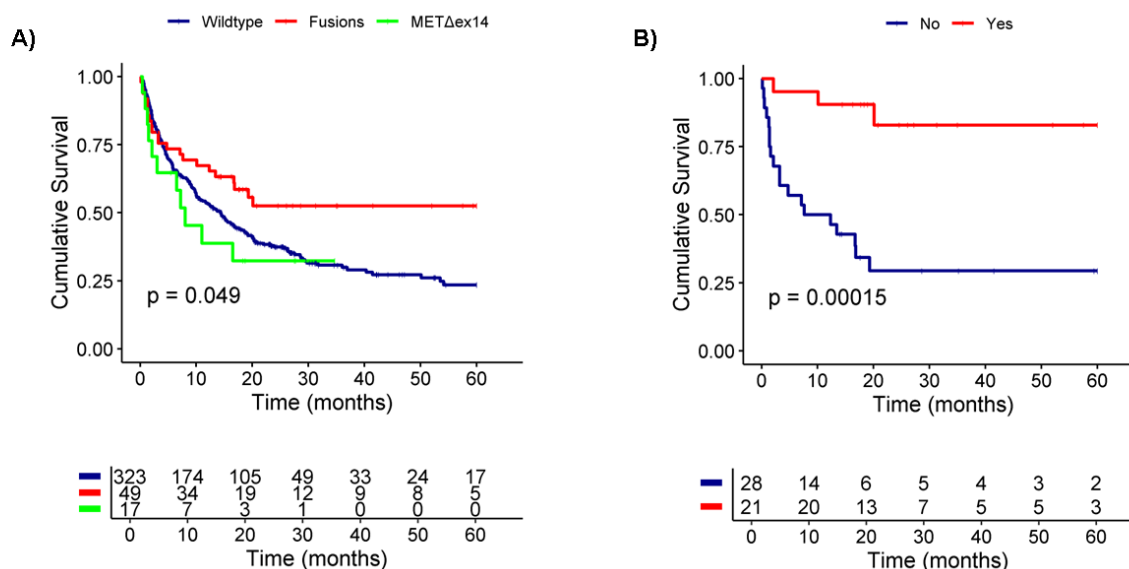


Figure 4- Kaplan-Meier representing the 5-year survival for patients. A) Comparison between Wildtype patient's vs Fusions vs METΔex14. B) Comparison between patients harboring fusions who received any TKI vs patients harboring fusions who received other treatment

We further performed a multivariate Cox regression analysis (Table 10). We observed that worse performance status ($p < 0.0001$, HR:2.2, 1.68 – 3.09), and advanced stage at diagnosis ($p < 0.0001$, HR:4.77, 95% CI: 2.76 – 8.23) were independently associated with a higher risk of death. On the other hand, TKI treatment in any line was independently associated with a lower risk of death ($p = 0.001$, HR:0.3, 95% CI: 0.15 – 0.63). The presence of fusions was not significantly associated with the outcome, probably due to the fact that most patients who presented fusions were treated with TKIs.

Table 9 – Multivariate Logistic regression analysis for Fusions and *METΔex14* clinical associations.

Characteristics		HR	95% CI		p-value
			Lower	Upper	
ECOG PS	0-1	Ref	Ref	Ref	Ref
	2-4	2.29	1.69	3.10	<0.0001
Stage at diagnosis	I/II	Ref	Ref	Ref	Ref
	III	1.80	0.95	3.39	0.069
	IV	4.69	2.72	8.08	0.0001
TKI treatment	No	Ref	Ref	Ref	Ref
	Yes	0.27	0.14	0.51	<0.0001

HR, Hazard Ratio; CI, Confidence Interval; TKI, Tyrosine Kinase Inhibitor; ECOG PS, Performance Status Eastern Cooperative Oncology Group

Discussion

In this study, we used multiplex techniques to assess the frequency of key fusion genes and *METΔex14* alterations in patients diagnosed with lung cancer who were referred for routine molecular analysis at Barretos Cancer Hospital in Brazil, and we associated their frequency with patient's clinicopathological features and outcome.

We reported that *ALK* fusions were identified in 9.1% (n=38/416) of patients, *METΔex14* in 4.1% (n=17/416), *RET* fusions in 1.4% (n=6/416), *ROS1* fusions in 0.7% (n=3/416), and *NTRK1* and *NTRK3* in 0.3% (n=1/416) each one. Worldwide, the frequency of *ALK* fusions in NSCLC cases ranges from 4-10%, *RET* fusions from 1-3%, *ROS1* fusions from 1-2%, and *NTRK* fusions in less than 1% [3,6,7,16–18]. Furthermore, *METΔex14* have been reported in about 3-4% [5,17,19]. In Brazil, studies reported *ALK* fusions in a range from 4-10% of NSCLC patients, while another Brazilian study reported in NSCLC patients 2.4% of *RET* fusions, 2.2% of *ROS1* fusions, 0.6% of *NTRK* fusions, and 4.7% of *MET* alterations, similar to our results [20–22].

Moreover, we observed that gene fusions were independently associated with patients with no history of tobacco consumption and low expression of PD-L1. Previous studies reported fusions associated with younger and never-smoker patients [8,23–26]. We also noted a higher frequency of *ALK* fusions in never-smoker patients in Brazil [15]. Although our results showed absence of an independently association with younger patients, in the univariate analysis we observed patients with fusions with a median age at diagnosis of 57 years. A recent review reported that gene fusions have poor response to immunotherapy treatments, probably due to their low expression of PD-L1[8]. Considering the presence of *METΔex14*, we observed this alteration independently associated with patients with no history of tobacco consumption and high expression of PD-L1, in agreement with the literature [5,27].

Additionally, we observed patients harboring fusions, exhibited a better five-year overall survival than other patients. We also reported that patients harboring *METΔex14* had worse overall survival. These results may be due to the subset of patients with fusions that received TKI treatments. Previous studies have reported the efficacy of TKI treatments for patients with NSCLC harboring *ALK*, *RET*, *ROS1*, and *NTRK* genes fusions[8,9,28–33]. *METΔex14* also may be benefited of targeted therapies such as

capmatinib and tepotinib, yet patients in our cohort were not treated with these agents [5,27].

In conclusion, *ALK/RET/ROS1/NTRK1-3* fusions and *METexΔ14* are present in almost 16% of Brazilian lung cancer patients. Furthermore, these patients benefit from targeted therapies.

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5.6 Frequência de mutações no gene *EGFR* e expressão de PD-L1 em pacientes com Câncer de Pulmão de não Pequenas Células diagnosticados em estadiamentos iniciais.

Este artigo buscou descrever a frequência mutacional do gene *EGFR* e da expressão de PD-L1 em pacientes do Hospital de Amor de Barretos diagnosticados com câncer de pulmão de não pequenas células em estadiamentos iniciais. A associação entre esses biomarcadores e as características clinicopatológicas dos pacientes foi explorada no artigo.

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, chi-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 (*EGFR*m). *EGFR* tyrosine kinase inhibitors (*EGFR*-TKis) are currently the standard of care for *EGFR*m 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 ($\geq 50\%$)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 $\geq 1\%$ 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 patients, 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 $500 \times$ 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 $\geq 50\%$.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 $\geq 1\%$).

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 *EGFRm*. PD-L1 expression was described as TPS. PD-L1 expression was categorized as $<1\%$, $1-49\%$, and $\geq 50\%$ ³³ and dichotomized as negative (TPS $<1\%$) and positive (TPS $\geq 1\%$).

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. *EGFRm*), and PD-L1 expression (PD-L1 $<1\%$ vs. $1-49\%$ vs. $\geq 50\%$).

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	<i>n</i> (%)
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 ^e (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

Table 1. Continued

Variables	Parameters	<i>n</i> (%)
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).

^aMedian age.

^bSelf-reported ancestry according to Brazilian Institute of Geography and Statistics (IBGE).

^cWeight loss <10% and >10% of total body weight in the last 6 months.

^dMutated cases at diagnosis, which includes 43 cases harboring sensitizing mutations and 5 cases harboring resistance mutations.

^eOne 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 EGFRm 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 EGFRm, whereas among those without relapse, 24% were EGFRm (*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 EGFRm 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
<i>EGFR</i> 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.
		IIB	0.44	0.09-1.96	.282
		IIIB	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

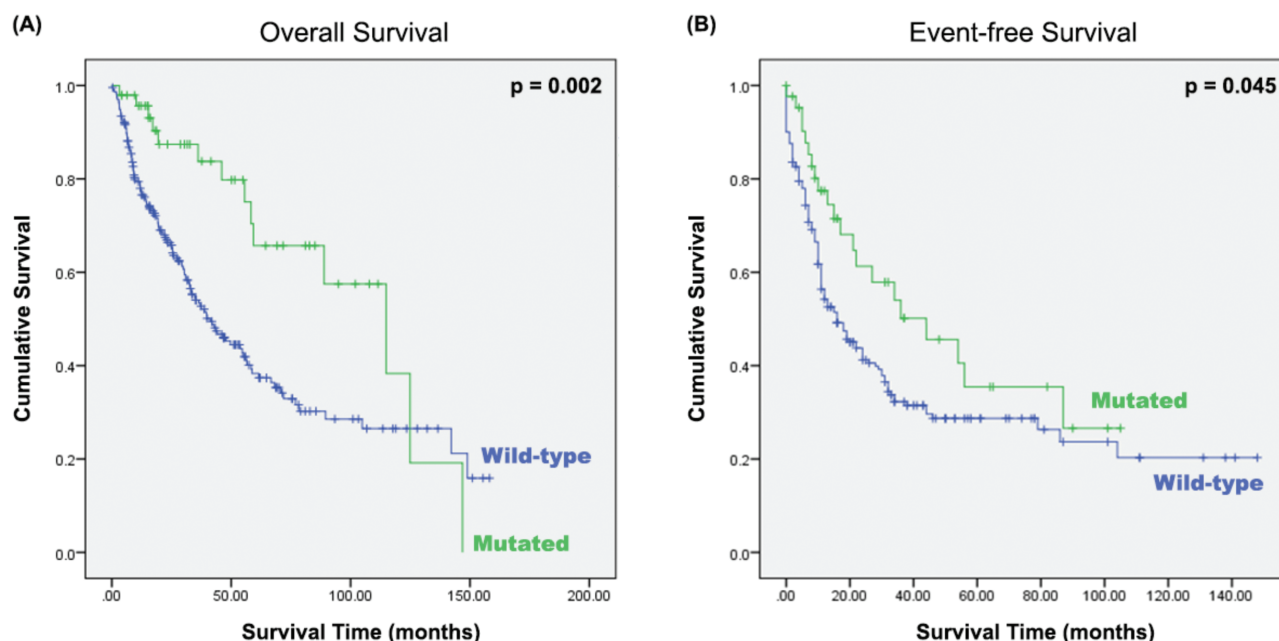


Figure 1. Kaplan-Meier curves for overall survival (OS) and event-free survival (EFS) of patients with NSCLC according to *EGFR* mutational status. (A) *EGFR* mutational status overall survival. Median OS: *EGFR* wild-type = 41.2 months; Median OS: *EGFR*m positive = 114.9 months. (B) *EGFR* mutational 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.

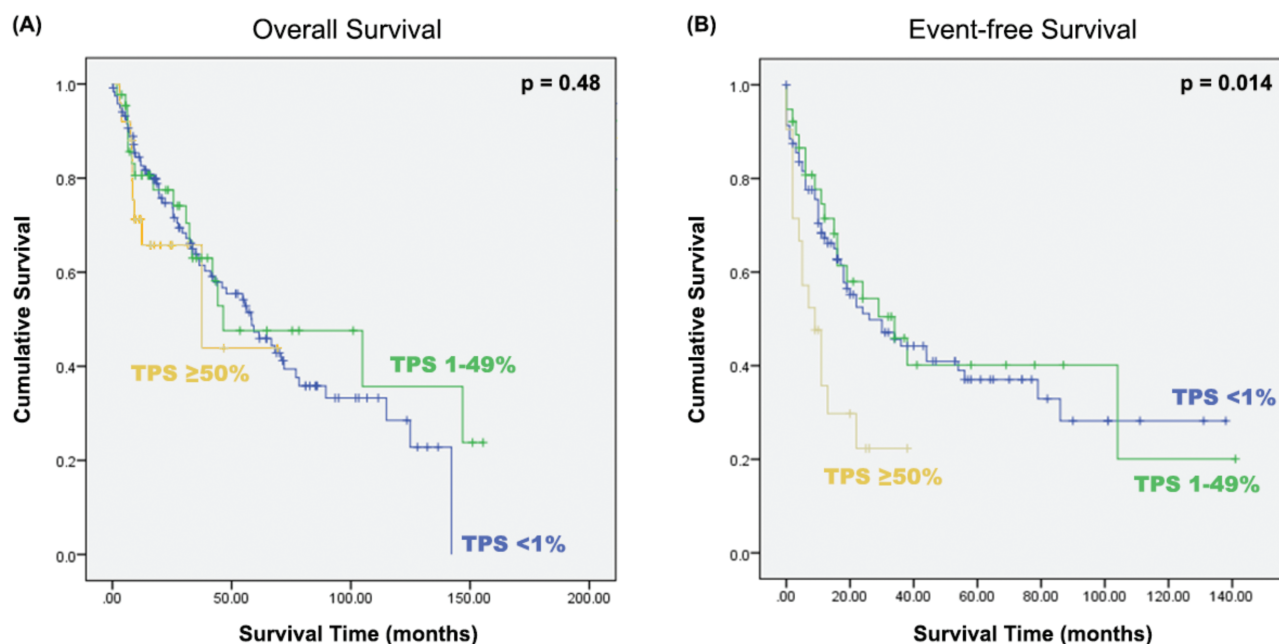


Figure 2. Kaplan-Meier curves for overall survival (OS) and event-free survival (EFS) of patients with NSCLC according to PD-L1 positivity. (A) PD-L1 expression stratified according to TPS categories overall survival. Median OS: TPS <1% = 50.4 months; Median OS: TPS 1%-49% = 46.5 months; ≥50% = 37.5 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 *EGFR*m 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

^aSelf-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 *EGFR*^m 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, Boehringer 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.d.S., A.J., E.C.A.d.S., V.D.d.S. Collection and/or assembly of data: I.A.P., A.L.V.d.S., I.V.S., J.M.D., I.V.S., L.C.S., E.A.F.d.S., M.F.B.F., G.D.J.P., I.S.N., F.E.d.P., G.N.B., M.F.S.G., G.M.S.C., M.O.d.S. Data analysis and interpretation: L.F.L., I.A.P., R.d.O.C., M.A.d.O., 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

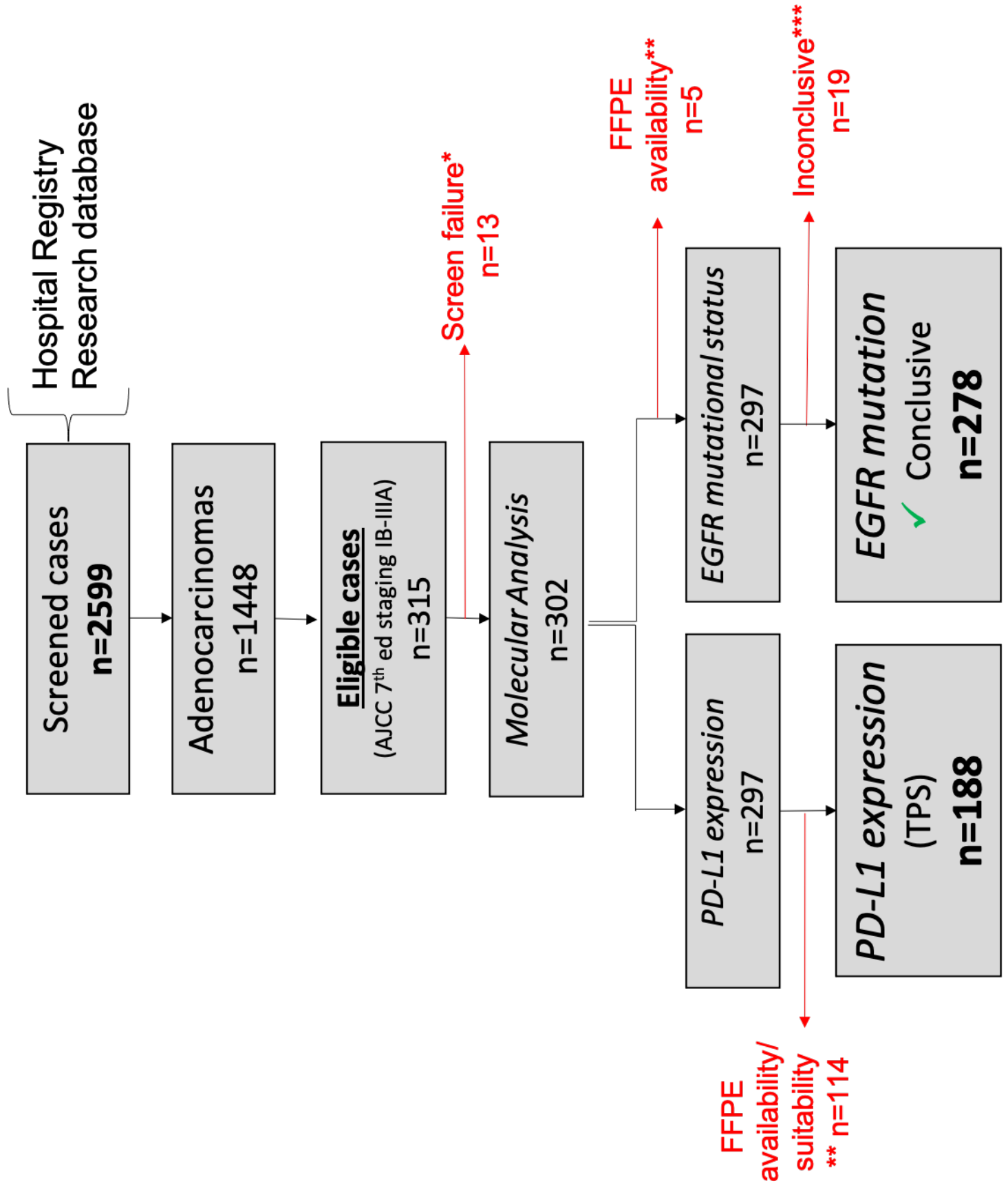
Supplementary material is available at *The Oncologist* online.

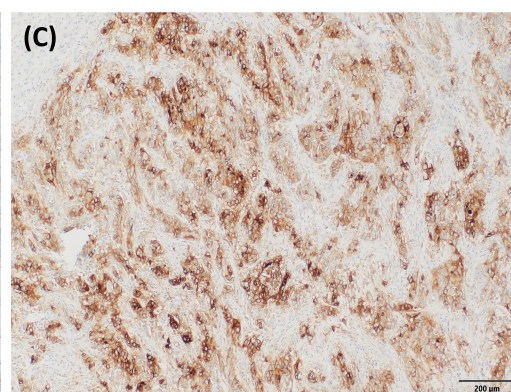
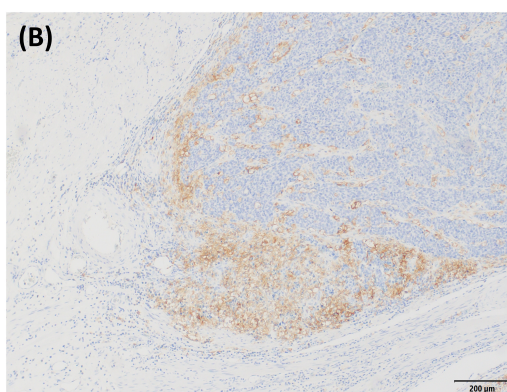
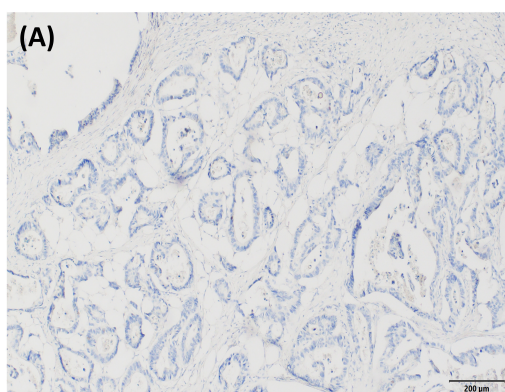
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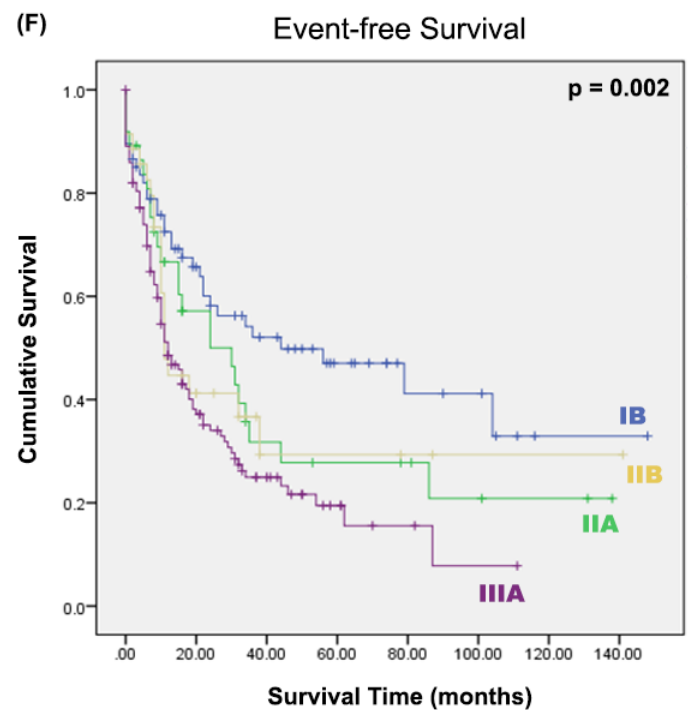
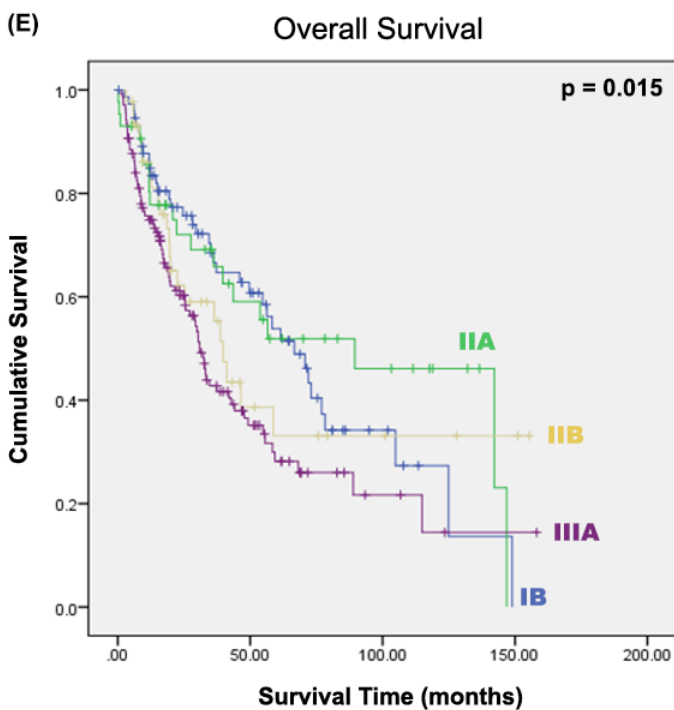
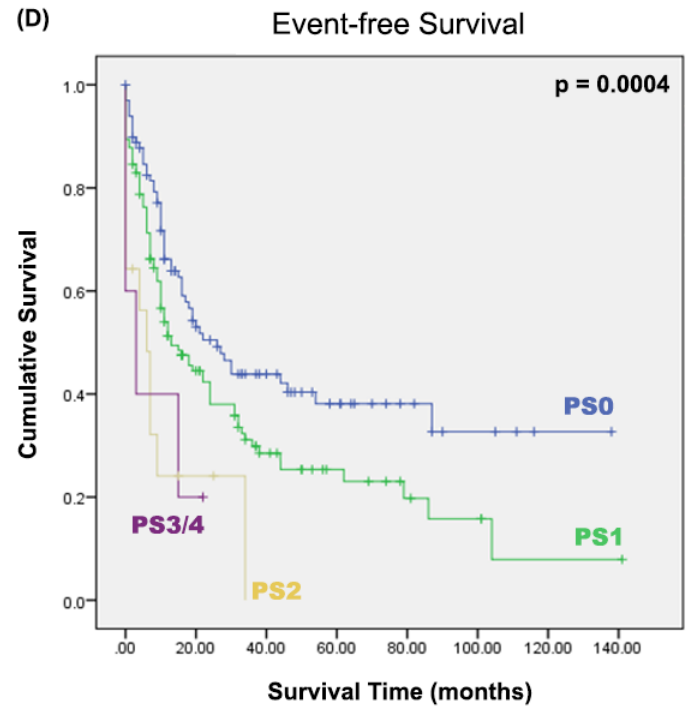
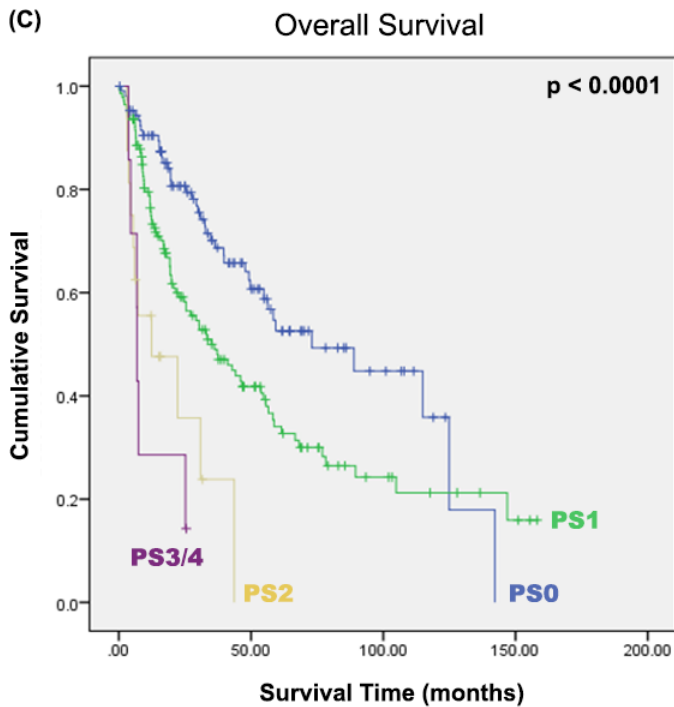
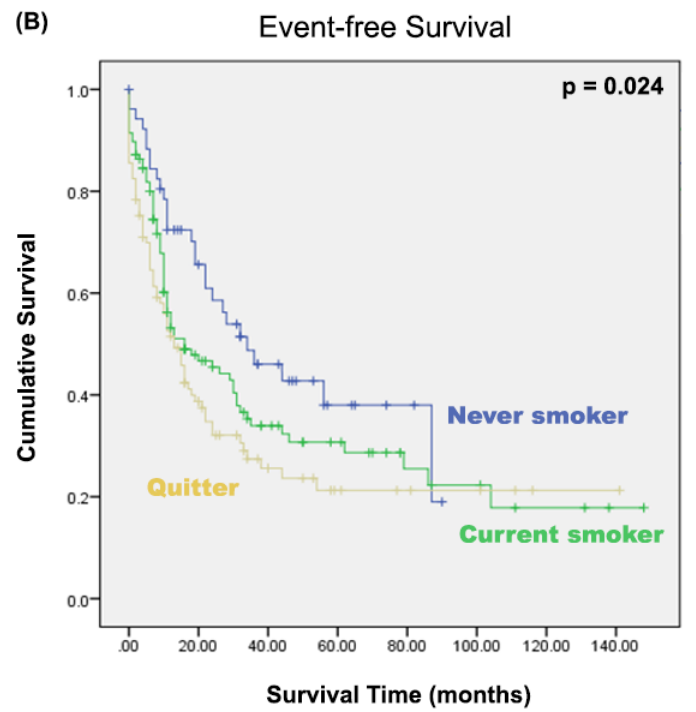
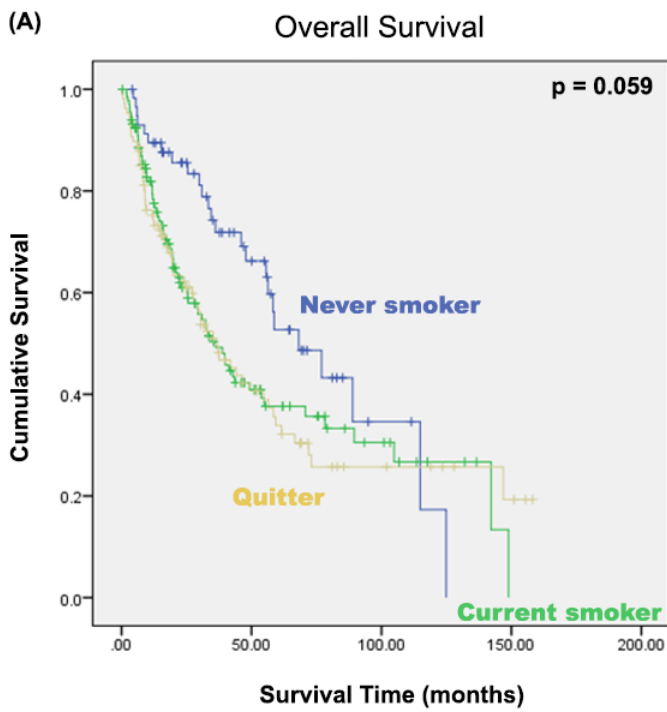
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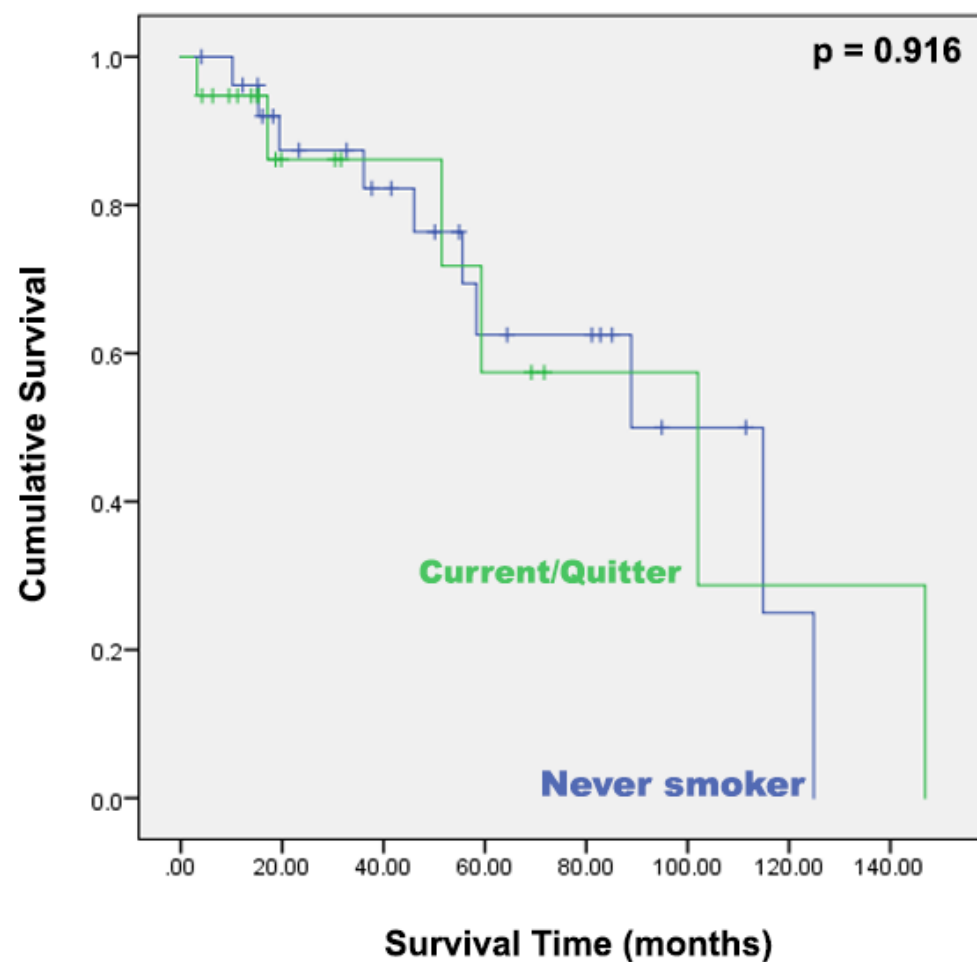






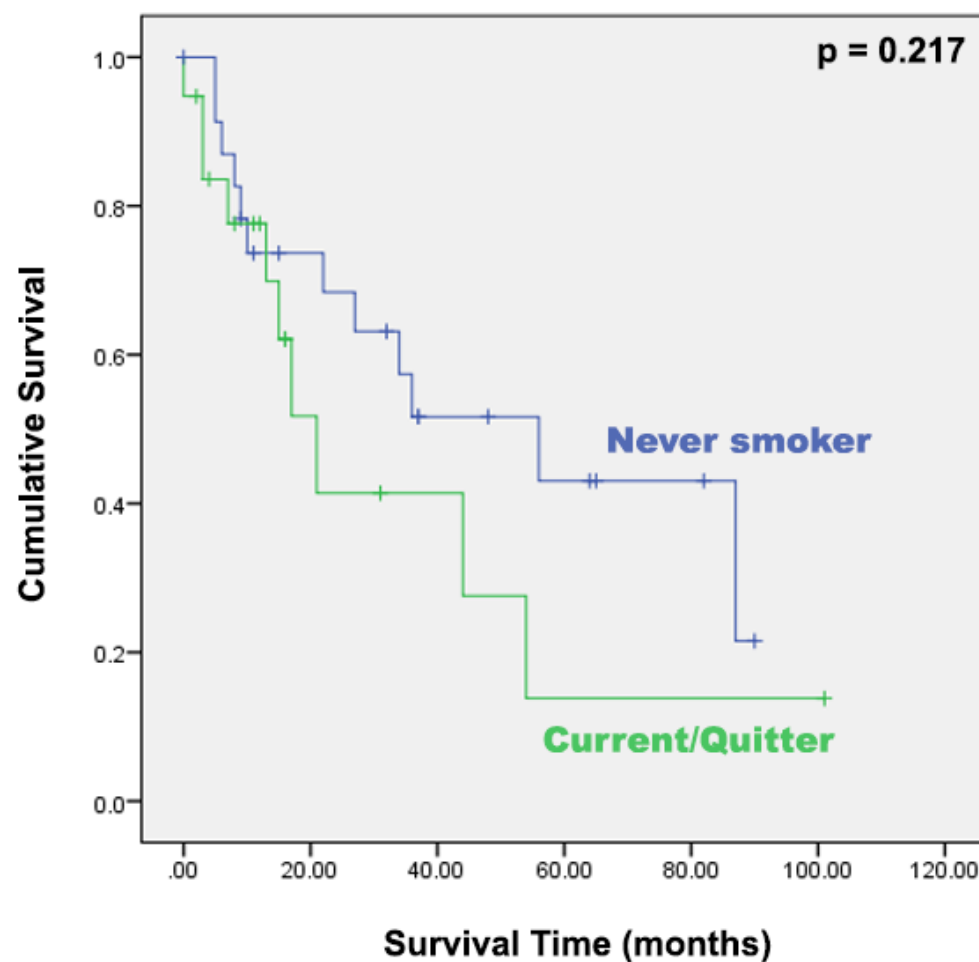
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Overall Survival



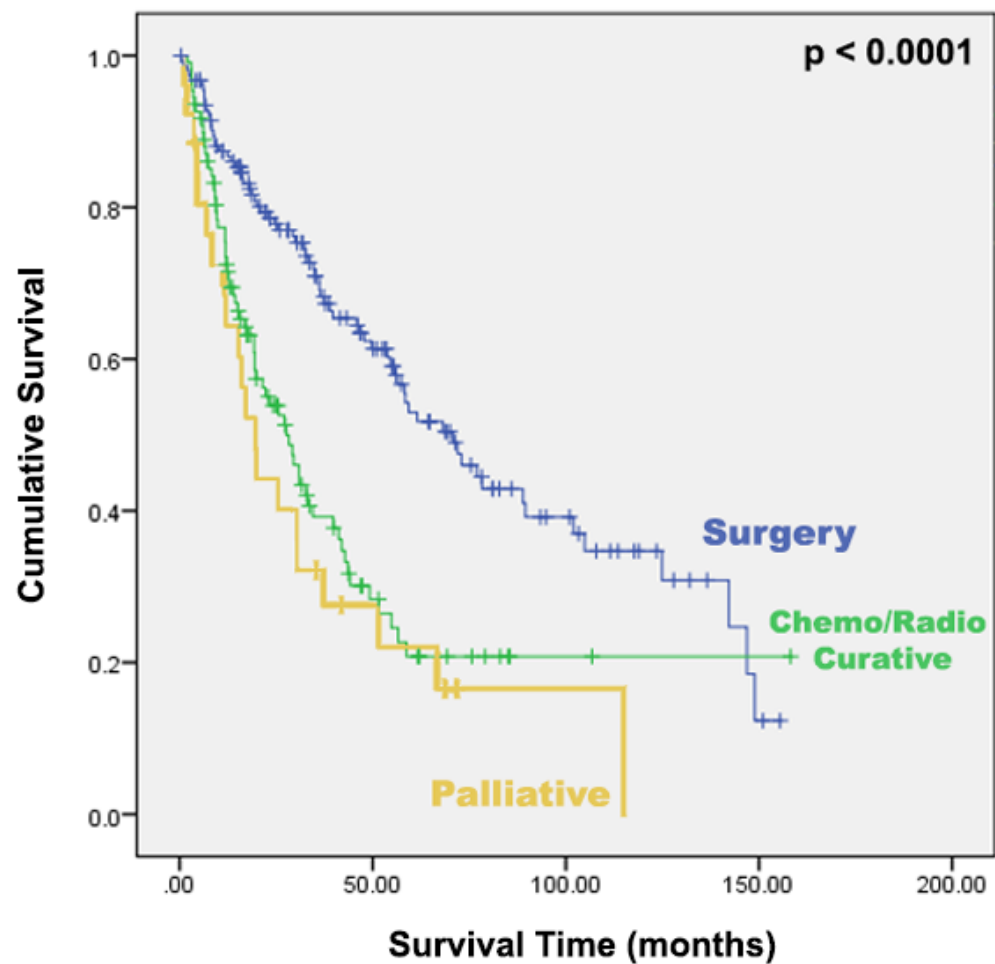
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Event-free Survival



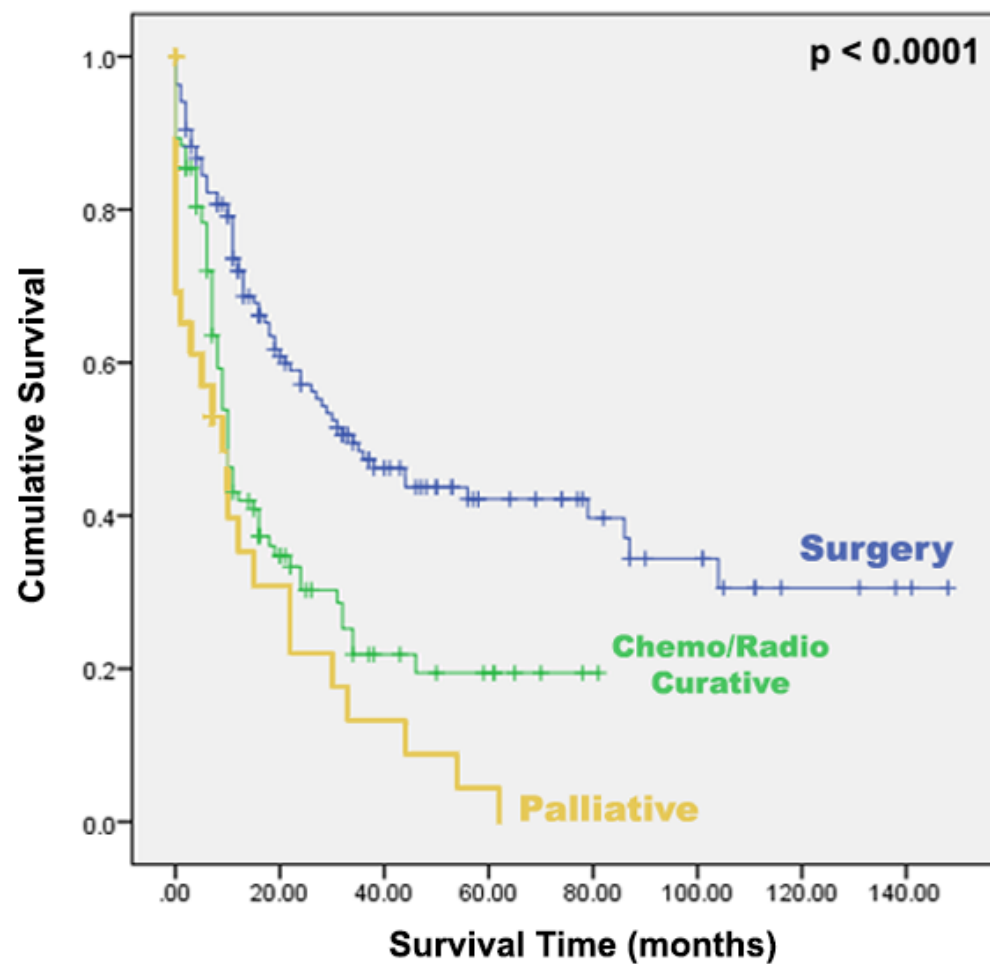
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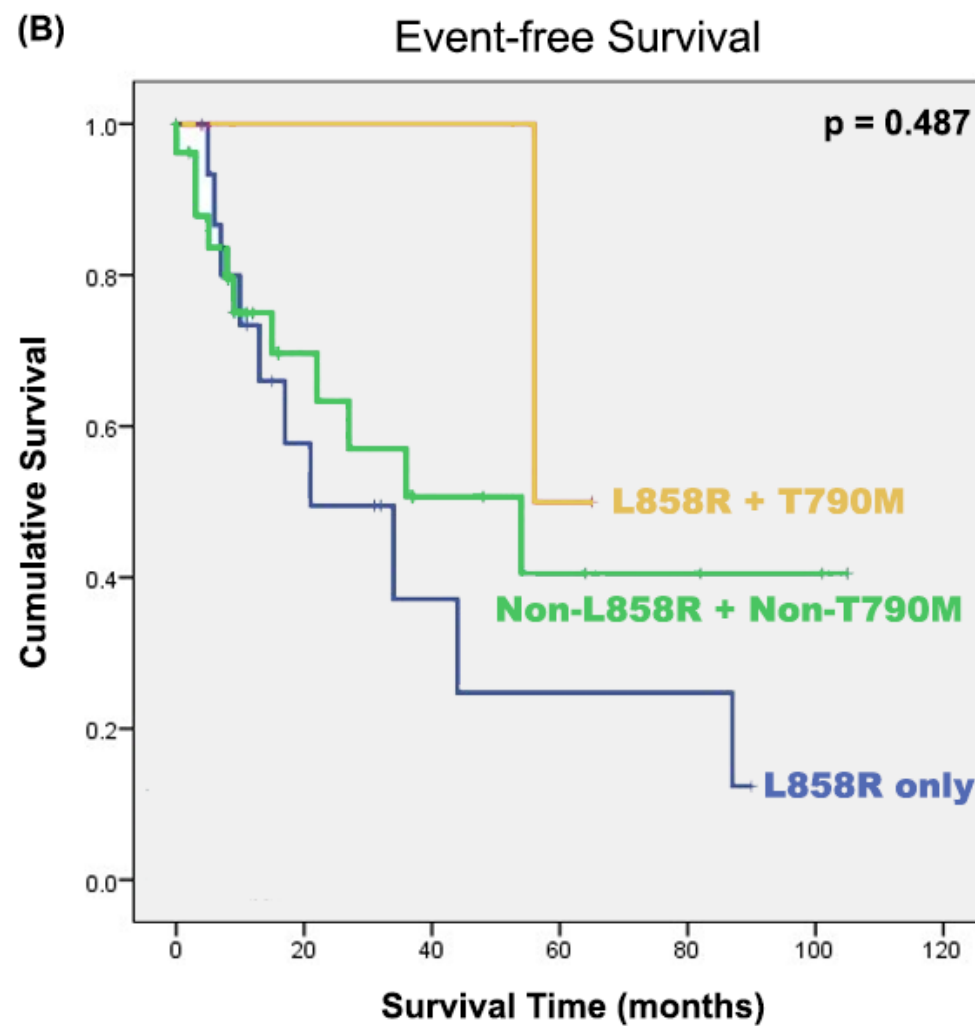
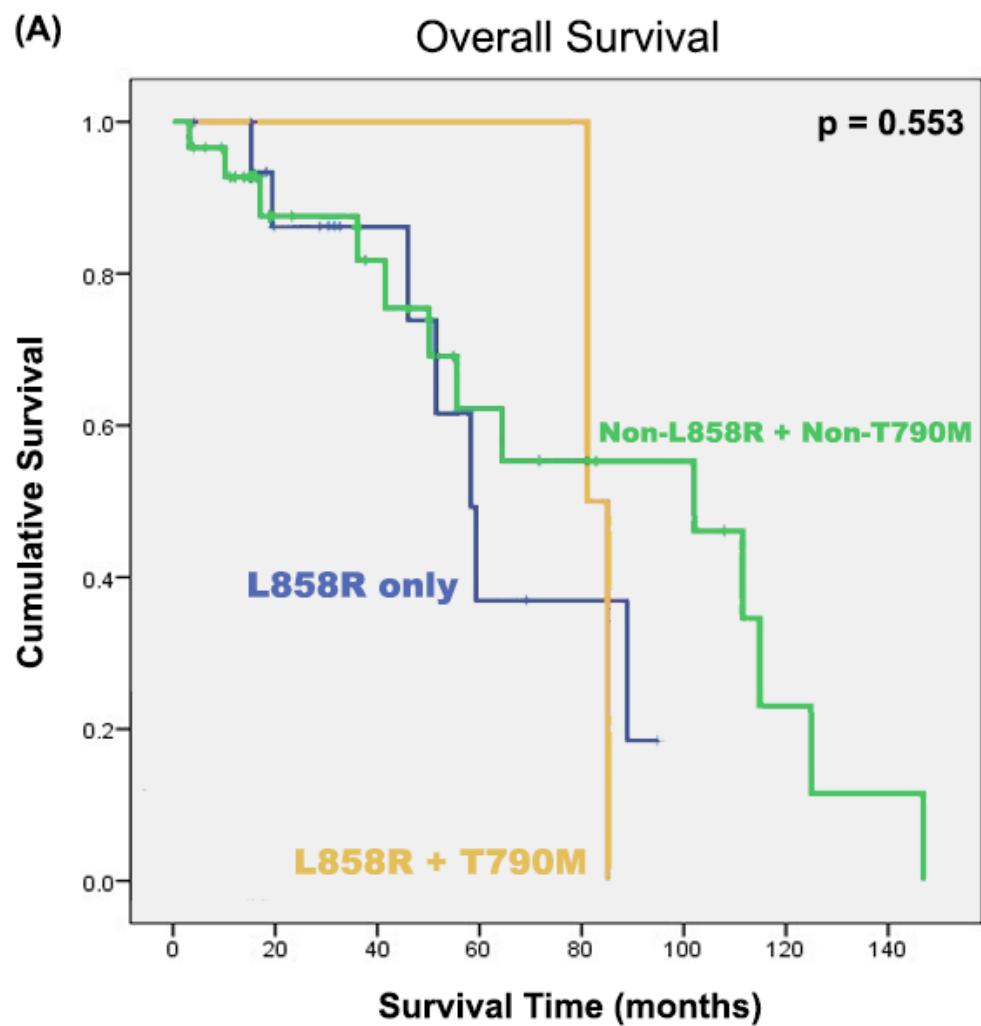
Overall Survival



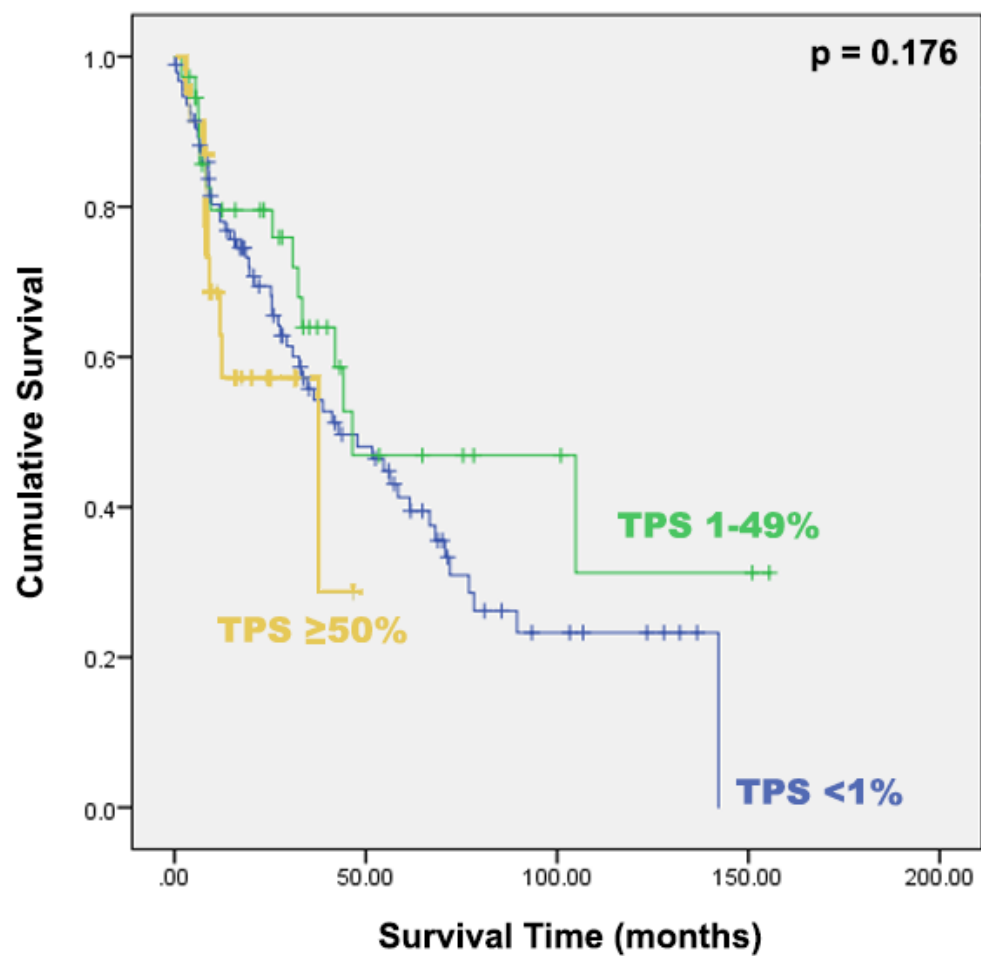
(B)

Event-free Survival

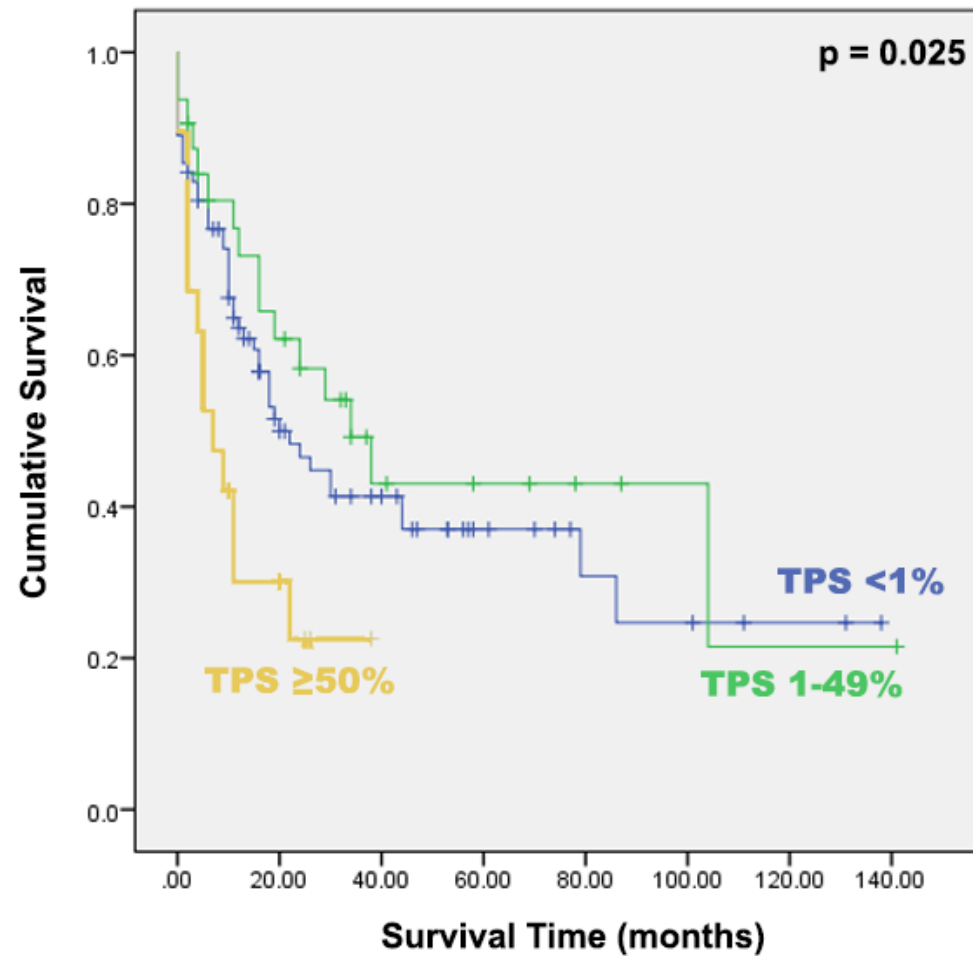




(A) Overall Survival



(B) Event-free Survival



Supplementary Figure Captions

Supplementary Figure 1. Sampling workflow for the current series. *Screen failure: Metastatic cases (n=3), Locally advanced IIIB (n=1), Early-stage IA (n=2), squamous cell carcinoma (n=7). **unsuccessful sample procurement, long-term storage or less than 100 tumor cells. ***highly degraded samples. FFPE, formalin-fixed paraffin-embedded tissues.

Supplementary Figure 2. Representative staining for PD-L1 expression according to TPS. (A) TPS <1% (0%). (B) TPS 1-49% (30%). (C) TPS ≥ 50% (90%). All figures were captured under 100xMagnification.

Supplementary Figure 3. Kaplan-Meier curves for overall survival (OS) and event-free survival (EFS) of NSCLC patients according to tobacco smoking (A and B), performance status (PS) at admission (C and D) disease staging (E and F). (A) Tobacco smoking - overall survival. Median OS: Never smoker = 68.2 months; Median OS: Current smoker = 36.2 months; Median OS: Quitter = 36.4 months. (B) Tobacco smoking - event-free survival. Median EFS: Never smoker = 34 months; Median EFS: Current smoker = 16 months; Median EFS: Quitter = 13 months. (C) Performance status (PS) - overall survival. Median OS: PS 0 = 73 months; Median OS: PS 1 = 35.1 months; Median OS: PS 2 = 12.4 months; Median OS: PS 3/4 = 6.9 months. (D) Performance status (PS) - event-free survival. Median EFS: PS 0 = 26 months; Median EFS: PS 1 = 13 months; Median EFS: PS 2 = 6 months; Median EFS: PS 3/4 = 3 months. (E) Disease staging - overall survival. Median OS: IB = 66.7 months; Median OS: IIA = 89.5 months; Median OS: IIB = 39.8 months; Median OS: IIIA = 30.9 months. (F) Disease staging - event-free survival. Median EFS: IB = 44 months; Median EFS: IIA = 24 months; Median EFS: IIB = 11 months; Median EFS: IIIA = 12 months. Survival time is presented in months; p values are related to Log-rank test results.

Supplementary Figure 4. Kaplan-Meier curves for overall survival (OS) and event-free survival (EFS) of *EGFR*^m patients only according to smoking history. (A) Overall survival. Median OS: never smokers = 88.9 months; Median OS: quitters/current smokers = 102 months (B) Event-free survival. Median EFS: never smokers = 56 months; Median EFS: quitters/current smokers = 21 months. Survival time is presented in months; p values are related to Log-rank test results.

Supplementary Figure 5. Kaplan-Meier curves for overall survival (OS) and event-free survival (EFS) of NSCLC patients according to initial treatment type. (A) Overall survival. Median OS: surgery = 70.7 months; Median OS: chemo/radio with curative intent = 27.6 months; Median OS: palliative care = 19.6 months (B) Event-free survival. Median EFS: surgery = 34 months; Median EFS: chemo/radio with curative intent = 10 months; Median EFS: palliative care = 9 months. Survival time is presented in months; p values are related to Log-rank test results.

Supplementary Figure 6. Kaplan-Meier curves for overall survival (OS) and event-free survival (EFS) of NSCLC patients according to mutation type. (A) Overall survival. Median OS: p.(Leu858Arg) only = 58.3 months; Median OS: p.(Leu858Arg) + p.(Thr790Met) = 81.1 months; Median OS: non-p.(Leu858Arg) + non-p.(Thr790Met) = 102 months (B) Event-free survival. Median EFS: p.(Leu858Arg) only = 21 months; Median EFS: p.(Leu858Arg) + p.(Thr790Met) =

56 months; Median EFS: non- p.(Leu858Arg) + non-p.(Thr790Met) = 54 months. Survival time is presented in months; p values are related to Log-rank test results.

Supplementary Figure 7. Kaplan-Meier curves for overall survival (OS) and event-free survival (EFS) of NSCLC *EGFR* wild-type patients according to PD-L1 positivity. (A) PD-L1 expression stratified according to TPS categories overall survival. Median OS: TPS <1% = 42.8 months; Median OS: TPS 1-49% = 46.5 months; $\geq 50\%$ = 37.5 months. (B) PD-L1 expression stratified according to TPS categories event-free survival. Median EFS: TPS <1% = 20 months; Median EFS: TPS 1-49% = 34 months; $\geq 50\%$ = 7 months. Survival time is presented in months; p values are related to Log-rank test results.

Supplementary Tables

<i>Variables</i>	<i>Parameters</i>	<i>GFR</i>
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mutations detected in the present study according to exon distribution.

Exon/Mutation	n (%)	n (%) Total
18		
p.(Glu709_Thr710delinsAsp)	1 (50)	2 (3.9)
p.(Gly719Ala)	1 (50)	
19		
p.(Lys745_Alal750del)	1 (4.3)	23 (45.1)
p.(Glu746_Alal750del)	7 (30.4)	
p.(Glu746_Ser752delinsVal)	2 (8.7)	
p.(Leu747_Alal750delinsPro)*	1 (4.3)	
p.(Leu747_Ser752del)	2 (8.7)	
p.(Leu747_Pro753delinsSer)	2 (8.7)	
p.(Leu747_Thr751del)	1 (4.3)	
p.(Leu747_Lys754delinsGly)	1 (4.3)	
Non-specified deletion	6 (26.1)	
20		
p.(Ala767_Val769dup)	1 (16.7)	6 (11.8)
p.(Ser768Ile)	1 (16.7)	
p.(Ser768_Val769delinsIleLeu)	1 (16.7)	
p.(Gly779Phe)	1 (16.7)	
p.(Thr790Met)*	2 [#] (33.3)	
21		
p.(Glu829Gln)	1 (5)	20 (39.2)
p.(Leu858Arg)*	19 (95)	

*These mutations may appear simultaneously with other EGFR mutations.

[#]These cases carried the p.(Thr790Met) mutation concomitantly to the p.(Leu858Arg) mutation at diagnosis.

		EGFR			p-value
		n	WT (%)	Mutated (%)	
<i>Age</i> ¹	≤ 66 years	157	86	14	0.112
	> 66 years	121	78.5	21.5	
<i>Sex</i>	Male	129	89.1	10.9	0.011
	Female	149	77.2	22.8	
<i>Self-reported race</i> ²	White/Brazilian	202	82.7	17.3	0.225
	Brown	28	85.7	14.3	
	Black	17	88.2	11.8	
	Yellow	3	33.3	66.7	
	Not well defined	27	81.5	18.5	
	No information	1	100	0	
<i>Smoking</i>	Never smoker	57	52.6	47.4	<0.0001
	Current Smoker	119	95.8	4.2	
	Quitter	99	85.9	14.1	
<i>Disease staging</i>	IB	67	76.1	23.9	0.378
	IIA	41	87.8	12.2	
	IIB	40	87.5	12.5	
	IIIA	130	83.1	16.9	
<i>Stage T</i>	T1a	5	100	0	0.059
	T1b	14	71.4	28.6	
	T2a	101	75.2	24.8	
	T2b	44	88.6	11.4	
	T3	75	90.7	9.3	
	T4	38	81.6	18.4	
	Tx	1	100	0	
<i>Stage N</i>	N0	149	81.9	18.1	0.323
	N1	45	84.4	15.6	
	N2	81	84	16	
	N3	1	0	100	
	Nx	2	100	0	
<i>PS ECOG</i>	0	100	77	23	0.053
	1	130	88.5	11.5	
	2	16	75	25	
	3/4	7	100	0	
<i>Loss of weight</i> ³	No	148	81.1	18.9	0.274
	< 10 %	59	88.1	11.9	
	> 10%	34	91.2	8.8	
<i>PD-L1 expression</i> ⁴	TPS <1%	118	79.7	20.3	0.332
	TPS 1-49%	43	86	14	
	TPS ≥50%	25	92	8	

Supplementary Table 2. Univariate analysis of the association between clinicopathological characteristics and *EGFR* mutational status.

n, number of patients; *PS ECOG*, performance status ECOG (Eastern Cooperative Oncology Group); *TPS*, Tumor Proportion Score (TPS).¹Median age; ²Self-reported race according to Brazilian Institute of Geography and Statistics (IBGE). ³ Loss of weight <10% and >10% of total body weight in the last 6 months. ⁴PD-L1 expression as a dichotomized variable was also analyzed according to *EGFR* mutational status: positive (TPS ≥ 1%) vs. negative (TPS < 1%) (p=0.161).

Supplementary Table 3. Detailed information about evolution after initial treatment in *EGFR*-mutated and *EGFR* wild-type patients.

Evolution after initial treatment		EGFR Status		
		WT (%)	Mutated (%)	
No	96	73 (76.0)	23 (24.0)	0.062
Yes**	157	135 (86.0)	22 (14.0)	

n, number of patients; WT, wild-type. *It does not include patients that evolved to death due to external causes or comorbidities and those that the cause of death was not reported (undetermined). ** Includes patients that evolved to cancer-related death.

Supplementary Table 4. Summary of treatment regimens after initial treatment evolution in *EGFR*-mutated and *EGFR* wild-type patients.

Variables	Parameters	n	EGFR Status	
			WT (%)	Mutated (%)
Palliative	Chemo	43	43 (100)	0 (0)
	RT	31	30 (96.8)	1 (3.2)
	Surgery	1	100 (0)	0 (0)
	Surgery + Chemo	1	0 (0)	1 (100)
	Chemo + RT	4	4 (100)	0 (0)
	TKI	17	2 (11.76)	15 (88.24)
	Chemo + TKI	2	0 (0)	2* (100)
	Immuno	3	3 (100)	0 (0)
	Chemo + Immuno + RT	1	1 (100)	0 (0)
	Other	10	10 (100)	0 (0)

n, number of patients; WT, wild-type; Chemo, chemotherapy; RT, radiotherapy; TKI, tyrosine kinase inhibitor; Immuno, immunotherapy. *One patient received palliative TKI as initial treatment. No statistical associations were conducted for these data due to sample size per group.

Supplementary Table 5. *EGFR* mutated TKi-treated patients according to exon distribution and TKi.

Exon/Mutation	TKI					
	Gefitinib	Erlotinib*	Osimertinib	Erlotinib + Osimertinib	Erlotinib + Gefitinib	Erlotinib + Gefitinib + Osimertinib
19						
p.(Glu746_Ala750del)	1				1	1
p.(Glu746_Ser752delinsVal)		1				
p.(Leu747_Ala750delinsPro)				1		
p.(Leu747_Ser752del)		1				
p.(Leu747_Thr751del)		1				
Non-specified deletion		1	1			
20						
p.(Thr790Met)				2		
21						
p.(Glu829Gln)		1		1		
p.(Leu858Arg)	1	3		2	1	

*No statistical associations were conducted for these data due to sample size per group. *One patient started treatment with gefitinib but switched to erlotinib due to a skin rash.*

Supplementary Table 6. Univariate analysis of the association between clinicopathological characteristics and *PD-L1* positivity.

Variables	Parameters	PD-L1 expression			p-value
		n	Negative (%)	Positive (%)	
Age ¹	≤ 66 years	107	59.8	40.2	0.287
	> 66 years	81	67.9	32.1	
Sex	Male	89	61.8	38.2	0.762
	Female	99	64.7	35.3	
Self-reported race ²	White/Brazilian	135	66.7	33.3	0.345
	Brown	19	68.4	31.6	
	Black	11	45.4	54.6	
	Yellow	3	66.7	33.3	
	Not well defined	19	47.4	52.6	
Smoking	Never smoker	41	78.0	22.0	0.101
	Current Smoker	80	60.0	40.0	
	Quitter	66	59.1	40.9	
Disease staging	IB	49	79.6	20.4	<0.001
	IIA	27	88.9	11.1	
	IIB	28	39.3	60.7	
	IIIA	84	53.6	46.4	
Stage T	T1	13	84.6	15.4	<0.001
	T2a	66	78.8	21.2	
	T2b	33	63.6	36.4	
	T3	52	46.2	53.8	
	T4	24	45.8	54.2	
Stage N	N0	104	65.4	34.6	0.364
	N1	30	70.0	30.0	
	N2+	52	55.8	44.2	
PS ECOG	0	71	67.6	32.4	0.154
	1	90	62.2	37.8	
	≥2	11	45.5	54.5	
Loss of weight ³	No	104	72.1	27.9	<0.001
	< 10 %	40	37.5	62.5	
	> 10%	23	60.9	39.1	
EGFR mutational Status	Wild-type	154	61.0	39.0	0.161
	Mutated	32	75.0	25.0	

Supplementary Table 7. Univariate analysis of the association between clinicopathological characteristics and *PD-L1 expression according to Tumor Proportion Score (TPS)*.

Variables	Parameters	<i>PD-L1 expression</i>				p-value
		n	< 1% (%)	1-49% (%)	≥50% (%)	
Age ¹	≤ 66 years	107	59.8	25.2	15	0.533
	> 66 years	81	67.9	21	11.1	
Sex	Male	89	61.8	21.3	16.9	0.385
	Female	99	64.6	25.3	10.1	
Self-reported race ²	White/Brazilian	135	66.7	20.7	12.6	0.171
	Brown	19	68.4	31.6	0	
	Black	11	45.5	18.2	36.4	
	Yellow	3	66.7	33.3	0	
	Not well defined	19	47.4	31.6	21.1	
	No information	1	0	100	0	
Smoking	Never smoker	41	78	17.1	4.9	0.174
	Current Smoker	80	60	27.5	12.5	
	Quitter	66	59.1	22.7	18.2	
Disease staging	IB	49	79.6	12.2	8.2	<0.0001
	IIA	27	88.9	11.1	0	
	IIB	28	39.3	39.3	21.4	
	IIIA	84	53.6	28.6	17.9	
Stage T	T1a	3	100	0	0	0.029
	T1b	10	80	10	10	
	T2a	66	78.8	12.1	9.1	
	T2b	33	63.3	24.2	12.1	
	T3	52	46.2	34.6	19.3	
	T4	24	45.8	37.5	16.7	
Stage N	N0	104	64.5	25	9.6	0.25
	N1	30	70	23.3	6.7	
	N2	51	54.9	21.6	23.5	
	N3	1	100	0	0	
PS ECOG	0	71	67.6	18.3	14.1	0.407
	1	90	62.2	24.4	13.3	
	2	9	33.3	33.3	33.3	
	3/4	2	100	0	0	
Loss of weight ³	No	104	72.1	18.3	9.6	0.003
	< 10 %	40	37.5	37.5	25	
	> 10%	23	60.9	21.7	17.4	
EGFR mutational Status	Wild-type	154	61	24	14.9	0.332
	Mutated	32	75	18.8	6.3	

n, number of patients; PS ECOG, performance status ECOG (Eastern Cooperative Oncology Group); TPS, Tumor Proportion Score (TPS).¹Median age; ²Self-reported race according to Brazilian Institute of Geography and Statistics (IBGE). ³ Loss of weight <10% and >10% of total body weight in the last 6 months

Supplementary Table 8. Multivariate analysis of the association between clinicopathological characteristics and event-free survival.

Variables	Parameters	n	HR	95%CI	p-value
PS ECOG	0	99	1	Ref.	Ref
	1	123	1.547	1.095–2.186	0.013
	2	14	3.548	1.811–6.948	<0.001
	3 or 4	5	3.162	0.909–7.079	0.075
Disease Staging	IB	60	1	Ref.	Ref.
	IIA	32	1.379	0.792–2.400	0.256
	IIB	31	1.292	0.723–2.308	0.387
	IIIA	118	1.927	1.261–2.944	0.002

* 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).

5.7 Perfil molecular de pacientes nunca fumantes diagnosticados com adenocarcinoma de pulmão.

Este trabalho descreveu a frequência dos genes contemplados no painel “*TruSight Tumor 15*” e das fusões nos genes *ALK/RET/ROS1/NTRK1,2,3* e *METΔex14* por painel “Elements” para nCounter em pacientes brasileiros não fumadores, diagnosticados com adenocarcinoma de pulmão no Hospital de Amor de Barretos.

Molecular profile of driver genes in lung adenocarcinomas of Brazilian patients who have never smoked: implications for targeted therapies

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Abstract

Introduction: Lung cancer in never-smoker (LCINS) patients accounts for 20% of lung cancer cases, and its biology remains poorly understood, particularly in genetically admixed populations. We elucidated the molecular profile of driver genes in Brazilian LCINS.

Methods: The mutational and gene fusion status of 119 lung adenocarcinomas from self-reported never-smoker patients, was assessed using targeted sequencing (NGS), nCounter, and immunohistochemistry. A panel of 46 ancestry-informative markers determined patients' genetic ancestry.

Results: The most frequently mutated gene was *EGFR* (49.6%), followed by *TP53* (39.5%), *ALK* (12.6%), *ERBB2* (7.6%), *KRAS* (5.9%), *PIK3CA* (1.7%), and less than 1% alterations in *RET*, *NTRK1*, *METΔex14*, *PDGFRA*, and *BRAF*. Except for *TP53* and *PIK3CA*, all other alterations were mutually exclusive. Genetic ancestry analysis revealed a predominance of European (71.1%), and a higher African ancestry was associated with *TP53* mutations.

Conclusion: Brazilian LCINS exhibited a similar molecular profile to other populations, except the increased *ALK* and *TP53* alterations. Importantly, 73% of these patients have actionable alterations that are suitable for targeted treatments.

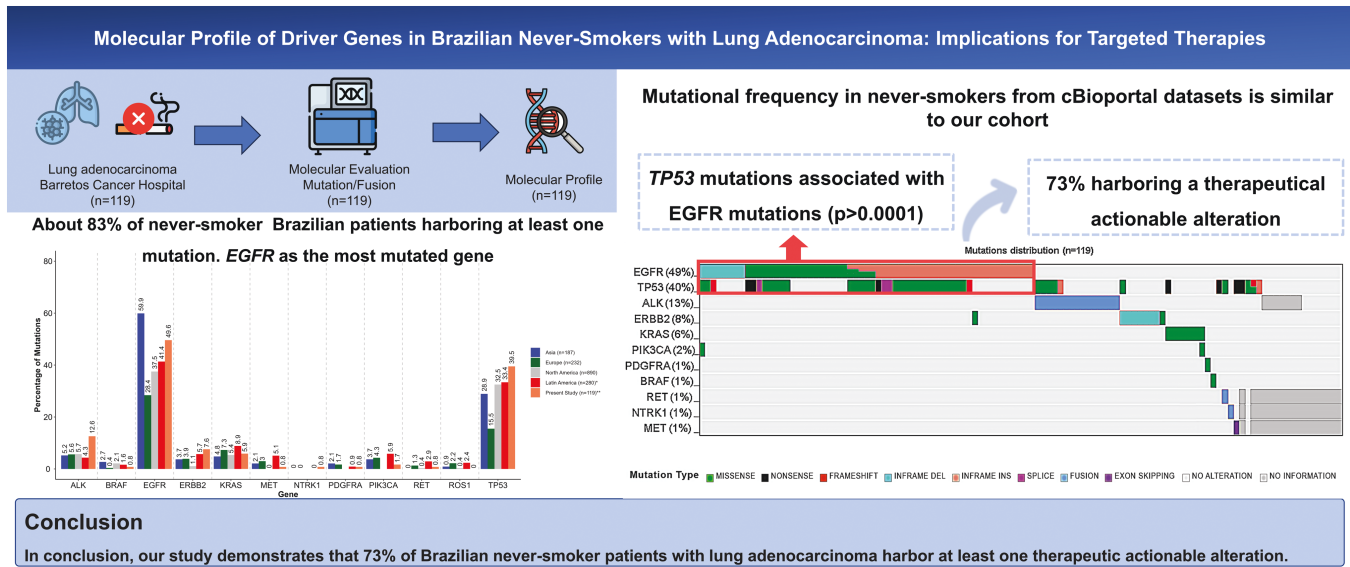
Key words: lung adenocarcinoma; molecular profile; driver mutations; never smoker; Latin America.

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Graphical Abstract



Implications for Practice

The identification of mutations in lung adenocarcinomas is crucial for deciding the best clinical management for the patients. Here, we observed 73% with at least one actionable alteration, with EGFR mutations reaching approximately 50% of patients. Therefore, these patients could be benefited by treatments with targeted drugs. A better understanding of the molecular profile in never-smoker patients from Brazil may improve the management of patients.

Introduction

Lung cancer in patients who have never smoked (LCINS) accounts for 20% of lung cancer cases and remains under-explored despite its increasing worldwide incidence.¹⁻⁴ Lung cancer in never-smokers shows a better prognosis compared to ever-smokers.¹⁻⁵

Lung cancer biology varies between never-smokers and smokers.^{1,3,6,7} Lung adenocarcinomas in never-smoker patients exhibit a higher frequency of *EGFR*, *PIK3CA*, and *ERBB2* mutations.^{1,6} *EGFR* mutations are notably more common, at variance with *KRAS* mutations, which are associated with tobacco exposure.^{2,4} Moreover, LCINS are more likely to harbor actionable variants, including not only *EGFR* mutations but also *ALK* translocation, impacting patients' clinical management.^{1,3}

Patient ethnicity also influences molecular profiles, with *EGFR* mutations more prevalent in Asians and *KRAS* mutations in Europeans.^{3,8,9} In admixed populations like Brazil, these profiles vary, and are poorly investigated.¹⁰ Therefore, we aimed to elucidate the molecular features of Brazilian LCINS.

Materials and methods

Study population

A series of 119 self-declared, never-smoker patients with lung adenocarcinoma (97 primary and 22 following treatment) from Barretos Cancer Hospital (BCH, Barretos, SP, Brazil) was evaluated. The local IRB approved the study (CAAE 05744712.3.0000.5437).

Mutation detection

Tumor mutational analysis was performed in FFPE sections using the commercial panel TruSight Tumor 15 (Illumina, San Diego, CA, USA) on a MiSeq instrument. The read alignment and variant calling were performed with Sophia DDM software version 4.2 (Sophia Genetics SA, Lausanne, Switzerland). Variants were filtered out as previously described, and pathogenic variants were retained.^{11,12} Actionable mutations (Tier I and II) were determined as reported.¹

Fusion detection

ALK fusions were evaluated in 95.0% ($n = 113/119$) of cases by immunohistochemistry using the Ventana *ALK* (D5F3) CDx Assay (Roche, Basel, Switzerland) in an automated equipment.¹¹

Detection of mRNA *ALK/RET/ROS1/NTRK1,2,3* fusion transcripts and *MET*Δex14 (*MET* exon 14 skipping) was assessed in patients with *EGFR* and *KRAS* wild-type tumors ($n=61$) by the nCounter Elements XT (NanoString Technologies, Seattle, WA, USA) custom panel for *ALK*, *RET*, *ROS1*, and *NTRK1/2/3* fusion detection by specific partner and 3' and 5' disbalance, and *MET*Δex14, using probes designed in-house.¹³ Twenty-four cases were inconclusive due to unavailable biological material. All analyses were performed in R environment v3.4.1.

Genetic ancestry

The genetic ancestry background was evaluated using a set of 46 ancestry-informative markers, including INDELs for European (EUR), African (AFR), Asian (ASN), and Native American (AME).^{8,12}

Statistical analysis

We described categorical variables using percentages and continuous variables using the medians. Fisher's exact test and χ^2 test were conducted for the association between the *EGFR* and *TP53* mutations and genetic ancestry in IBM SPSS software Version 25 (IBM, Armonk, NY, USA) with a statistical significance limit of .05.

Results

Characterization of patients

The clinicopathological features of the 119 LCINS are summarized in [Supplementary Table S1](#). Genetic ancestry was determined in 90.0% ($n = 107/119$) of the cases, following the proportion of 71.0% of EUR, 15.9% of AFR, 6.1% of ASN, and 7.9% of AME ([Supplementary Figure S1](#)).

Molecular profile

Among the 119 lung adenocarcinomas, 83.2% ($n = 99/119$) harbored at least one pathological mutation ([Figure 1A](#)). Among the 99 mutated cases, 54.5% carried one mutation, 39.4% 2, 4.1% 3, and 2.0% 4 ([Supplementary Table S2](#)). *EGFR* was the most mutated gene (49.6%, $n = 59/119$), followed by *TP53* 39.5% ($n = 47/119$), and *ALK* fusions in 12.6% ($n = 15/119$; [Figure 1A](#)). The genes *ERBB2*, *KRAS*, *PIK3CA*, *RET*, *BRAF*, *PDGFRA*, *NTRK1*, and *MET* showed fewer genetic alterations ([Figure 1A](#)). No alterations were observed on *AKT1*, *FOXL2*, *GNA11*, *GNAQ*, *KIT*, *NRAS*, *ROS1*, and *NTRK2/3* hotspot regions. Apart from *TP53* and *PIK3CA*, all the alterations were mutually exclusive ([Figure 1A](#)). Eighty-seven patients (73.1%) harbor actionable mutations ([Supplementary Table S2](#)).

Among *EGFR* mutations, exon 19 deletions were present in 49.2% of cases, followed by exon 21 (28.8%), and less frequently in exon 20 (7.6%) and 18 (1.7%; [Figure 1B](#); [Supplementary Table S2](#)). Three tumors (following treatment) harbored the p.(Thr790Met) resistance mutation, and 2 presented additionally the p.(Cys797Ser) variant. Concerning *TP53*, the most common variant was the p.(Arg337His) (12.8%), followed by the p.(Arg273Cys) (6.4%) and the p.(Val216Leu) (4.3%; [Figure 1C](#); [Supplementary Table S2](#)). One patient harbored 2 *TP53* variants ([Supplementary Table S2](#)). *TP53* mutations were associated with African ancestry ($P = .002$; [Supplementary Table S3](#)).

Exon 20 insertion p.(Tyr772_Ala775dup) accounted for 66.7% of *ERBB2* mutations ([Supplementary Table S2](#)). The most common variant of the *KRAS* gene was p.(Gly12Asp) (42.8%, $n = 3/7$), and the variants observed in the *PIK3CA*, *BRAF*, and *PDGFRA* genes were at hotspot regions ([Supplementary Table S2](#)).

ALK fusions were observed in 15 patients (12.6%), both by immunohistochemistry and nCounter, the latter allowing to identify *EML4* as the fusion partner in 73.3% of the cases. *RET* and *NTRK1* fusions were identified by 3'-5' disbalance in one patient each, ([Figure 1A](#); [Supplementary Table S2](#)).

Co-occurring mutations

EGFR and *TP53* mutations significantly co-occurred in 27.7% ($n = 33/119$) of cases ($P < .0001$; [Figure 1A](#); [Supplementary Table S4](#)). The Brazilian founder *TP53* mutation p.(Arg337His) variant was mostly concurrent with *EGFR* mutations ([Supplementary Table S2](#)). *EGFR* variants

also co-occurred with *PIK3CA* and *ERBB2* mutations. *TP53* mutations co-occurred with 1/3 of *ALK* fusions. The *PIK3CA* variants also co-occurred with the *KRAS* and *TP53* ([Figure 1A](#)).

Discussion

The present study interrogated the molecular profile of driver genes of LCINS from a single Brazilian institution. Overall, we found that 73% of cases harbor actionable molecular alterations, in accordance with the literature.^{1,4,5}

EGFR mutations occurred in half of our cases, in agreement with other populations, being higher than European and lower than Asian populations ([Figure 2](#); [Supplementary Table S5](#)).^{1,2,4,6} Similar to our results, *EGFR*-TKi sensitizing exon 19 deletions are found in 50% of patients diagnosed with lung adenocarcinoma, while exon 20 insertions are less common globally.^{1,6,8} *TP53* was our second most mutated gene (39.5%). This frequency is higher compared to studies of LCINS in Asia, Europe, North America, and Latin America ([Figure 2](#); [Supplementary Table S5](#)).⁶ We observed an association between *TP53* mutations and higher African ancestry, similar to our recent study on ever- and never-smoker patients with lung adenocarcinoma.¹² Notably, the most frequent *TP53* mutation was the Brazilian germline variant p.(Arg337His), often concurrent with *EGFR* mutations. Previous lung cancer studies reported higher co-occurrence of *EGFR* and *TP53* mutations, mainly with the Brazilian founder mutation.¹⁴⁻¹⁷

We observed 7.6% of *ERBB2* mutations in our LCINS, consistent with 2%-13% reported in other populations ([Figure 2](#); [Supplementary Table S5](#)).^{1-4,6} In our series, only 5.9% of patients with lung adenocarcinoma had *KRAS* mutations, in accordance with other LCINS studies (4.4%-18%). Interestingly, *ALK* fusions in our LCINS series were significantly more frequent (12.6%) than reported globally (3%-8%).^{2,4,6} Finally, as previously reported, we identified less frequent alterations (1%-2%) in the genes *PIK3CA*, *PDGFRA*, *BRAF*, *RET*, *NTRK1*, and *MET*, similar to other populations^{1,6,18} ([Figure 2](#); [Supplementary Table S5](#)).

Thus, we observe in the Brazilian LCINS population an overall similarity in the frequencies of driver genes reported worldwide. The exception was our higher frequency of *ALK* fusions and *TP53* mutations, which could potentially be due to the significant presence of African ancestry, or founder *TP53* p.(Arg337His) variant in the Brazilian population ([Figure 2](#); [Supplementary Table S5](#)). Further studies are needed to validate and extend these findings.

Concluding, the molecular profile of Brazilian LCINS resembles that of other populations worldwide, and 73% of patients could be eligible for personalized treatments.

Acknowledgments

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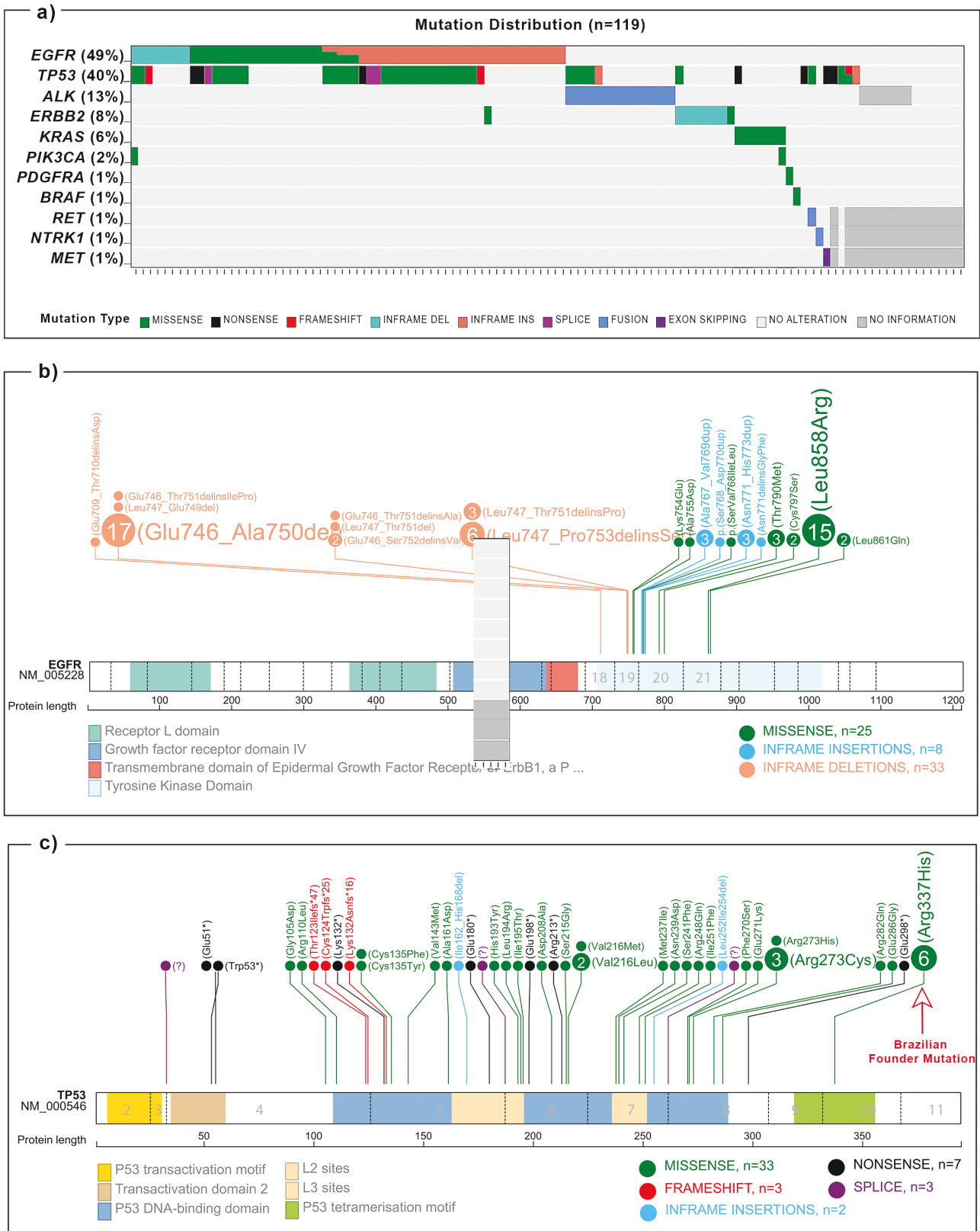


Figure 1. Representative figure of the mutations identified in the LCINS samples included in the study ($n = 119$). Only mutated genes are shown; (a) distribution of mutations through samples; lollipop plots depicting the distribution of (b) *EGFR* ($n = 66$); (c) *TP53* ($n = 48$). Figures were created using tools in <https://proteinpaint.stjude.org/>.

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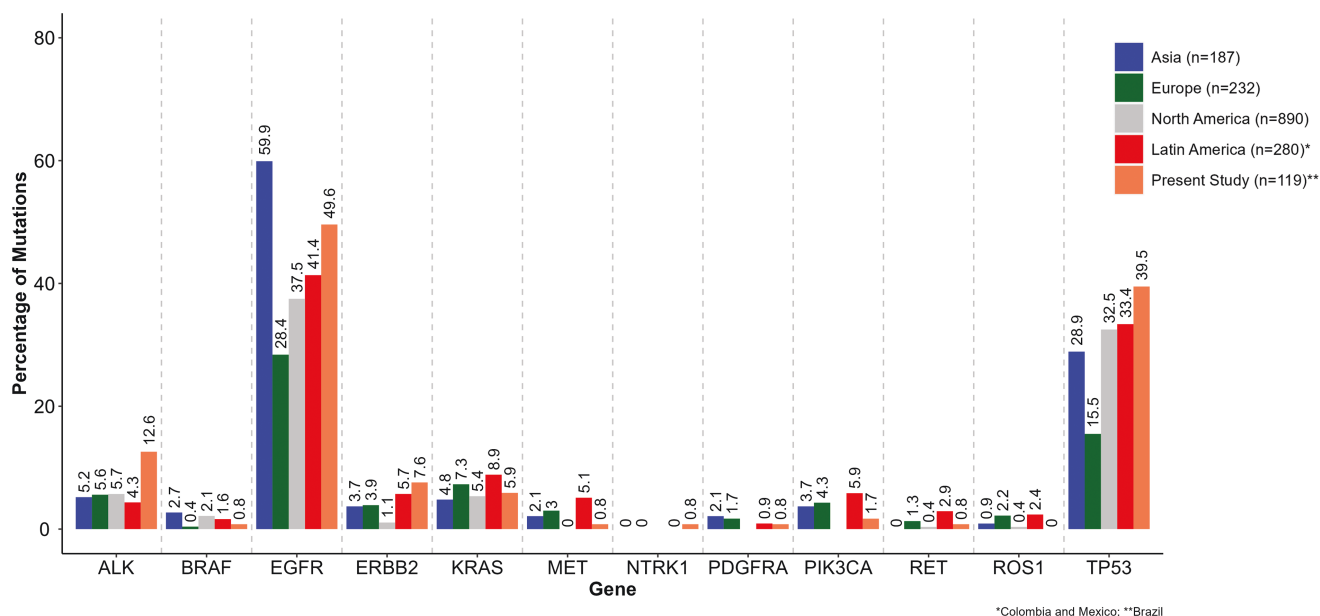


Figure 2. Mutational frequency comparison between studies with never-smoker patients with lung adenocarcinoma according to the geographic region of the study ($n = 1708$).

undergraduate research project scholarship. Funding sources have no contribution to filling out authorship for the present study. We thank all members of the GTOP group (Translational Group of Pulmonary Oncology-Barretos Cancer Hospital, Brazil) for scientific discussion and suggestions.

Author contributions

Rodrigo de Oliveira Cavagna: conception/design, data analysis and interpretation, manuscript writing, final approval of manuscript. Flávia Escremim de Paula: data analysis and interpretation, provision of study material or patients, final approval of manuscript. Gustavo Noriz Berardinelli: data analysis and interpretation, provision of study material or patients, final approval of manuscript. Murilo Queiroz de Almeida Bonatelli: data analysis and interpretation, provision of study material or patients, final approval of manuscript. Iara Santana: data analysis and interpretation, provision of study material or patients, final approval of manuscript. Eduardo Caetano Albino da Silva: data analysis and interpretation, provision of study material or patients, final approval of manuscript. Gustavo Ramos Teixeira: data analysis and interpretation, provision of study material or patients, final approval of manuscript. Beatriz Garbe Zaniolo: collection and/or assembly of data, final approval of manuscript. Josiane Mourão Dias: collection and/or assembly of data, final approval of manuscript. Flávio Augusto Ferreira da Silva: collection and/or assembly of data, provision of study material or patients, final approval of manuscript. Carlos Eduardo Baston Silva: collection and/or assembly of data, provision of study material or patients, final approval of manuscript. José Elias Miziara: collection and/or assembly of data, final approval of manuscript. Marcela Gondim Borges Guimarães: collection and/or assembly of data, provision of study material or patients, final approval of manuscript. Camila Pinto Barone: collection and/or assembly of data, provision of study material or patients, final approval of manuscript. Alexandre Arthur Jacinto: collection and/or assembly

of data, final approval of manuscript. Rachid Eduardo Noleto da Nóbrega Oliveira: collection and/or assembly of data, final approval of manuscript. Pedro de Marchi: collection and/or assembly of data, final approval of manuscript. Miguel Angel Molina: data analysis and interpretation, final approval of manuscript. Leticia Ferro Leal: conception/design, data analysis and interpretation, manuscript writing, final approval of manuscript. Rui Manuel Reis: conception/design, data analysis and interpretation, manuscript writing, final approval of manuscript.

Conflicts of interest

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In3Bio Ltd. Consulting fees: Atheneum. Honoraria for presentations: Spanish Lung Cancer Group (SLCG). Receipt of equipment: Astrazeneca. L.F.L.—research grant from AstraZeneca do Brasil.

Data availability

The data that support the findings of this study are available from Dr. Rui Manuel Reis, but restrictions apply to the availability of these data, because of patients' personal data. De-identified data are, however, available from the authors upon request.

Supplementary material

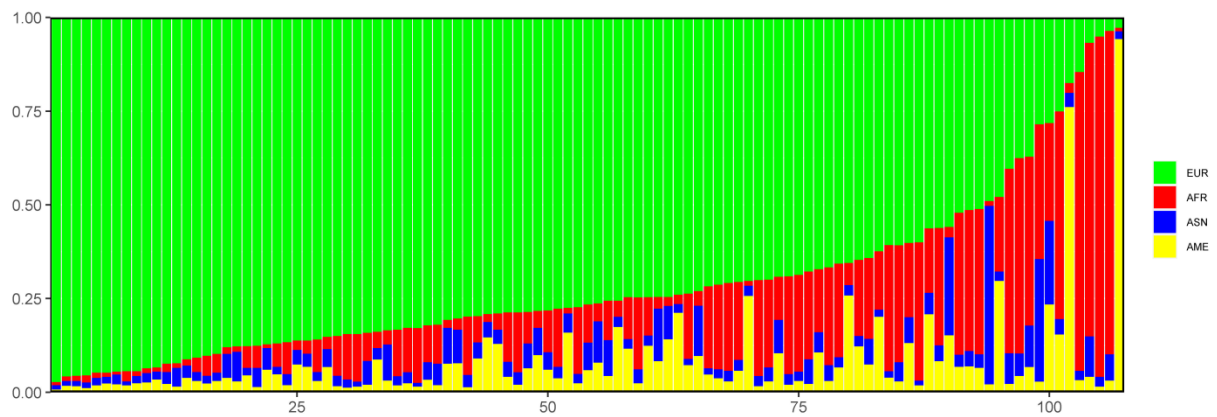
Supplementary material is available at *The Oncologist* online.

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Supplementary Figure Caption

Supplementary Figure 1 - Genetic ancestry proportions from lung adenocarcinomas (n=107). Figure shows the ancestry proportions in the X axis and the ancestry proportion of the populations for each patient in the Y axis. AFR, African (red); EUR, European (green); ASN, Asian (blue); AME, Native American (yellow).



		ADENOCARCINOMA (n=119)	
Characteristics	Parameters	n	(%)
Age (year)	Median (range)	60	(19 – 86)
	<=60	62	(52.1)
	>60	57	(47.9)
Sex	Female	77	(64.7)
	Male	42	(35.3)
Self-reported skin color*	White	69	(58.0)
	Non-White	26	(21.8)
	Missing	24	(20.2)
ECOG PS	0	27	(22.7)
	1	51	(42.9)
	2	23	(19.3)
	3/4	7	(5.9)
	Missing	11	(9.2)
Weight loss**	No	42	(35.3)
	<=10% of weight	36	(30.2)
	>10% of weight	22	(18.5)
	Missing	19	(16.0)
Stage at diagnosis***	I/II/III	29	(24.4)
	IV	86	(72.3)
	Missing	4	(3.4)
Metastasis at diagnosis	No	29	(24.4)
	Yes, CNS	29	(24.4)
	Yes, Others	56	(47.1)
	Missing	5	(4.2)
Vital status	Alive – no disease	8	(6.7)
	Alive – active disease	35	(29.4)
	Death - cancer	72	(60.5)
	Death - others	3	(2.5)
	Loss of follow-up	1	(0.8)

*According to IBGE (Instituto Brasileiro de Geografia e Estatística); **Weight of loss 6 months prior to diagnosis. ***According to AJCC 7th edition.

Supplementary Table 2 - Description of molecular alterations per patient.

mutation/patient	patient	gene	refseq	chromosome	start	aachange	class	REF	ALT	Actionable Mutation
1	2	EGFR	NM_005228	7	55259515	p.(Leu858Arg)	Missense	T	G	Yes
1	3	EGFR	NM_005228	7	55242467	p.(Glu746_Ser752delinsVal)	ProteinDel	AATTAAGAGAAGCAACATC	T	Yes
1	5	EGFR	NM_005228	7	55259515	p.(Leu858Arg)	Missense	T	G	Yes
2	10	EGFR	NM_005228	7	55242464	p.(Glu746_Ala750del)	ProteinDel	AGGAATTAAGAGAAGC	A	Yes
	10	TP53	NM_000546	17	7577129	p.(Phe270Ser)	Missense	A	G	No
1	11	EGFR	NM_005228	7	55248998	p.(Ala767_Val769dup)	ProteinIns	A	ATGGCCAGCG	Yes
2	15	EGFR	NM_005228	7	55259515	p.(Leu858Arg)	Missense	T	G	Yes
	15	TP53	NM_000546	17	7574017	p.(Arg337His)	Missense	C	T	No
1	16	ERBB2	NM_004448	17	37880981	p.(Tyr772_Ala775dup)	ProteinIns	A	AGCATACGTGATG	Yes
1	21	ALK		2		EML4 exon 2::ALK exon 20	Fusion			Yes
1	32	EGFR	NM_005228	7	55241678	p.(Glu709_Thr710delinsAsp)	ProteinDel	AAAC	A	Yes
1	36	EGFR	NM_005228	7	55259515	p.(Leu858Arg)	Missense	T	G	Yes
1	39	EGFR	NM_005228	7	55249010	p.(Asn771_His773dup)	ProteinIns	G	GACAACCCCC	Yes
1	40	EGFR	NM_005228	7	55259524	p.(Leu861Gln)	Missense	T	A	Yes
1	41	ERBB2	NM_004448	17	37880981	p.(Tyr772_Ala775dup)	ProteinIns	A	AGCATACGTGATG	Yes
1	42	EGFR	NM_005228	7	55259515	p.(Leu858Arg)	Missense	T	G	Yes
1	52	EGFR	NM_005228	7	55259515	p.(Leu858Arg)	Missense	T	G	Yes
1	53	EGFR	NM_005228	7	55242464	p.(Glu746_Ala750del)	ProteinDel	AGGAATTAAGAGAAGC	A	Yes
1	59	EGFR	NM_005228	7	55242465	p.(Glu746_Ala750del)	ProteinDel	GGAATTAAGAGAAGCA	G	Yes
1	66	ALK		2		EML4 exon 13::ALK exon 20	Fusion			Yes
2	70	EGFR	NM_005228	7	55248998	p.(Ala767_Val769dup)	ProteinIns	A	ATGGCCAGCG	Yes
	70	TP53	NM_000546	17	7578533	p.(Lys132Asnfs*16)	Frameshift	TTC	T	No
2	74	EGFR	NM_005228	7	55259524	p.(Leu861Gln)	Missense	T	A	Yes
	74	TP53	NM_000546	17	7574017	p.(Arg337His)	Missense	C	T	No
2	75	EGFR	NM_005228	7	55242469	p.(Leu747_Pro753delinsSer)	ProteinDel	TTAAGAGAAGCAACATCTC	T	Yes
	75	TP53	NM_000546	17	7574017	p.(Arg337His)	Missense	C	T	No
2	97	TP53	NM_000546	17	7577120	p.(Arg273His)	Missense	C	T	No
	97	ALK		2		EML4 exon 13::ALK exon 20	Fusion			Yes
2	99	EGFR	NM_005228	7	55242469	p.(Leu747_Pro753delinsSer)	ProteinDel	TTAAGAGAAGCAACATCTC	T	Yes
	99	TP53	NM_000546	17	7574017	p.(Arg337His)	Missense	C	T	No
2	100	EGFR	NM_005228	7	55242464	p.(Glu746_Ala750del)	ProteinDel	AGGAATTAAGAGAAGC	A	Yes
	100	TP53	NM_000546	17	7577530	p.(Ile251Phe)	Missense	T	A	No
2	102	EGFR	NM_005228	7	55259515	p.(Leu858Arg)	Missense	T	G	Yes
	102	TP53	NM_000546	17	7578536	p.(Lys132*)	Nonsense	T	A	No
1	103	ALK		2		EML4 exon 6::ALK exon 20	Fusion			Yes
1	106	ERBB2	NM_004448	17	37880981	p.(Tyr772_Ala775dup)	ProteinIns	A	AGCATACGTGATG	Yes
1	112	ALK		2		EML4 exon 13::ALK exon 20	Fusion			Yes
1	113	ERBB2	NM_004448	17	37880981	p.(Tyr772_Ala775dup)	ProteinIns	A	AGCATACGTGATG	Yes
1	114	EGFR	NM_005228	7	55259515	p.(Leu858Arg)	Missense	T	G	Yes
3	118	EGFR	NM_005228	7	55242469	p.(Leu747_Pro753delinsSer)	ProteinDel	TTAAGAGAAGCAACATCTC	T	Yes
	118	EGFR	NM_005228	7	55242494	p.(Ala755Asp)	Missense	C	A	Yes
	118	TP53	NM_000546	17	7578265	p.(Ile195Thr)	Missense	A	G	No
1	122	TP53	NM_000546	17	7578257	p.(Glu198*)	Nonsense	C	A	No
2	127	EGFR	NM_005228	7	55242464	p.(Glu746_Ala750del)	ProteinDel	AGGAATTAAGAGAAGC	A	Yes
	127	TP53	NM_000546	17	7577127	p.(Glu271Lys)	Missense	C	T	No
1	142	EGFR	NM_005228	7	55242464	p.(Glu746_Ala750del)	ProteinDel	AGGAATTAAGAGAAGC	A	Yes
1	146	EGFR	NM_005228	7	55259515	p.(Leu858Arg)	Missense	T	G	Yes
2	151	EGFR	NM_005228	7	55242469	p.(Leu747_Thr751delinsPro)	ProteinDel	TTAAGAGAAGCAA	C	Yes
	151	TP53	NM_000546	17	7579536	p.(Glu51*)	Nonsense	C	A	No
1	152	EGFR	NM_005228	7	55242469	p.(Leu747_Thr751delinsPro)	ProteinDel	TTAAGAGAAGCAA	C	Yes
1	157	KRAS	NM_004985	12	25398285	p.(Gly12Cys)	Missense	C	A	Yes
1	158	ALK		2		(unknown partner)	Fusion			Yes
1	162	ERBB2	NM_004448	17	37880981	p.(Tyr772_Ala775dup)	ProteinIns	A	AGCATACGTGATG	Yes
1	171	EGFR	NM_005228	7	55259515	p.(Leu858Arg)	Missense	T	G	Yes

2	179	EGFR	NM_005228	7	55242465	p.(Glu746_Ala750del)	ProteinDel	GGAATTAAGAGAAGCA	G	Yes
	179	TP53	NM_000546	17	7578226	p.(Asp208Ala)	Missense	T	G	No
1	191	EGFR	NM_005228	7	55242465	p.(Glu746_Ala750del)	ProteinDel	GGAATTAAGAGAAGCA	G	Yes
2	193	EGFR	NM_005228	7	55242464	p.(Glu746_Ala750del)	ProteinDel	AGGAATTAAGAGAAGC	A	Yes
	193	TP53	NM_000546	17	7578203	p.(Val216Met)	Missense	C	T	No
1	196	ALK		2		EML4 exon 2::ALK exon 20	Fusion			Yes
1	206	KRAS	NM_004985	12	25398284	p.(Gly12Ala)	Missense	C	G	No
2	207	EGFR	NM_005228	7	55242467	p.(Leu747_Thr751del)	ProteinDel	AATTAAGAGAAGCAAC	A	Yes
	207	TP53	NM_000546	17	7577081	p.(Glu286Gly)	Missense	T	C	No
2	218	TP53	NM_000546	17	7577538	p.(Arg248Gln)	Missense	C	T	No
	218	RET		10		Fusion (unknown partner)	Fusion			Yes
1	226	ALK		2		(unknown partner)	Fusion			Yes
1	241	KRAS	NM_004985	12	25398284	p.(Gly12Asp)	Missense	C	T	No
1	244	EGFR	NM_005228	7	55249002	p.(Ser768_Asp770dup)	ProteinIns	C	CCAGCGTGGA	Yes
2	249	EGFR	NM_005228	7	55242465	p.(Glu746_Ala750del)	ProteinDel	GGAATTAAGAGAAGCA	G	Yes
	249	TP53	NM_000546	17	7577121	p.(Arg273Cys)	Missense	G	A	No
2	251	TP53	NM_000546	17	7577121	p.(Arg273Cys)	Missense	G	A	No
	251	ALK		2		EML4 exon 13::ALK exon 20	Fusion			Yes
2	257	EGFR	NM_005228	7	55242469	p.(Leu747_Pro753delinsSer)	ProteinDel	TTAAGAGAAGCAACATCTC	T	Yes
	257	TP53	NM_000546	17	7579591	p.(?)	Splice	C	A	No
2	273	EGFR	NM_005228	7	55249010	p.(Asn771_His773dup)	ProteinIns	G	GACAACCCCC	Yes
	273	TP53	NM_000546	17	7577566	p.(Asn239Asp)	Missense	T	C	No
2	289	TP53	NM_000546	17	7578423	p.(Ile162_His168del)	ProteinDel	CATGTGCTGTGACTGCTGTGTAG	C	No
	289	ALK		2		EML4 exon 6::ALK exon 20	Fusion			Yes
2	290	TP53	NM_000546	17	7578212	p.(Arg213*)	Nonsense	G	A	No
	290	MET		7		Exon 14 skipping	Fusion			Yes
1	298	ALK		2		(unknown partner)	Fusion			Yes
1	311	EGFR	NM_005228	7	55242464	p.(Glu746_Ala750del)	ProteinDel	AGGAATTAAGAGAAGC	A	Yes
4	321	EGFR	NM_005228	7	55242465	p.(Glu746_Ala750del)	ProteinDel	GGAATTAAGAGAAGCA	G	Yes
	321	EGFR	NM_005228	7	55249071	p.(Thr790Met)	Missense	C	T	Yes
	321	EGFR	NM_005228	7	55249092	p.(Cys797Ser)	Missense	G	C	Yes
	321	TP53	NM_000546	17	7577570	p.(Met237Ile)	Missense	C	G	No
3	325	EGFR	NM_005228	7	55242469	p.(Leu747_Pro753delinsSer)	ProteinDel	TTAAGAGAAGCAACATCTC	T	Yes
	325	EGFR	NM_005228	7	55249071	p.(Thr790Met)	Missense	C	T	Yes
	325	TP53	NM_000546	17	7578272	p.(His193Tyr)	Missense	C	T	No
4	327	EGFR	NM_005228	7	55242465	p.(Glu746_Ala750del)	ProteinDel	GGAATTAAGAGAAGCA	G	Yes
	327	EGFR	NM_005228	7	55249092	p.(Cys797Ser)	Missense	G	C	Yes
	327	EGFR	NM_005228	7	55249071	p.(Thr790Met)	Missense	C	T	Yes
	327	TP53	NM_000546	17	7578503	p.(Val143Met)	Missense	C	T	No
2	329	EGFR	NM_005228	7	55259515	p.(Leu858Arg)	Missense	T	G	Yes
	329	TP53	NM_000546	17	7579529	p.(Trp53*)	Nonsense	C	T	No
2	331	EGFR	NM_005228	7	55242469	p.(Leu747_Thr751delinsPro)	ProteinDel	TTAAGAGAAGCAA	C	Yes
	331	TP53	NM_000546	17	7574017	p.(Arg337His)	Missense	C	T	No
1	336	BRAF	NM_004333	7	140453136	p.(Val600Glu)	Missense	A	T	Yes
1	338	PDGFRA	NM_006206	4	55144149	p.(Leu660Val)	Missense	T	G	No
1	346	KRAS	NM_004985	12	25398284	p.(Gly12Asp)	Missense	C	T	No
1	352	ERBB2	NM_004448	17	37880997	p.(Gly776delinsLeuCys)	ProteinIns	G	TTGT	Yes
1	358	ALK		2		EML4 exon 13::ALK exon 20	Fusion			Yes
1	369	TP53	NM_000546	17	7574017	p.(Arg337His)	Missense	C	T	No
2	372	EGFR	NM_005228	7	55242467	p.(Glu746_Ser752delinsVal)	ProteinDel	AATTAAGAGAAGCAACATC	T	Yes
	372	TP53	NM_000546	17	7578526	p.(Cys135Phe)	Missense	C	A	No
1	390	TP53	NM_000546	17	7577518	p.(Leu252Ile254del)	ProteinDel	TGATGGTGAG	T	No
2	391	EGFR	NM_005228	7	55242464	p.(Glu746_Ala750del)	ProteinDel	AGGAATTAAGAGAAGC	A	Yes
	391	TP53	NM_000546	17	7579315	p.(Cys124Trpfs*25)	Frameshift	G	GC	No
2	395	ERBB2	NM_004448	17	37880981	p.(Tyr772_Ala775dup)	ProteinIns	A	AGCATACGTGATG	Yes
	395	TP53	NM_000546	17	7579373	p.(Gly105Asp)	Missense	C	T	No
2	397	TP53	NM_000546	17	7577046	p.(Glu298*)	Nonsense	C	A	No
	397	KRAS	NM_004985	12	25398284	p.(Gly12Val)	Missense	C	A	No
2	404	EGFR	NM_005228	7	55259515	p.(Leu858Arg)	Missense	T	G	Yes
	404	TP53	NM_000546	17	7577498	p.(?)	Splice	C	T	No

2	408	EGFR	NM_005228	7	55259515	p.(Leu858Arg)	Missense	T	G	Yes
	408	TP53	NM_000546	17	7578268	p.(Leu194Arg)	Missense	A	C	No
3	416	EGFR	NM_005228	7	55248998	p.(Ala767_Val769dup)	ProteinIns	A	ATGGCCAGCG	Yes
	416	PIK3CA	NM_006218	3	178936091	p.(Glu545Lys)	Missense	G	A	No
	416	TP53	NM_000546	17	7578526	p.(Cys135Tyr)	Missense	C	T	No
1	426	NTRK1		1		Fusion (unknown partner)	Fusion			Yes
2	427	TP53	NM_000546	17	7577093	p.(Arg282Gln)	Missense	C	T	No
	427	ALK		2		(unknown partner)	Fusion			Yes
1	433	ALK		2		EML4 exon 13::ALK exon 20	Fusion			Yes
2	440	EGFR	NM_005228	7	55242464	p.(Glu746_Ala750del)	ProteinDel	AGGAATTAAGAGAAGC	A	Yes
	440	ERBB2	NM_004448	17	37879604	p.(Gly660Asp)	Missense	G	A	Yes
2	447	TP53	NM_000546	17	7579318	p.(Thr123Ilefs*47)	Frameshift	AG	A	No
	447	TP53	NM_000546	17	7577121	p.(Arg273Cys)	Missense	G	A	No
1	454	TP53	NM_000546	17	7578392	p.(Glu180*)	Nonsense	C	A	No
1	457	ERBB2	NM_004448	17	37880219	p.(Leu755Pro)	Missense	TT	CC	Yes
1	564	KRAS	NM_004985	12	25398284	p.(Gly12Val)	Missense	C	A	No
1	566	EGFR	NM_005228	7	55259515	p.(Leu858Arg)	Missense	T	G	Yes
2	596	PIK3CA	NM_006218	3	178936091	p.(Glu545Lys)	Missense	G	A	No
	596	KRAS	NM_004985	12	25398284	p.(Gly12Asp)	Missense	C	T	No
2	602	EGFR	NM_005228	7	55259515	p.(Leu858Arg)	Missense	T	G	Yes
	602	TP53	NM_000546	17	7578448	p.(Ala161Asp)	Missense	G	T	No
1	692	EGFR	NM_005228	7	55242465	p.(Glu746_Ala750del)	ProteinDel	GGAATTAAGAGAAGCA	G	Yes
1	789	EGFR	NM_005228	7	55242466	p.(Glu746_Thr751delinsAla)	ProteinDel	GAATTAAGAGAAGCAA	G	Yes
2	1128	EGFR	NM_005228	7	55242464	p.(Glu746_Ala750del)	ProteinDel	AGGAATTAAGAGAAGC	A	Yes
	1128	TP53	NM_000546	17	7579358	p.(Arg110Leu)	Missense	C	A	No
2	1803	EGFR	NM_005228	7	55249005	p.(SerVal768IleLeu)	Missense	GCG	TCT	Yes
	1803	TP53	NM_000546	17	7578203	p.(Val216Leu)	Missense	C	A	No
1	1892	EGFR	NM_005228	7	55249013	p.(Asn771delinsGlyPhe)	ProteinIns	AA	GGGTT	Yes
2	1938	EGFR	NM_005228	7	55242469	p.(Leu747_Pro753delinsSer)	ProteinDel	TTAAGAGAAGCAACATCTC	T	Yes
	1938	TP53	NM_000546	17	7578290	p.(?)	Splice	C	A	No
2	1942	TP53	NM_000546	17	7577559	p.(Ser241Phe)	Missense	G	A	No
	1942	ALK		2		EML4 exon 6::ALK exon 20	Fusion			Yes
2	1950	EGFR	NM_005228	7	55242465	p.(Glu746_Thr751delinsIlePro)	ProteinDel	GGAATTAAGAGAAGCAA	AATTC	Yes
	1950	TP53	NM_000546	17	7578203	p.(Val216Leu)	Missense	C	A	No
1	2157	EGFR	NM_005228	7	55249010	p.(Asn771_His773dup)	ProteinIns	G	GACAACCCCC	Yes
3	2242	EGFR	NM_005228	7	55242465	p.(Leu747_Glu749del)	ProteinDel	GGAATTAAGA	G	Yes
	2242	EGFR	NM_005228	7	55242490	p.(Lys754Glu)	Missense	A	G	Yes
	2242	TP53	NM_000546	17	7578206	p.(Ser215Gly)	Missense	T	C	No

Supplementary Table 3 – Association between most frequent molecular alterations and genetic ancestry background (n=119).

TP53 status						EGFR status					ALK status*					ERBB2 status				
Ancestry	Wild-type		Mutated		p-value	Wild-type		Mutated		p-value	Wild-type		Mutated		p-value	Wild-type		Mutated		p-value
Tertiles	n	%	n	%		n	%	n	%		n	%	n	%		n	%	n	%	
African																				
Low	29	82.9	6	17.1	0.002	23	65.7	12	34.3	0.063	26	83.9	5	16.1	0.320	31	88.6	4	11.4	0.632
Intermedium	19	52.8	17	47.2		16	44.4	20	55.6		29	82.9	6	17.1		33	91.7	3	8.3	
High	16	44.4	20	55.6		14	38.9	22	61.1		32	94.1	2	5.9		34	94.4	2	5.6	
Missing	8		4			7		5			9		2			12		0		
Asian																				
Low	25	71.4	10	28.6	0.250	18	51.4	17	48.6	0.912	30	93.8	2	6.3	0.421	31	88.6	4	11.4	0.629
Intermedium	20	54.1	17	45.9		17	45.9	20	54.1		29	85.3	5	14.7		35	94.6	2	5.4	
High	19	54.3	16	45.7		18	51.4	17	48.6		28	82.4	6	17.6		32	91.4	3	8.6	
Missing	8		4			7		5			9		2			12		0		
European																				
Low	17	48.6	18	51.4	0.139	17	48.6	18	51.5	1	32	94.1	2	5.9	0.096	31	88.6	4	11.4	0.177
Intermedium	25	59.5	17	40.5		21	50.0	21	50.0		31	77.5	9	22.5		41	97.6	1	2.4	
High	22	73.3	8	26.7		15	50.0	15	50.0		24	92.3	2	7.7		26	86.7	4	13.3	
Missing	8		4			7		5			9		2			12		0		
Native American																				
Low	22	56.4	17	43.6	0.562	21	53.8	18	46.2	0.831	30	83.3	6	16.7	0.807	36	92.3	3	7.7	1
Intermedium	16	55.2	13	44.8		14	48.3	15	51.7		25	89.3	3	10.7		26	89.7	3	10.3	
High	26	66.7	13	33.3		18	46.2	21	53.8		32	88.9	4	11.1		36	92.3	3	7.7	
Missing	8		4			7		5			9		2			12		0		

*ALK status (n=111). Inconclusive samples were not considered here. P-value = χ^2 test

Supplementary Table 4 – Association between *EGFR* mutations and *TP53* mutations (n=119).

TP53 status					
EGFR status	Wild-type		Mutated		p-value
	n	%	n	%	
Wild-type	46	76.7	14	23.3	<0.0001
Mutated	26	44.1	33	55.9	

Supplementary Table 5 - Mutational frequency in patients with no history of tobacco consumption diagnosed with lung cancer (n=1708).

Studies with never smoker patients and molecular data	Number of samples	Geographic Region		Ancestry				EGFR		TP53		ERBB2		KRAS		BRAF		PDGFRA		PIK3CA		ALK		RET		NTRK1		ROS1		MET			
				Asian	African	European	Admixed/Native American	NA	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Lung Adenocarcinoma (MSK, J Thorac Oncol 2020) ¹	138	USA	North America	0	0	0	0	138	62.8	86	26.8	37	3.6	5	9.4	13	2.9	4	0.7	1	2.9	4	2.9	4	5.8	8	0	0	2.2	3	5.8	8	
Lung Cancer in Never Smokers (NCI, Nature Genetics 2021) ¹	232	-	European	4	2	226	0	0	28.4	66	15.5	36	3.9	9	7.3	17	0.4	1	1.7	4	4.3	10	5.6	13	1.3	3	0	0	2.2	5	3	7	
Lung Adenocarcinoma (TCGA, Nature 2014) ¹	32	TCGA	North America	0	0	0	0	32	31.3	10	25.0	8	9.4	3	18.8	6	0	0	0	0	0	6.3	2	3.1	1	0	0	0	6.3	2	12.5	4	
Lung Adenocarcinoma (MSK Mind,Nature Cancer 2022) ¹	22	USA	North America	0	0	0	0	22	31.8	7	54.5	12	13.6	3	13.6	3	4.5	1	4.5	1	18.2	4	4.5	1	0	0	0	0	0	0	4.5	1	
Lung Adenocarcinoma (Broad, Cell 2012) ¹	27	USA	North America	5	1	19	0	2	37.0	10	18.5	5	3.7	1	7.4	2	0	0	0	0	7.4	2	NA	NA	NA	NA	NA	NA	NA	NA	3.7	1	
Lung Adenocarcinoma (MSK, Science 2015) ¹	6	USA	North America	0	0	0	0	6	0.0	0	33.3	2	0.0	0	0	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	0	0	
Lung Adenocarcinoma (OncoSG, Nat Genet 2020) ¹	187	China	Asian	187	0	0	0	0	59.9	112	28.9	54	3.7	7	4.8	9	2.7	5	2.1	4	3.7	7	5.2	6	0	0	0	0	0.9	1	2.1	4	
Lung Adenocarcinoma (MSK, NPJ Precision Oncology 2021) ¹	110	USA	North America	0	0	0	0	110	59.1	65	26.4	29	5.5	6	7.3	8	0.9	1	0	0	3.6	4	5.5	6	7.3	8	0	0	1.8	2	5.5	6	
Lung Adenocarcinoma Met Organotropism (MSK, Cancer Cell 2023) ¹	253	USA	North America	72	11	115	12	6	58.5	148	37.5	95	4.0	10	9.1	23	0.4	1	0.8	2	7.1	18	8.3	20	4.1	10	0	0	2.1	5	4.3	11	
Non-Small Cell Cancer (MSK, Cancer Discov 2017) ¹	302	USA	North America	0	0	0	0	302	50.3	152	45.0	136	6.0	18	5.3	16	4.3	13	1.3	4	7.6	23	7.3	22	3	9	0	0	6.6	20	4.6	14	
Carrot-Zhang et al. (Cancer Discovery 2021)	280	Colombia and Mexico	Latin America	0	0	0	280	0	37.5	105	32.5	91	1.07	3	5.36	15	2.14	6	NA	NA	NA	5.71	16	0.36	1	NA	NA	0.36	1	0	0	0	
Present Study	119	Brazil	Latin America	0	0	0	119	0	49.6	59	39.5	47	7.6	9	5.9	7	0.8	1	0.8	1	1.7	2	12.6	15	0.8	1	0.8	1	0	0	0.8	1	

NA, not available; ¹Accoding to where the study was conducted when patients' origins were not available; ²Data available in cBioportal dataset.

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5.8 Perfil Molecular dos pacientes com Câncer de Pulmão de não Pequenas Células.

Este artigo busca descrever o perfil molecular dos pacientes com câncer de pulmão de não pequenas células em pacientes brasileiros usando dados de mundo real do Hospital de Amor de Barretos.

Association of the molecular profile with clinicopathological features of a large Brazilian lung cancer patient population from a single institution

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Abstract

INTRODUCTION: The driver alterations profile directly impacts lung cancer patients' clinical management and can significantly change among ethnic groups. We aimed to evaluate the molecular profile of a large Brazilian patient cohort with non-small cell lung cancer (NSCLC) using real-world data from a single center in Brazil and explore its association with clinicopathological features. **METHODS:** Oncogenic mutations and fusions in 1133 NSCLC patients were assessed by NGS and RNA-based approaches, respectively. The genetic ancestry was assessed using 46-ancestry-informative markers. Additionally, the molecular profile was associated with patients' clinicopathological features. **RESULTS:** Most patients originated from the Southeast region of Brazil (65.2%), and 71.7% exhibited a European ancestry proportion. Overall, 87% of patients harbor at least driver alteration, with the *TP53* gene being the most commonly mutated (57.9%) followed by the *KRAS* gene (25.3%), *EGFR* (20.5%), *ALK* fusions (5.9%), *PIK3CA* (3.3%), *ERBB2* (2.9%), *BRAF* (2.5%), *METΔex14* (1.4%). The *NRAS*, *RET* fusions, *ROS1* fusions, *KIT*, and *NTRK1-3* fusions were mutated in less than 1% of patients. No mutations were found in the *AKT1*, *FOXL2*,

GNA11, *GNAQ*, *PDGFRA*, and *NTRK2* genes. The multivariate logistic regression revealed several independent associations, namely: the presence of *TP53* mutations with males, ever-smokers, non-adenocarcinoma histology, CNS metastasis, and low European ancestry; the presence of *EGFR* mutations with females, never-smokers, CNS metastasis, and low European ancestry; the presence of *KRAS* mutations with ever-smokers and adenocarcinoma histology; the presence of *ALK* fusions with younger, never-smoker patients, *PI3KCA* mutations with older patients and squamous histology, and *BRAF* mutations with females. **Conclusion:** The great majority of Brazilian lung cancer patients exhibited at least one oncogenic alteration, with targeted therapies potentially benefiting around 35% of cases. We found a significant association of ethnicity with some of the frequency of key driver genes. The present study offers a comprehensive understanding of the impact of molecular profiles in Brazilian lung cancer patients.

Keywords: Lung Cancer, Brazil, Molecular Profile, Mutations, Fusions

Introduction

Lung cancer is the most common and deadly tumor in the world, according to the IARC (International Agency for Research on Cancer)[1]. The NSCLC (Non-small cell lung cancer) is the most frequent histological type, representing 85% of cases[2]. In the last decades, targeted therapies have revolutionized the clinical management of patients diagnosed with lung cancer [2–6].

NSCLC is genetically heterogeneous with alterations in many other driver genes, such as *EGFR* mutations, *BRAF V600E* mutation, *ERBB2* insertions, *KRAS* mutations, and rearrangements of *ALK*, *RET*, *ROS1*, *NTRK1-2-3* genes[7–11]. Due to this plethora of molecular events, a complete understanding of the molecular profile is crucial for better clinical management of the patients [2]. Patients harboring *EGFR* mutations will benefit from treatment with *EGFR* inhibitors. In contrast, patients with *KRAS* mutations are resistant to *EGFR* inhibitors, but with the recent development of *KRAS* inhibitors, they can also be therapeutically tackled. Furthermore, patients harboring *BRAF V600E* mutations may benefit from treatments with Dabrafenib and Trametinib[2,3,12,13]. Knowing the molecular profile is crucial for patient treatment, as the frequency of mutated genes in lung cancer can vary significantly based on factors such as smoking history and the patient's population of origin [14–16].

The molecular profile of lung tumors in mixed populations, such as Brazil, is poorly understood. Here, we aim to describe and better comprehend the molecular profile of lung tumors in a large number of Brazilian patients diagnosed in a single institution in Brazil.

Materials and Methods

Study Population

We evaluated a retrospective cohort of 1133 Brazilian lung cancer patients diagnosed and recommended for routine molecular testing using a Next Generation Sequencing (NGS) targeted panel at Barretos Cancer Hospital (Barretos, São Paulo, Brazil) between 2018 and 2023. The clinicopathological and molecular data were collected from patients' medical records. The Institutional Review Board approved the study protocol (CAAE 05744712.3.0000.5437) and waived written informed consent due to the study's retrospective nature.

DNA/RNA Isolation

DNA and RNA isolation were performed from FFPE (Formalin-Fixed Paraffin-embedded) tumor samples sectioned on slides with a thickness of 10µm. One slide was stained with hematoxylin and eosin (H&E) and evaluated by a pathologist for identification, sample adequacy assessment, and selection of tumor area. DNA was isolated using the commercial kit QIAamp DNA Micro Kit (Qiagen, Hilden, Germany) according to the manufacturer's specifications. DNA concentration and purity were evaluated by NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA) and by Qubit 2.0 Fluorometer (Thermo Fisher Scientific) with Qubit dsDNA HS assay kit (Thermo Fisher Scientific). RNA was isolated using the RNeasy FFPE Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA concentration and purity were evaluated by NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA).

Mutation detection

The mutational analysis of genes comprehended in the TruSight Tumor 15 (TST15) panel (Illumina, San Diego, CA, USA) was assessed by NGS on the MiSeq instrument, according to the manufacturer's instruction as previously described [17]. The read alignment and variant calling were performed with BaseSpace BWA Enrichment version 2.1 (Illumina, San Diego, CA, USA) and Sophia DDM® software version 4.2 (Sophia

Genetics SA, Saint Sulpice, Switzerland). Variants were filtered out according to the following criteria: intronic (except splicing variants), synonymous single nucleotide variants (SNVs), populational frequency >1%, poorly read depth < 500, allele frequency <5%, poorly read depth < 500, and non-pathogenic variants according to Sophia DDM®.

A subset of patients (n=81/1133) was also analyzed by real-time PCR using the COBAS platform (ROCHE, Basilea, Switzerland) as described [18]. COBAS DNA Sample Preparation Kit (ROCHE, Basilea, Switzerland) was used for manual sample preparation, followed by the Cobas z 480 analyzer for automated amplification and detection following the COBAS EGFR Mutation Test v2 kit (ROCHE, Basilea, Switzerland).

Classification of variants

The retained variants were classified based on oncogenicity, using the scoring system proposed by the most recent guidelines as follows: Oncogenic, Likely-Oncogenic, VUS (variant of undetermined significance), Likely-benign, and Benign [19]. Then, these variants were classified according to the clinical impact into four categories (TIERS): actionable oncogenic and likely-oncogenic variants with targeted therapies (TIER I), oncogenic and likely-oncogenic variants with potential clinical significance (TIER II), VUS variants with unknown clinical significance (TIER III), and likely benign and benign variants (TIER IV)[20].

Gene fusions and *MET*Δ*ex14* detection

Several methodologies were used to assess the presence of *ALK*, *ALK/RET/ROS1/NTRK1,2,3* fusion, and *MET*Δ*ex14* (MET exon 14 skipping), mainly in patients with *EGFR* and *KRAS* wild-type tumors.

The detection of mRNA *ALK/RET/ROS1/NTRK1,2,3* fusion transcripts and *MET*Δ*ex14* was done by the RNA-based Archer panel by NGS, the RNA-based Real-Time PCR Idylla technique, and the nCounter Elements XT (NanoString Technologies, Seattle, WA, USA) custom panel for *ALK*, *RET*, *ROS1*, and *NTRK1/2/3* fusion detection by specific

partner and 3' and 5' disbalance, and *METΔex14* [11,21–23]. All analyses were performed in R environment v3.4.1.

Moreover, *ALK* fusions were evaluated in 81.3% (n=922/1133) of cases by immunohistochemistry (IHC) using the Ventana *ALK* (D5F3) CDx Assay (Roche, Basel, Switzerland) in an automated equipment (BenchMark Ventana Ultra™) [24]. *NTRK* fusions were evaluated in 12.7% (n=144/1133) of cases by IHC expression on an automated staining system (BenchMark Ventana Ultra™) and classified as previously described [11,25]. *RET* and *ROS1* fusions detection was performed in 0.1% (n=2/1133) and 17.7% (n=201/1133) of cases, respectively, using commercial probes (ZytoLight SPEC *RET* Dual Color Break Apart, ZytoLight SPEC *ROS1* Dual Color Break Apart), and FISHView 7.0 software (Applied Spectral Imaging) was employed for the analysis as previously described [9].

Genetic Ancestry analysis

The genetic ancestry background was evaluated using a set of 46 ancestry-informative markers, including INDELs for European (EUR), African (AFR), Asian (ASN), and Native American (AME) as previously reported by our group [26,27].

Statistical Analysis

For statistical analysis, the percentage was used to describe categorical variables, and medians were used to describe continuous variables. As previously described, individual ancestry proportions were defined as categorical variables and divided into tertiles (low, intermedium, and high)[26]. For univariate analysis, the Fisher's exact test, Qui-square test, and logistic regression were used for the association analysis between the clinicopathological and molecular data. All tests were made in the software IBM SPSS Statistics Version 25 (IBM, Armonk, Nova York, USA) with a limit of statistical significance of 0.05

RESULTS

Demographic, clinicopathological, and molecular features of patients

A total of 1133 patients diagnosed with lung cancer were included in the study and had the hotspot region of 15 genes assessed by the TruSight Tumor 15 panel, with their variants classified according to oncogenicity. Demographically, considering the patients' geographic place of birth, all Brazilian states were represented in our cohort, with the majority from the Southeast region (68.2%; n=678/995) (**Figure 1**). Additionally, data on the place of origin were available for 99.9% (n=1132/1133) of the patients, with most being from the Southeast region (65.2%; n=737/1132) (**Figure 1**).

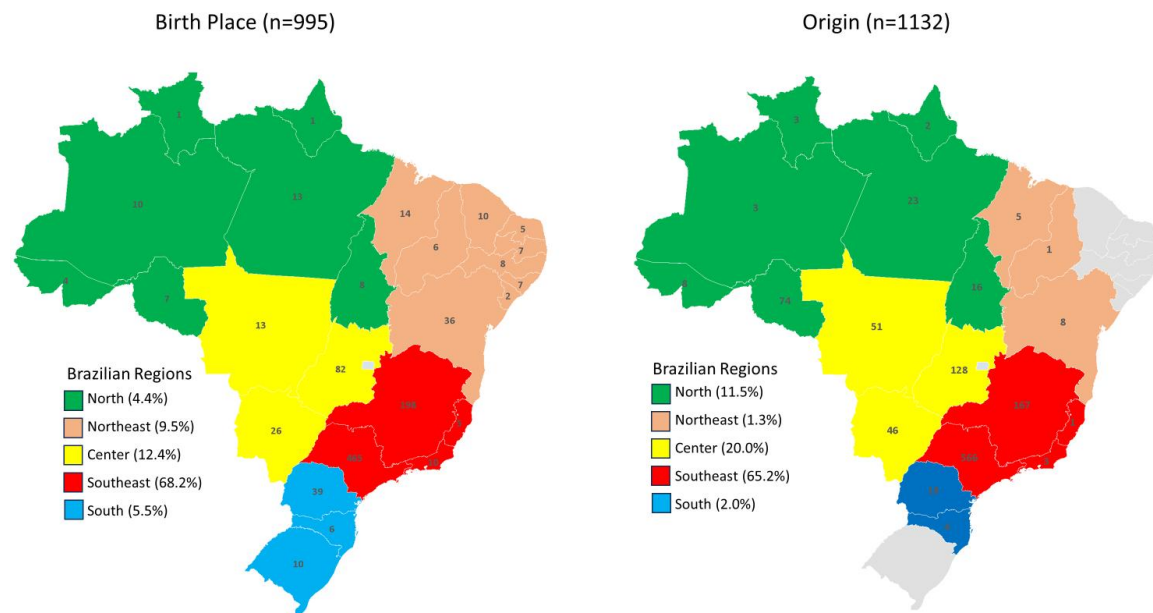


Figure 1 - Geographic distribution of patients according to origin (n=1132) and place of birth (n=995) across the states and regions of Brazil. States without patients are represented in gray.

Among 1133 patients diagnosed with lung cancer, the median age at diagnosis was 64 years (**Table 1**). The majority were male (55.1%; n=624/1133), self-identified as White (62.6%; n=709/1133), and were either current or former smokers (69.9%; n=792/1133). Clinically, 46.0% (n=521/1133) of the patients experienced weight loss within six months before diagnosis, 59.4% (n=673/1133) had a performance status of 0

or 1 at diagnosis, 80.4% (n=911/1133) were diagnosed with lung adenocarcinoma, 64.3% (n=728/1133) were at stage IV at diagnosis, and 22.6% (n=256/1133) presented with central nervous system metastases.

Table 1 – Clinicopathological characteristics of the Lung Cancer patients (n=1133).

Characteristic	Parameters	n	%
Age at diagnosis (years)	Median (Min-Max)	64.0 (19.0 – 94.0)	
	≤ 64	589	52.0
	> 64	544	48.0
Sex	Female	509	44.9
	Male	624	55.1
Self-declared skin color*	White	709	62.6
	Non-White	232	20.5
	N.I.	192	16.9
Smoking status	Never smoker	297	26.2
	Former smoker	372	32.8
	Current smoker	420	37.1
	N.I.	44	3.9
Weight loss within 6 months prior to diagnosis	No	386	34.1
	Yes	521	46.0
	N.I.	226	19.9
ECOG Performance Status	0-1	673	59.4
	2-4	313	27.6
	N.I.	147	13.0
Histology	Adenocarcinoma	911	80.4
	Squamous	65	5.7
	NSCLC SOE	119	10.5
	Outros**	38	3.4
Stage at diagnosis	I/II	193	17.0
	III	181	16.0
	IV	728	64.3
	N.I.	31	2.7
Metastasis at diagnosis	No	375	33.1
	Yes, CNS	256	22.6
	Yes, others	474	41.8
	No	28	2.5

N.I., no information available; NSCLC NOS, Non-Small Cell Lung Cancer not otherwise specified; CNS, Central Nervous System; *According to the IBGE (Brazilian Institute of Geography and Statistics); **Includes Large Cell Carcinoma, Adenosquamous Carcinoma, Sarcomatoid Carcinoma, and Large Cell Neuroendocrine Carcinoma.

At the molecular level, 87.0% of the patients exhibited at least one oncogenic alteration (**Figure 2**), and about 35% of patients carried at least one actionable mutation (TIER I) (**Supplementary Table 1**). The *TP53* gene was the most commonly mutated, with at least one mutation identified in 57.9% (n=657/1133)(Supplementary Figure 1) of the

patients, followed by the *KRAS* gene, mutated in 25.3% (n=289/1133)(Supplementary Figure 2), *EGFR*, mutated in 20.5% (n=232/1133)(supplementary Figure 3), *ALK*, with fusions identified in 5.9% (n=67/1133), *PIK3CA*, mutated in 3.3% (n=37/1133), *ERBB2*, mutated in 2.9% (n=33/1133), *BRAF*, mutated in 2.5% (n=29/1133), *MET*, altered in 2.5% (n=28/1133; being *MET* Δ ex14, identified in 1.4%; n=16/1133), *NRAS*, mutated in 0.6% (n=7/1133), *RET*, with fusions identified in 0.5% (n=6/1133), *ROS1*, with fusions identified in 0.3% (n=4/1133), *KIT*, mutated in 0.1% (n=1/1133), *NTRK1*, with fusions identified in 0.1% (n=1/1133), and *NTRK3*, with fusions identified in 0.1% (n=1/1133) of the patients. No mutations were found in the *AKT1*, *FOXL2*, *GNA11*, *GNAQ*, *PDGFRA*, and *NTRK2* genes.

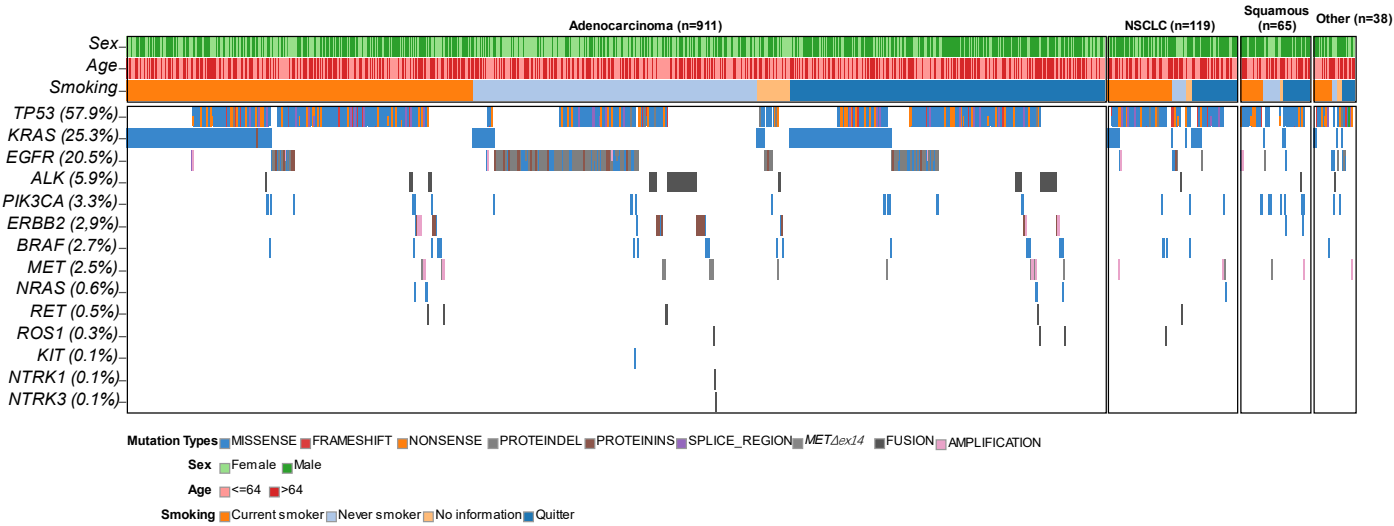


Figure 2 – Molecular profile of Brazilian lung cancer patients (n=1133). Only variants classified as oncogenic and likely-oncogenic were considered.

Co-occurring mutations

In patients with *EGFR* mutations (n=232), the most commonly co-occurring mutations identified were in the *TP53* gene (60.3%; n=140/232), followed by *PIK3CA* mutations (3.4%; n=8/232). Similarly, considering *KRAS* mutations (n=289), the most frequent co-occurring mutations were in *TP53* and *PIK3CA* (50.5%; n=146/289 and 3.5%; n=10/289, respectively). *BRAF* mutations (n=31) commonly co-occurred with *TP53* and *PIK3CA* (35.5%; n=11/31 and 9.7%; n=3/31, respectively). *ERBB2* alterations (n=33) were most commonly co-occurred with *TP53* mutations (45.5%; n=15/33). For patients harboring gene fusions and *MET* Δ ex14, no co-occurred mutations were identified, except

for the *TP53* mutations with the following frequency: 26.9% (n=18/67) with *ALK* fusions, 50% (n=2/4) with *ROS1* fusions, 100% (n=2/2 and n=6/6) with *NTRK* and *RET* fusions, and 68.8% (n=11/16) with *MET*Δex14. Detailed co-occurring mutations are described in Supplementary Table 1.

Genetic Ancestry Analysis

The genetic ancestry was possible in 942 patients, with the following ancestry proportions: 71.7% European, 14.6% African, 7.4% Native American, and 6.1% Asian (Figure 3). When dividing the patients' ancestry results and birthplace information (n=995), we observed a significant difference between the North region and other regions of Brazil (Figure 4), with a higher proportion of Native American ancestry (20%) and a lower proportion of European (52%) compared to the other regions. Also, different ancestry proportions may be observed between all regions.

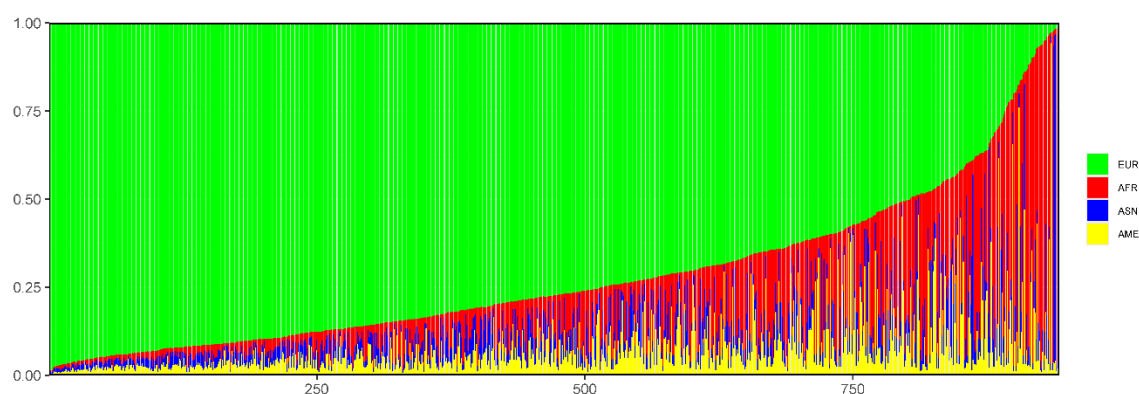


Figure 3- Distribution of ancestry proportions for each proportional group (n=942). The X-axis represents each patient, while the Y-axis represents the proportion of ancestry.

Distribution of ancestry according to patients' birth region

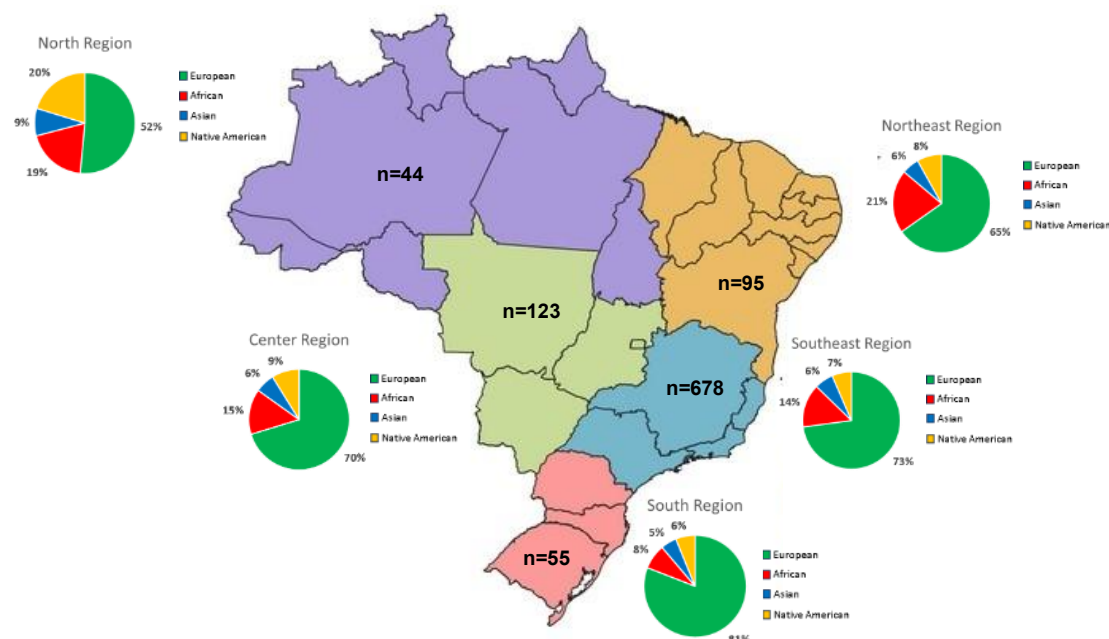


Figure 4- Distribution of ancestry proportions for each Brazilian region based on the patients' birth place (n=995).

Clinicopathological and molecular associations

Next, we compared the clinicopathological characteristics of the patients with the genes with alterations observed in higher than 2% of cases (Chi-square test, Fisher's Exact Test, and U-Mann-Whitney). All associations are summarized in **Table 2**.

We observed that mutations in the *TP53* gene were significantly associated with male patients ($p < 0.0001$), ever-smokers ($p < 0.0001$), non-adenocarcinoma histology ($p < 0.0001$), higher ECOG PS ($p = 0.015$), advanced stages of disease at diagnosis ($p = 0.004$), CNS metastasis ($p = 0.017$), high Asian ancestry ($p = 0.018$), and low European ancestry ($p = 0.011$). *KRAS* gene mutations were significantly associated with ever-smokers ($p < 0.00001$), and adenocarcinoma histology ($p < 0.0001$). Regarding *EGFR* gene mutations, we found a significant association with female patients ($p < 0.0001$), never-smokers ($p < 0.0001$), adenocarcinoma histology ($p < 0.0001$), and CNS metastases ($p < 0.0001$). *ALK* gene fusions were associated with younger patients at diagnosis ($p < 0.0001$), never smokers ($p < 0.0001$), better ECOG PS ($p = 0.025$), and adenocarcinoma histology ($p = 0.020$). Furthermore, *PIK3CA* gene mutations were significantly associated

with older patients at diagnosis ($p=0.007$), squamous cell histology ($p<0.0001$), and stage III at diagnosis ($p=0.030$), *BRAF* gene mutations were significantly associated with older and female patients ($p=0.045$ and $p=0.029$, respectively), *MET* gene alterations were significantly associated with higher ECOG performance status, and no significant association was observed for *ERBB2* alterations.

Table 2 – Univariate associations of molecular and clinicopathological features for the most frequently altered genes (n=1133).

Characteristics	Parameters	TP53					EGFR					KRAS					ALK-fusions				
		Wildtype (n=473)		Mutant (n=660)		p-value	Wildtype (n=901)		Mutant (n=232)		p-value	Wildtype (n=844)		Mutant (n=289)		p-value	Negative (n=980)		Positive (n=67)		p-value
		n	%	n	%		n	%	n	%		n	%	n	%		n	%	N	%	
Age at diagnosis (years)	Median (Min-Max)	65 (19 – 94)		63 (31 – 94)		0.226	64 (19 – 94)		63 (31 – 94)		0.046	64 (19 – 94)		63 (31 – 94)		0.327	64 (20 – 94)		57 (19 – 85)		p<0.0001
	≤ 64	239	40.6	350	59.4		468	79.5	121	20.5		431	73.2	158	26.8		507	93.9	33	6.1	
	> 64	234	43.0	310	57.0	0.372	433	79.6	111	20.4	0.941	413	75.9	131	24.1	0.321	473	93.3	34	6.7	0.694
Sex	Female	244	47.9	265	52.1		369	72.5	140	27.5		378	74.3	131	25.7		446	93.5	31	6.5	
	Male	229	36.7	395	63.3	<0.0001	532	85.3	92	14.7	<0.0001	466	74.7	158	25.3	0.873	534	93.7	36	6.3	0.904
Self-declared skin color*	White	300	42.3	409	57.7		565	79.7	144	20.3		538	75.9	171	24.1		623	93.4	44	6.6	
	Non-White	91	39.2	141	60.8	0.407	191	82.3	41	17.7	0.380	165	71.1	67	28.9	0.148	195	94.7	11	5.3	0.516
	N.I.	82		110			145		47			141		51			172		12		
Smoking status	Never smoker	181	60.9	116	39.1		154	51.9	143	48.1		275	92.6	22	7.4		238	86.9	36	13.1	
	Former smoker	148	29.5	224	60.2	<0.0001	319	85.8	53	14.2	<0.0001	262	70.4	110	29.6	<0.0001	321	93.6	22	6.4	<0.0001
	Current smoker N.I.	124	39.8	296	70.5		393	93.6	27	6.4		272	64.8	148	35.2		389	98.2	7	1.8	
Weight loss within 6 months prior to diagnosis	No	175	45.3	211	54.7		294	76.2	92	23.8		284	73.6	102	26.4		338	93.9	22	6.1	
	Yes	207	39.7	314	60.3	0.103	424	81.4	97	18.6	0.058	386	74.1	135	25.9	0.879	451	93.0	34	7.0	0.676
	N.I.	91		135			183		43			174		52			191		56		
ECOG Performance Status	0-1	301	44.7	372	55.3		536	79.6	137	20.4		507	75.3	166	24.7		571	91.8	51	8.2	
	2-4	114	36.4	199	63.6	0.015	252	80.5	61	19.5	0.798	224	71.6	89	28.4	0.212	281	95.9	12	4.1	0.025
	N.I.	58		89			113		34			113		34			128		4		
Histology	Adenocarcinoma	412	45.2	499	54.8		698	76.6	213	23.4		654	71.8	257	28.2		782	92.4	64	7.6	
	Squamous	18	27.7	47	72.3		60	92.3	5	7.7		61	93.8	4	6.2		49	98.0	1	2.0	
	NSCLC SOE Others*	30	25.2	89	74.8	<0.0001	111	93.3	8	6.7	<0.0001	97	81.5	22	18.5	<0.0001	114	99.1	1	0.9	0.020
Stage at diagnosis**	I/II	101	52.3	92	47.7		149	77.2	44	22.8		147	76.2	23.8	23.8		170	96.0	7	4.0	
	III	67	37.0	114	63.0	0.004	161	89.0	20	11.0	0.002	135	74.6	25.4	25.4	0.881	154	93.9	10	6.1	0.266
	IV N.I.	294	40.4	432	59.6		562	77.2	166	22.8		541	74.3	25.7	25.7		632	92.7	50	7.3	
Metastasis at diagnosis	No	169	45.1	206	54.9		310	82.7	65	17.3		283	75.5	92	24.5		325	95.3	16	4.7	
	Yes, CNS	88	34.4	168	65.6	0.017	185	72.3	71	27.7	0.005	192	75.0	64	25.0	0.890	223	92.1	19	7.9	0.238
	Yes, others N.I.	207	43.7	267	59.3		380	80.2	94	19.8		351	74.1	25	25.9		410	92.8	32	7.2	
Asian Ancestry	Low	127	40.8	184	59.2		244	78.5	67	21.5		237	76.2	74	23.8		267	94.3	16	5.7	
	Intermedium	148	46.5	170	53.5	0.018	262	82.4	56	17.6	0.465	241	75.8	77	24.2	0.287	285	93.1	21	6.9	0.717
	High N.I.	111	35.5	202	64.5		253	80.8	60	19.2		223	71.2	90	28.8		265	92.7	21	7.3	
African Ancestry	Low	87		104			142		49			143		48			163		9		
	Intermedium	138	45.0	169	55.0	0.133	250	81.4	57	18.6		217	70.7	90	29.3	0.190	277	96.2	311	3.8	
	High N.I.	133	41.0	191	59.0		267	82.4	57	17.6	0.312	246	75.9	78	24.1		276	91.4	26	8.6	0.050
European Ancestry	Low	115	37.0	196	63.0		242	77.8	69	22.2		238	76.5	73	23.5		264	92.6	21	7.4	
	Intermedium	87		104			142		49			143		48			163		9		
	High N.I.	109	34.8	204	65.2	0.011	242	77.3	71	22.7	0.187	242	77.3	71	22.7	0.187	264	93.6	18	6.4	0.013
Native American Ancestry	Low	131	41.5	185	58.5		262	82.9	54	17.1		262	82.9	54	17.1		268	90.2	29	9.8	
	Intermedium	146	46.6	167	53.4		255	81.5	58	18.5		255	91.5	58	18.5		285	96.3	11	3.7	
	High N.I.	87		104			142		49			143		48			163		9		
Native American Ancestry	Low	129	43.4	168	56.6		241	81.1	56	18.9		229	77.1	68	22.9		256	93.8	17	6.2	
	Intermedium	144	43.8	185	56.2	0.070	268	81.5	61	18.5	0.723	237	72.0	92	28.0	0.352	290	93.5	20	6.5	0.886
	High N.I.	113	35.8	203	64.1		250	79.1	66	20.9		235	74.4	81	25.6		271	92.8	21	7.2	
	N.I.	87		104			142		49			143		48			163		9		

N.I., no information available; NSCLC NOS, Non-Small Cell Lung Cancer not otherwise specified; CNS, Central Nervous System; *Includes Large Cell Carcinoma, Adenosquamous Carcinoma, Sarcomatoid Carcinoma, and Large Cell Neuroendocrine Carcinoma. **According to the IBGE (Brazilian Institute of Geography and Statistics); Chi-square and FISHER test employed in categorical variables; U-Mann-Whitney test employed in continuous variables.

Table 2 (continued) – Univariate associations of molecular and clinicopathological features for the most frequently altered genes (n=1133).

Characteristics	Parameters	PIK3CA					ERBB2					BRAF					MET				
		Wildtype (n=1096)		Mutant (n=37)		p-value	Wildtype (n=1100)		Mutant (n=33)		p-value	Wildtype (n=1102)		Mutant (n=31)		p-value	Negative (n=1105)		Positive (n=28)		p-value
		n	%	n	%		n	%	n	%		n	%	n	%		n	%	n	%	
Age at diagnosis (years)	Median (Min-Max)	64 (19 – 94)		69 (39 – 89)		0.007	64 (19 – 94)		64 (41 – 87)		0.910	64 (19 – 94)		68 (40 – 83)		0.045	64 (19 – 94)		66.5 (45 – 86)		0.155
	≤ 64	571	96.9	18	3.1		574	97.5	15	2.5		572	97.1	17	2.9		577	98.0	12	2.0	
	> 64	525	96.5	19	3.5	0.739	526	96.7	18	3.3	0.483	530	97.4	14	2.6	0.856	528	97.1	16	2.9	0.345
Sex	Female	493	96.9	16	3.1		491	96.5	18	3.5		489	96.1	20	3.9		498	97.8	11	2.2	
	Male	603	96.6	21	3.4	0.868	609	97.6	15	2.4	0.289	613	98.2	11	1.8	0.029	607	97.3	17	2.7	0.571
Self-declared skin color*	White	688	97.0	21	3.0		691	97.5	18	2.5		688	97.0	21	3.0		690	97.3	19	2.7	
	Non-White	224	96.6	8	3.4	0.827	222	95.7	10	4.3	0.183	227	97.8	5	2.2	0.648	226	97.4	6	2.6	1
	N.I.	184		8			187		5			187		5			189		3		
Smoking status	Never smoker	289	97.3	8	2.7		283	95.3	14	4.7		291	98.0	6	2.0		293	98.7	4	1.3	
	Former smoker	358	96.2	14	3.8		365	98.1	7	1.9		362	97.3	10	2.7		362	97.3	10	2.7	
	Current smoker	409	97.4	11	2.6	0.596	410	97.6	10	2.4	0.067	409	97.4	11	2.6	0.857	407	96.9	13	3.1	0.322
Weight loss within 6 months prior to diagnosis	N.I.	40		4			42		2			40		4			43		1		
	No	374	96.9	12	3.1		372	96.4	14	3.6		378	97.9	8	2.1		376	97.4	10	2.6	
	Yes	508	97.5	13	2.5	0.683	510	97.9	11	2.1	0.218	506	97.1	15	2.9	0.525	508	97.5	13	2.5	1
ECOG Performance Status	N.I.	214		12			218		8			218		8			221		5		
	0-1	652	96.9	21	3.1		651	96.7	22	3.3		656	97.5	17	2.5		661	98.2	12	1.8	
	2-4	300	95.8	13	4.2	0.454	307	98.1	6	1.9	0.304	303	96.8	10	3.2	0.676	300	95.8	13	4.2	0.032
Histology	N.I.	144		3			142		5			143		4			144		3		
	Adenocarcinoma	889	97.6	22	2.4		880	96.6	3.4	3.4		885	97.1	26	2.9		889	97.6	22	2.4	
	Squamous	55	84.6	10	15.4		63	96.9	3.1	3.1		65	100	0	0		64	98.5	1	1.5	
Stage at diagnosis**	NSCLC SOE	116	97.5	3	2.5	<0.0001	119	100	0	0	0.112	115	96.6	4	3.4	0.549	116	97.5	3	2.5	0.594
	Others*	36	94.7	2	5.3		38	100	0	0		37	97.4	1	2.6		36	94.7	2	5.3	
	I/II	188	97.4	5	2.6		186	96.4	7	3.6		188	97.4	5	2.6		191	99.0	2	1.0	
Metastasis at diagnosis	III	170	93.9	11	6.1	0.030	174	96.1	7	3.9	0.499	179	98.9	2	1.1	0.334	175	96.7	6	3.3	0.322
	IV	711	97.7	17	2.3		710	97.5	18	2.5		705	96.8	23	3.2		710	97.5	18	2.5	
	N.I.	27		4			30		1			30		1			29		2		
Asian Ancestry	No	358	95.5	17	4.5		361	96.3	14	3.7		368	98.1	7	1.9		368	98.1	7	1.9	
	Yes, CNS	249	97.3	7	2.7	0.119	250	97.7	6	2.3	0.489	248	96.9	8	3.1	0.466	252	98.4	4	1.6	0.297
	Yes, others	464	97.9	10	2.1		462	97.5	12	2.5		459	96.8	15	3.2		459	96.8	15	3.2	
African Ancestry	N.I.	25		3			27		1			27		1			26		2		
	Low	298	95.8	13	4.2		299	96.1	12	3.9		300	96.5	11	3.5		302	97.1	9	2.9	
	Intermediate	310	97.5	8	2.5	0.203	308	96.9	10	3.1	0.217	314	98.7	4	1.3	0.127	312	98.1	6	1.9	0.662
European Ancestry	High	307	98.1	6	1.9		308	98.4	5	1.6		302	96.5	11	3.5		304	97.1	9	2.9	
	N.I.	181		10			185		6			186		5			187		4		
	Low	296	96.4	11	3.6		302	98.4	5	1.6		302	98.4	5	1.6		299	97.4	8	2.6	
Native American Ancestry	Intermediate	317	97.8	7	2.2	0.571	313	96.6	11	3.4	0.304	314	96.9	10	3.1	0.332	312	96.3	12	3.7	0.137
	High	302	97.1	9	2.9		300	96.5	11	3.5		300	96.5	11	3.5		307	98.7	4	1.3	
	N.I.	181		10			185		6			186		5			187		4		
European Ancestry	Low	306	97.8	7	2.2		301	96.2	12	3.8		301	96.2	12	3.8		309	98.7	4	1.3	
	Intermediate	307	97.2	9	2.8	0.630	308	97.5	8	2.5	0.465	308	97.5	8	2.5	0.355	306	96.8	10	3.2	0.261
	High	302	96.5	11	3.5		306	97.8	7	2.2		307	98.1	6	1.9		303	96.8	10	3.2	
Native American Ancestry	N.I.	181		10			185		6			186		5			187		4		
	Low	286	96.3	11	3.7		285	96.0	12	4.0		289	97.3	8	2.7		290	97.6	7	2.4	
	Intermediate	319	97.0	10	3.0	0.404	321	97.6	8	2.4	0.358	321	97.6	8	2.4	0.852	319	97.0	10	3.0	0.787
	High	310	98.1	6	1.9		309	97.8	7	2.2		306	96.8	10	3.2		309	97.8	7	2.2	
	N.I.	181		10			185		6			186		5			187		4		

N.I., no information available; NSCLC NOS, Non-Small Cell Lung Cancer not otherwise specified; CNS, Central Nervous System; *Includes Large Cell Carcinoma, Adenosquamous Carcinoma, Sarcomatoid Carcinoma, and Large Cell Neuroendocrine Carcinoma. **According to the IBGE (Brazilian Institute of Geography and Statistics); Chi-square and FISHER test employed in categorical variables; U-Mann-Whitney test employed in continuous variables.

To further perform the multivariate analysis using the Logistic Regression test, with variables with a p-value ≤ 0.2 in the univariate analysis. *TP53* mutations were independently associated with male and ever-smoker patients, non-adenocarcinoma histology, CNS metastasis, and low European Ancestry (Table 3). *EGFR* mutations were independently associated with female patients, never-smokers, and CNS metastasis (Table 3). *KRAS* mutations were independently associated with ever-smoker patients and adenocarcinoma histology (Table 3). *ALK* fusions were associated with younger patients at diagnosis and never-smoker patients (Table 3). Finally, *PIK3CA* mutations were independently associated with older patients at diagnosis and with the squamous histology (Table 4). *BRAF* mutations were independently associated with older and female patients (Table 4). Finally, *MET* alterations were independently associated with higher ECOG performance status at diagnosis (Table 4). *ERBB2* mutations had no variable independently associated.

Table 3 – Independent association with patients' features for mutations in *TP53*, *EGFR*, *KRAS*, and fusions in the *ALK* gene.

Characteristics	Parameters	TP53 mutations				EGFR mutations				KRAS mutations				ALK-fusions			
		95% CI				95% CI				95% CI				95% CI			
		OR	Min	Max	p-value	OR	Min	Max	p-value	OR	Min	Max	p-value	OR	Min	Max	p-value
Sex	Female	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Not available				Not available			
	Male	1.34	1.00	1.76	0.048	0.60	0.43	0.85	0.004								
Smoking	Never	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
	Quitter	1.95	1.36	2.81	<0.0001	0.20	0.13	0.29	<0.0001	5.84	3.57	9.55	<0.0001	0.67	0.37	1.23	0.203
	Current	3.67	2.55	5.27	<0.0001	0.07	0.04	0.12	<0.0001	7.74	4.77	12.55	<0.0001	0.14	0.06	0.32	<0.0001
Histology	Adenocarcinoma	Ref	Ref	Ref	Ref	Not available				Ref	Ref	Ref	Ref	Not available			
	Squamous	3.28	1.63	6.60	0.001					0.15	0.05	0.42	<0.0001				
	NSCLC SOE	2.03	1.21	3.39	0.007					0.42	0.25	0.71	0.001				
	Others*	1.16	0.46	2.90	0.751					0.33	0.12	0.88	0.028				
Metastasis at diagnosis	No	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Not available				Not available			
	Yes, CNS	1.73	1.17	2.56	0.006	1.88	1.21	2.90	0.004								
	Yes, Others	1.06	0.76	1.47	0.708	1.03	0.69	1.53	0.862								
European ancestry	High	Ref	Ref	Ref	Ref	Not available				Not available				Not available			
	Intermedium	1.39	0.98	1.96	0.062												
	Low	1.84	1.29	2.62	0.001												
Age at diagnosis	Younger x Older**	Not available				Not available				Not available				0.95	0.92	0.97	<0.0001

OR, Odds Ratio, CI, Confidence Interval, Min, Minimum value, Max, Maximum value.; *Includes Large Cell Carcinoma, Adenosquamous Carcinoma, Sarcomatoid Carcinoma, and Large Cell Neuroendocrine Carcinoma, **Continuous variable.

Table 4 – Independent association with patients' features for mutations in the *PIK3CA*, *BRAF* and *MET* gene.

Characteristics	Parameters	PIK3CA mutations				BRAF mutations				MET alterations			
		OR	95% CI		p-value	OR	95% CI		p-value	OR	95% CI		p-value
			Min	Max			Min	Max			Min	Max	
Age at diagnosis	Younger x Older*	1.04	1.01	1.08	0.010	1.04	1.00	1.07	0.24	Not available			
Histology	Adenocarcinoma	Ref	Ref	Ref	Ref	Not available				Not available			
	Squamous	7.43	3.33	16.16	<0.0001								
	NSCLC SOE	1.00	0.29	3.41	0.996								
	Other**	2.38	0.53	10.63	0.254								
Sex	Female	Not available				2.53	1.19	5.39	0.015	Not available			
	Male	Not available				Ref	Ref	Ref	Ref				
ECOG PS	0-1	Not available				Not available				Ref	Ref	Ref	Ref
	2-4	Not available								2.38	1.07	5.29	0.032

OR, Odds Ratio, CI, Confidence Interval, Min, Minimum value, Max, Maximum value. *Continuous variable, **Includes Large Cell Carcinoma, Adenosquamous Carcinoma, Sarcomatoid Carcinoma, and Large Cell Neuroendocrine Carcinoma,

Discussion

The present study evaluated the molecular profile of driver genes of lung tumors from a series of 1133 patients diagnosed with lung cancer in a single Brazilian institution. Overall, we found that 87% of cases harbored oncogenic molecular alterations.

The most frequent mutated gene was *TP53*, mutated in 57.9% (n=657/1133) of the patients. Similarly, we reported *TP53* mutations in about 60% of lung adenocarcinoma patients from a subset of 446 NSCLC, with higher frequency in squamous histology (81%)[27]. Our frequency is also similar to other studies in the literature since the worldwide frequency for *TP53* mutations in lung adenocarcinoma ranges from 29% to 60%[7,27,28] and about 81% for lung squamous cell carcinomas [29]. In this study, *TP53* mutations were associated with male patients, ever-smokers, squamous histology, CNS metastasis, and lower European ancestry. *TP53* mutations have been previously associated with male patients, the presence of CNS metastasis, tobacco consumption, and squamous histology [27,29–34]. In contrast, we observed an association with lower European ancestry, while a previous study showed an association of *TP53* mutations with African population and ancestry [27,35].

KRAS was the second most mutated gene in our series, representing 25.3% (n=287/1133) of patients. This frequency is in agreement with previous studies [7,10,16,36–38]. We observed *KRAS* mutations associated with tobacco consumption and adenocarcinoma histology, as previously reported in the literature [10,39,40]. However, we observed no association between *KRAS* mutations and genetic ancestry; studies reported a lower frequency of *KRAS* mutations in Asian patients and a higher frequency in European patients [7,16,26,38]. In Brazil, *KRAS* mutations are presented in about 25% of patients diagnosed with NSCLC, mainly lung adenocarcinoma [10,26,37].

EGFR mutations were identified in 20.5% (n=232/1133) of patients in this study, in agreement with previous studies in Brazil [37,40–42]. Worldwide, *EGFR* mutations are more frequent in Asian patients and lower frequent in European patients with lung cancer, and associated with higher genetic ancestry in patients [16,26,43]. Recently, *EGFR* mutations have also been associated with Native American ancestry [15]. In our study, *EGFR* mutations were

associated with female patients, never-smokers, CNS metastasis, and lower European ancestry, which is in agreement with previous studies [14,18,26,35,43–45].

ALK fusions were identified in 5.9% (n=67/1133) of patients from this study, similar to previous studies in Brazil and worldwide [9,37,46–48]. *ALK* fusions were associated with younger and never-smoker patients with lung cancer, in agreement with previous studies [9,14,35,47]. We observed fusions in the *RET* gene, with a frequency of 0.5% (n=6/1133), and *ROS1*, in 0.3% (n=4/1133) of patients, similar to previous studies [9,49]. As we previously described, *NTRK1* and *NTRK3* fusion are observed in one patient each [11]. *MET*Δ*ex14* alteration was identified in 1.4% (n=16/1133) of patients, a lower frequency than in other studies [14,35,50]. Finally, *BRAF*, *PIK3CA*, *NRAS*, *KIT*, and *ERBB2* mutations were identified in 3% or less of patients, similar to previous studies worldwide [7,24,51]. In contrast, *PIK3CA* is described as more frequent (6%) in the TCGA (The Cancer Genome Atlas) [7], and in Brazil, a previous study reported *PIK3CA* in a higher frequency (8.8%) in lung tumors, such as *ERBB2* mutations (4.9%)[37].

Moreover, *TP53* and *PIK3CA* genes were the most frequent mutations co-occurring with *EGFR* and *KRAS* mutations, and *ALK* fusions were more frequent with *TP53* mutations. Previous studies in Brazil have reported *TP53* and *PIK3CA* as the most frequent mutations co-occurring with *EGFR* and *KRAS* mutations, such as *ALK* fusions with *TP53* mutations[37]. Also, we have previously reported the association of *TP53* mutations with *EGFR* mutations in Brazilian patients diagnosed with lung adenocarcinoma who never smoked[35]. Therefore, co-occurring mutations have been reported as important for treatment choices [2]. For example, *TP53* mutations have been associated with a shorter overall survival in patients harboring *EGFR* mutations treated with TKIs[2,52]. However, more studies focusing on the treatment impact of co-occurring mutations in Brazilian NSCLC patients are essential.

Our study has some limitations, such as its retrospective nature. Moreover, the low frequency of alterations in most genes (*RET*, *ROS1*, *NTRK*, *MET*, *ERBB2*, *BRAF*, *NRAS*, *PIK3CA*, *KIT*) hampers meaningful statistical analysis. Also, we used real-world data for our analysis, so a subset of patients was evaluated entirely for mutations and fusions.

Conclusion

The majority of Brazilian lung cancer patients (87%) harbor at least one oncogenic alteration, and targeted therapies could potentially benefit at least 35% of cases. However, further studies are required to investigate co-alterations and a broader range of less frequently mutated genes to comprehensively understand the molecular profile.

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Supplementary Material

Supplementary Table 1 - Oncogenic variants identified in the lung tumors

ID	Gene	Alteration	Type	TIER	Oncogenicity
1	TP53	p.Val157Phe	Missense	II	Oncogenic
2	EGFR	p.Leu858Arg	Missense	I	Oncogenic
3	EGFR	p.Glu746_Ser752delinsVal	ProteinDel	I	Oncogenic
5	EGFR	p.Leu858Arg	Missense	I	Oncogenic
6	TP53	p.Ala159Pro	Missense	II	Oncogenic
7	KRAS	p.Gly12Cys	Missense	I	Oncogenic
9	TP53	p.Arg158Gly	Missense	II	Oncogenic
10	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Phe270Ser	Missense	II	Likely Oncogenic
11	EGFR	p.Ala767_Val769dup	ProteinIns	I	Oncogenic
12	TP53	p.Arg248Trp	Missense	II	Oncogenic
13	KRAS	p.Gly12Val	Missense	II	Oncogenic
15	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Arg337His	Missense	II	Oncogenic
16	ERBB2	p.Tyr772_Ala775dup	ProteinIns	I	Oncogenic
17	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Arg273Leu	Missense	II	Oncogenic
18	KRAS	p.Gly13Cys	Missense	II	Oncogenic
19	EGFR	p.Leu747_Ala750delinsPro	ProteinDel	I	Oncogenic
20	EGFR	p.Asp770delinsGlyTyr	ProteinIns	I	Oncogenic
	TP53	p.Arg337His	Missense	II	Oncogenic
	PIK3CA	p.Glu542Lys	Missense	II	Oncogenic
21	ALK	EML4:ALK	Fusion	I	Oncogenic
24	KRAS	p.Gly12Val	Missense	II	Oncogenic
25	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Tyr220Cys	Missense	II	Oncogenic
	PIK3CA	p.His1047Leu	Missense	II	Likely Oncogenic
26	EGFR	p.Leu747_Pro753delinsSer	ProteinDel	I	Oncogenic
	PIK3CA	p.Glu542Lys	Missense	II	Oncogenic
27	TP53	p.Pro177Phe	Missense	II	Likely Oncogenic
	TP53	p.Arg213*	Nonsense	II	Oncogenic
28	KRAS	p.Gly13Cys	Missense	II	Oncogenic
	TP53	p.Arg156Gly	Missense	II	Likely Oncogenic
29	TP53	p.Ala159Pro	Missense	II	Oncogenic
31	KRAS	p.Gly12Val	Missense	II	Oncogenic
32	EGFR	p.Glu709_Thr710delinsAsp	ProteinDel	I	Oncogenic
34	EGFR	p.Thr790Met	Missense	I	Oncogenic
	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Val274Profs*24	Frameshift	II	Oncogenic
35	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Arg337His	Missense	II	Oncogenic
36	EGFR	p.Leu858Arg	Missense	I	Oncogenic
37	TP53	p.Gln331*	Nonsense	II	Oncogenic
38	EGFR	p.Leu747_Ser752del	ProteinDel	I	Oncogenic
	TP53	p.Asp281His	Missense	II	Oncogenic
39	EGFR	p.Asn771_His773dup	ProteinIns	I	Oncogenic
40	EGFR	p.Leu861Gln	Missense	I	Oncogenic
41	ERBB2	p.Tyr772_Ala775dup	ProteinIns	I	Oncogenic
42	EGFR	p.Leu858Arg	Missense	I	Oncogenic
43	KRAS	p.Gly12Cys	Missense	I	Oncogenic
44	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Arg267Glnfs*3	Frameshift	II	Oncogenic
46	KRAS	p.Gly12Val	Missense	II	Oncogenic
	TP53	p.Arg273Cys	Missense	II	Oncogenic
47	TP53	p.Tyr163Serfs*7	Frameshift	II	Oncogenic
48	ALK	EML4:ALK	Fusion	I	Oncogenic
49	KRAS	p.Gly12Asp	Missense	II	Oncogenic
	TP53	p.?	Splice_region	II	Oncogenic

50	TP53	p.Arg158Leu	Missense	II	Oncogenic
51	TP53	p.Arg196*	Nonsense	II	Oncogenic
	RET	KIF5B:RET	Fusion	I	Oncogenic
52	EGFR	p.Leu858Arg	Missense	I	Oncogenic
53	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
54	TP53	p.Ile251Serfs*94	Frameshift	II	Oncogenic
55	TP53	p.Arg337His	Missense	II	Oncogenic
	TP53	p.Pro250del	ProteinDel	II	Likely Oncogenic
56	TP53	p.Leu35Cysfs*9	Frameshift	II	Oncogenic
	TP53	p.Gln167*	Nonsense	II	Oncogenic
57	TP53	p.Lys139*	Nonsense	II	Oncogenic
58	TP53	p.Arg249Ser	Missense	II	Oncogenic
	TP53	p.Arg273Cys	Missense	II	Oncogenic
59	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
61	EGFR	p.Leu747_Thr751delinsPro	ProteinDel	I	Oncogenic
	TP53	p.Glu286Val	Missense	II	Likely Oncogenic
63	KRAS	p.Gly13Asp	Missense	II	Oncogenic
64	KRAS	p.Gly12Cys	Missense	I	Oncogenic
65	TP53	p.Val157Phe	Missense	II	Oncogenic
66	ALK	Unkown Partner	Fusion	I	Oncogenic
67	TP53	p.Gly245Val	Missense	II	Likely Oncogenic
68	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Pro190Leufs*57	Frameshift	II	Oncogenic
69	KRAS	p.Gly12Cys	Missense	I	Oncogenic
70	EGFR	p.Ala767_Val769dup	ProteinIns	I	Oncogenic
	TP53	p.Lys132Asnfs*16	Frameshift	II	Oncogenic
71	TP53	p.Arg248Trp	Missense	II	Oncogenic
72	TP53	p.Leu43fs*1	Frameshift	II	Oncogenic
	ALK	Unkown Partner	Fusion	I	Oncogenic
73	TP53	p.Leu130Val	Missense	II	Likely Oncogenic
74	EGFR	p.Leu861Gln	Missense	I	Oncogenic
	TP53	p.Arg337His	Missense	II	Oncogenic
75	EGFR	p.Leu747_Thr751del	ProteinDel	I	Oncogenic
	TP53	p.Arg337His	Missense	II	Oncogenic
76	TP53	p.His168Arg	Missense	II	Likely Oncogenic
78	ALK	Unkown Partner	Fusion	I	Oncogenic
82	TP53	p.?	Splice_region	II	Oncogenic
84	TP53	p.Leu348_Glu349delinsPhe*	Frameshift	II	Oncogenic
86	TP53	p.Tyr236Cys	Missense	II	Oncogenic
87	TP53	p.Arg282Pro	Missense	II	Likely Oncogenic
88	TP53	p.Arg273His	Missense	II	Oncogenic
92	TP53	p.Arg273Leu	Missense	II	Oncogenic
94	TP53	p.Val157Phe	Missense	II	Oncogenic
96	ALK	Unkown Partner	Fusion	I	Oncogenic
97	TP53	p.Arg273His	Missense	II	Oncogenic
98	KRAS	p.Gly12Cys	Missense	I	Oncogenic
99	EGFR	p.Leu747_Thr751del	ProteinDel	I	Oncogenic
	TP53	p.Arg337His	Missense	II	Oncogenic
100	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Ile251Phe	Missense	II	Likely Oncogenic
102	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Lys132*	Nonsense	II	Oncogenic
103	ALK	EML4:ALK	Fusion	I	Oncogenic
106	ERBB2	p.Tyr772_Ala775dup	ProteinIns	I	Oncogenic
108	TP53	p.Arg110Leu	Missense	II	Likely Oncogenic
109	TP53	p.?	Splice_region	II	Oncogenic
111	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.His179Tyr	Missense	II	Oncogenic
112	ALK	EML4:ALK	Fusion	I	Oncogenic
113	ERBB2	p.Tyr772_Ala775dup	ProteinIns	I	Oncogenic
114	EGFR	p.Leu858Arg	Missense	I	Oncogenic
115	TP53	p.Arg337Leu	Missense	II	Oncogenic
116	KRAS	p.Gly12Ser	Missense	II	Oncogenic

117	TP53	p.Cys275Tyr	Missense	II	Likely Oncogenic
118	EGFR	p.Ala755Asp	Missense	I	Oncogenic
	EGFR	p.Leu747_Pro753delinsSer	ProteinDel	I	Oncogenic
	TP53	p.Ile195Thr	Missense	II	Likely Oncogenic
119	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Gly266Glu	Missense	II	Likely Oncogenic
121	KRAS	p.Gly12Asp	Missense	II	Oncogenic
122	TP53	p.Glu198*	Nonsense	II	Oncogenic
123	TP53	p.His179Arg	Missense	II	Oncogenic
124	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Cys275Trp	Missense	II	Likely Oncogenic
125	TP53	p.Glu171Argfs*3	Frameshift	II	Oncogenic
126	KRAS	p.Gly12Val	Missense	II	Oncogenic
127	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Glu271Lys	Missense	II	Likely Oncogenic
128	TP53	p.?	Splice_region	II	Oncogenic
130	TP53	p.Arg273Leu	Missense	II	Oncogenic
131	TP53	p.Gly245Val	Missense	II	Oncogenic
133	EGFR	p.Leu861Gln	Missense	I	Oncogenic
	TP53	p.Glu336*	Nonsense	II	Oncogenic
134	TP53	p.Val274Phe	Missense	II	Oncogenic
135	KRAS	p.Gly12Val	Missense	II	Oncogenic
	TP53	p.Arg158Leu	Missense	II	Oncogenic
136	TP53	p.Arg158Leu	Missense	II	Oncogenic
	TP53	p.Lys164*	Nonsense	II	Oncogenic
137	TP53	p.Arg213Leu	Missense	II	Oncogenic
139	KRAS	p.Gly12Val	Missense	II	Oncogenic
140	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Met66Serfs*57	Frameshift	II	Oncogenic
	TP53	p.Arg248Leu	Missense	II	Oncogenic
142	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
143	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Arg337Leu	Missense	II	Oncogenic
144	KRAS	p.Gly12Asp	Missense	II	Oncogenic
145	TP53	p.Gly334Valfs*10	Frameshift	II	Oncogenic
146	EGFR	p.Leu858Arg	Missense	I	Oncogenic
147	TP53	p.His179Arg	Missense	II	Oncogenic
148	KRAS	p.Gly12Val	Missense	II	Oncogenic
149	EGFR	p.Glu709_Thr710delinsAsp	ProteinDel	I	Oncogenic
	TP53	p.Ile50*	Nonsense	II	Oncogenic
	TP53	p.Ile50*	Nonsense	II	Oncogenic
151	EGFR	p.Leu747_Thr751delinsPro	ProteinDel	I	Oncogenic
	TP53	p.Glu51*	Nonsense	II	Oncogenic
152	EGFR	p.Leu747_Ala750delinsPro	ProteinDel	I	Oncogenic
153	TP53	p.Val272Met	Missense	II	Oncogenic
	NRAS	p.Gln61His	Missense	II	Likely Oncogenic
154	KRAS	p.Gly12Cys	Missense	I	Oncogenic
156	EGFR	p.Glu709Val	Missense	I	Oncogenic
	EGFR	p.Leu858Arg	Missense	I	Oncogenic
157	KRAS	p.Gly12Cys	Missense	I	Oncogenic
158	ALK	Unkown Partner	Fusion	I	Oncogenic
159	TP53	p.Arg249Met	Missense	II	Oncogenic
160	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Gln331*	Nonsense	II	Oncogenic
162	ERBB2	p.Tyr772_Ala775dup	ProteinIns	I	Oncogenic
163	BRAF	p.Val600Glu	Missense	I	Oncogenic
164	TP53	p.Arg249Ser	Missense	II	Oncogenic
165	TP53	p.Gly245Cys	Missense	II	Oncogenic
166	KRAS	p.Gly12Cys	Missense	I	Oncogenic
167	TP53	p.Arg273Leu	Missense	II	Oncogenic
168	KRAS	p.Gly12Ala	Missense	II	Oncogenic
	PIK3CA	p.His1047Leu	Missense	II	Likely Oncogenic
169	KRAS	p.Gly12Cys	Missense	I	Oncogenic

170	KRAS	p.Gly12Val	Missense	II	Oncogenic
	TP53	p.Arg273Leu	Missense	II	Oncogenic
171	EGFR	p.Leu858Arg	Missense	I	Oncogenic
172	TP53	p.Gly244Cys	Missense	II	Oncogenic
173	TP53	p.His179Arg	Missense	II	Oncogenic
174	KRAS	p.Gly12Val	Missense	II	Oncogenic
175	KRAS	p.Gly12Val	Missense	II	Oncogenic
176	KRAS	p.Gly12Cys	Missense	I	Oncogenic
178	TP53	p.Ala159Pro	Missense	II	Oncogenic
179	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
180	TP53	p.Gly244Cys	Missense	II	Oncogenic
181	EGFR	p.Glu690Asp	Missense	I	Oncogenic
	TP53	p.Met237Ile	Missense	II	Oncogenic
	TP53	p.Ala276Asp	Missense	II	Likely Oncogenic
182	TP53	p.Tyr126*	Nonsense	II	Oncogenic
	RET	CCDC6:RET	Fusion	I	Oncogenic
183	TP53	p.Arg337His	Missense	II	Oncogenic
	TP53	p.Arg273His	Missense	II	Oncogenic
184	TP53	p.Cys277Phe	Missense	II	Likely Oncogenic
186	TP53	p.Gly334Val	Missense	II	Likely Oncogenic
187	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
189	TP53	p.Tyr126His	Missense	II	Likely Oncogenic
	MET	METΔex14	Exon Skipping	I	Oncogenic
191	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
192	TP53	p.Gly266Val	Missense	II	Likely Oncogenic
193	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Val216Met	Missense	II	Likely Oncogenic
194	KRAS	p.Gly13Cys	Missense	II	Oncogenic
	TP53	p.Ile195Phe	Missense	II	Oncogenic
195	KRAS	p.Gly12Ala	Missense	II	Oncogenic
	TP53	p.Gly245Ser	Missense	II	Oncogenic
196	ALK	EML4:ALK	Fusion	I	Oncogenic
197	KRAS	p.Gly12Cys	Missense	I	Oncogenic
198	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Ala276Gly	Missense	II	Likely Oncogenic
200	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Glu339*	Nonsense	II	Oncogenic
201	TP53	p.Asp281Glu	Missense	II	Oncogenic
202	TP53	p.Gly266*	Nonsense	II	Oncogenic
203	EGFR	p.Gly719Ala	Missense	I	Oncogenic
	TP53	p.Phe338Leufs*6	Frameshift	II	Oncogenic
204	KRAS	p.Gly12Ala	Missense	II	Oncogenic
205	ALK	Unkown Partner	Fusion	I	Oncogenic
206	KRAS	p.Gly12Ala	Missense	II	Oncogenic
207	EGFR	p.Leu747_Thr751del	ProteinDel	I	Oncogenic
	TP53	p.Glu286Gly	Missense	II	Likely Oncogenic
208	TP53	p.Arg273Cys	Missense	II	Oncogenic
209	MET	METΔex14	Exon Skipping	I	Oncogenic
210	EGFR	p.Val769Met	Missense	I	Oncogenic
	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
211	TP53	p.Val173Leu	Missense	II	Oncogenic
212	TP53	p.Arg249Met	Missense	II	Oncogenic
213	TP53	p.Arg280Gly	Missense	II	Oncogenic
214	BRAF	p.Val600Glu	Missense	I	Oncogenic
216	TP53	p.Met237Ile	Missense	II	Oncogenic
217	KRAS	p.Ala59Glu	Missense	II	Likely Oncogenic
218	TP53	p.Arg248Gln	Missense	II	Oncogenic
	RET	Unkown Partner	Fusion	I	Oncogenic
219	PIK3CA	p.Glu545Lys	Missense	II	Oncogenic
220	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Tyr163Cys	Missense	II	Oncogenic
221	TP53	p.?	Splice_region	II	Oncogenic
	ALK	Unkown Partner	Fusion	I	Oncogenic

222	TP53	p.Pro153Serfs*8	Frameshift	II	Oncogenic
	KRAS	p.Gly12Val	Missense	II	Oncogenic
223	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
224	TP53	p.Arg249Ser	Missense	II	Oncogenic
225	KRAS	p.Gly12Asp	Missense	I	Oncogenic
226	ALK	Unkown Partner	Fusion	I	Oncogenic
227	TP53	p.Tyr126Argfs*45	Frameshift	II	Oncogenic
228	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Gly245Cys	Missense	II	Oncogenic
229	TP53	p.Cys238Tyr	Missense	II	Oncogenic
231	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Gln136Glu	Missense	II	Likely Oncogenic
232	TP53	p.Arg248Leu	Missense	II	Oncogenic
233	MET	Amplification	CNV	II	Oncogenic
235	KRAS	p.Gly12Val	Missense	II	Oncogenic
236	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Ser185Argfs*23	Frameshift	II	Oncogenic
237	TP53	p.Cys124*	Nonsense	II	Oncogenic
238	TP53	p.Glu68Profs*51	Frameshift	II	Oncogenic
239	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Asn310Thrfs*35	Frameshift	II	Oncogenic
240	TP53	p.Arg333Valfs*12	Frameshift	II	Oncogenic
241	KRAS	p.Gly12Asp	Missense	II	Oncogenic
242	KRAS	p.Gly12Cys	Missense	I	Oncogenic
243	TP53	p.Ser90Profs*33	Frameshift	II	Oncogenic
	TP53	p.Gly105Arg	Missense	II	Likely Oncogenic
244	EGFR	p.Ser768_Asp770dup	ProteinIns	I	Oncogenic
245	KRAS	p.Gly12Val	Missense	II	Oncogenic
	TP53	p.Glu198*	Nonsense	II	Oncogenic
246	KRAS	p.Gly12Val	Missense	II	Oncogenic
	TP53	p.Glu343*	Nonsense	II	Oncogenic
247	KRAS	p.Gly12Val	Missense	II	Oncogenic
	BRAF	p.Asn581Ile	Missense	II	Likely Oncogenic
	TP53	p.Gly105Val	Missense	II	Oncogenic
	TP53	p.Arg175Leu	Missense	II	Likely Oncogenic
248	TP53	p.Arg156His	Missense	II	Likely Oncogenic
249	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Arg273Cys	Missense	II	Oncogenic
250	TP53	p.Val157Phe	Missense	II	Oncogenic
251	TP53	p.Arg273Cys	Missense	II	Oncogenic
	ALK	EML4:ALK	Fusion	I	Oncogenic
252	TP53	p.Val157Phe	Missense	II	Oncogenic
253	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Asp259Tyr	Missense	II	Likely Oncogenic
254	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Tyr163Cys	Missense	II	Oncogenic
255	TP53	p.Arg248Gly	Missense	II	Oncogenic
	TP53	p.Arg156His	Missense	II	Likely Oncogenic
256	TP53	p.Val157Phe	Missense	II	Oncogenic
257	EGFR	p.Leu747_Pro753delinsSer	ProteinDel	I	Oncogenic
	TP53	p.?	Splice_region	II	Oncogenic
258	KRAS	p.Gln61Leu	Missense	II	Oncogenic
259	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Val173Met	Missense	II	Oncogenic
260	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Arg337His	Missense	II	Oncogenic
261	KRAS	p.Gly12Val	Missense	II	Oncogenic
262	TP53	p.Tyr205His	Missense	II	Oncogenic
	MET	Amplification	CNV	II	Oncogenic
264	KRAS	p.Gly12Cys	Missense	I	Oncogenic
265	TP53	p.Met1Ilefs*9	Frameshift	II	Oncogenic
266	ALK	Unkown Partner	Fusion	I	Oncogenic
267	KRAS	p.Gly12Cys	Missense	I	Oncogenic

	TP53	p.Cys135Arg	Missense	II	Likely Oncogenic
268	TP53	p.Arg273His	Missense	II	Oncogenic
269	TP53	p.Tyr220Cys	Missense	II	Oncogenic
	ERBB2	Amplification	CNV	II	Oncogenic
270	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
271	KRAS	p.Gly12Val	Missense	II	Oncogenic
	TP53	p.Gln165*	Nonsense	II	Oncogenic
272	TP53	p.Met160Hisfs*12	Frameshift	II	Oncogenic
	KRAS	p.Gly12Ala	Missense	II	Oncogenic
273	EGFR	p.Asn771_His773dup	ProteinIns	I	Oncogenic
	TP53	p.Asn239Asp	Missense	II	Likely Oncogenic
275	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Arg209Lysfs*6	Frameshift	II	Oncogenic
276	KRAS	p.Gly13Asp	Missense	II	Oncogenic
278	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Lys132Asn	Missense	II	Oncogenic
279	KRAS	p.Gly12Val	Missense	II	Oncogenic
	TP53	p.Gly245Ser	Missense	II	Oncogenic
280	KRAS	p.Gly12Cys	Missense	I	Oncogenic
281	TP53	p.Arg283Pro	Missense	II	Likely Oncogenic
283	KRAS	p.Gly12Asp	Missense	II	Oncogenic
284	TP53	p.Lys132Asnfs*12	Frameshift	II	Oncogenic
286	KRAS	p.Gly12Val	Missense	II	Oncogenic
287	KRAS	p.Gly12Val	Missense	II	Oncogenic
288	KRAS	p.Gly12Asp	Missense	II	Oncogenic
289	TP53	p.Ile162_His168del	ProteinDel	II	Likely Oncogenic
	ALK	EML4:ALK	Fusion	I	Oncogenic
290	TP53	p.Arg213*	Nonsense	II	Oncogenic
	MET	METΔex14	Exon Skipping	I	Oncogenic
291	KRAS	p.Gly13Asp	Missense	II	Oncogenic
292	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Arg282Trp	Missense	II	Oncogenic
293	KRAS	p.Gly12Cys	Missense	I	Oncogenic
294	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Arg273His	Missense	II	Oncogenic
295	TP53	p.?	Splice_region	II	Oncogenic
296	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Arg158Leu	Missense	II	Likely Oncogenic
297	KRAS	p.Gly12Cys	Missense	I	Oncogenic
298	ALK	Unknown Partner	Fusion	I	Oncogenic
300	TP53	p.Arg337Cys	Missense	II	Oncogenic
302	BRAF	p.Val600Glu	Missense	I	Oncogenic
	TP53	p.Glu171Gly	Missense	II	Likely Oncogenic
303	TP53	p.Arg175His	Missense	II	Oncogenic
	TP53	p.Val143Met	Missense	II	Likely Oncogenic
304	KRAS	p.Gly12Cys	Missense	I	Oncogenic
306	TP53	p.Gly105Cys	Missense	II	Likely Oncogenic
	TP53	p.Gly245Phe	Missense	II	Likely Oncogenic
307	KRAS	p.Gly12Asp	Missense	II	Oncogenic
308	TP53	p.Arg337His	Missense	II	Oncogenic
	TP53	p.Ser215Arg	Missense	II	Oncogenic
310	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	PIK3CA	p.Glu545Lys	Missense	II	Oncogenic
311	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
312	EGFR	p.Leu858Arg	Missense	I	Oncogenic
313	TP53	p.Glu286Lys	Missense	II	Oncogenic
314	TP53	p.Tyr205Phe	Missense	II	Likely Oncogenic
315	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Ala161Ser	Missense	II	Likely Oncogenic
316	TP53	p.Cys176Ser	Missense	II	Oncogenic
318	EGFR	p.Leu747_Ala750delinsPro	ProteinDel	I	Oncogenic
319	TP53	p.Glu198*	Nonsense	II	Oncogenic
320	TP53	p.Glu286Asp	Missense	II	Likely Oncogenic

321	EGFR	p.Thr790Met	Missense	I	Oncogenic
	EGFR	p.Cys797Ser	Missense	I	Oncogenic
	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Met237Ile	Missense	II	Oncogenic
322	EGFR	p.Leu747_Ala750delinsPro	ProteinDel	I	Oncogenic
	TP53	p.Pro177Ser	Missense	II	Likely Oncogenic
323	TP53	p.Val157Phe	Missense	II	Oncogenic
324	TP53	p.Val172Phe	Missense	II	Likely Oncogenic
325	EGFR	p.Thr790Met	Missense	I	Oncogenic
	EGFR	p.Leu747_Pro753delinsSer	ProteinDel	I	Oncogenic
	TP53	p.His193Tyr	Missense	II	Likely Oncogenic
326	EGFR	p.Val769Met	Missense	I	Oncogenic
	EGFR	p.Val786Met	Missense	I	Oncogenic
	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Arg248Glyfs*97	Frameshift	II	Oncogenic
327	EGFR	p.Thr790Met	Missense	I	Oncogenic
	EGFR	p.Cys797Ser	Missense	I	Oncogenic
	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Val143Met	Missense	II	Likely Oncogenic
328	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Glu285Lys	Missense	II	Oncogenic
329	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Trp53*	Nonsense	II	Oncogenic
330	BRAF	p.Val600Glu	Missense	I	Oncogenic
	PIK3CA	p.Glu545Lys	Missense	II	Oncogenic
	TP53	p.Gly334Val	Missense	II	Likely Oncogenic
331	EGFR	p.Leu747_Ala750delinsPro	ProteinDel	I	Oncogenic
	TP53	p.Arg337His	Missense	II	Oncogenic
333	EGFR	p.Ala763_Tyr764insPheGlnGluAla	ProteinIns	I	Oncogenic
335	EGFR	p.Leu747_Ala750delinsPro	ProteinDel	I	Oncogenic
336	BRAF	p.Val600Glu	Missense	I	Oncogenic
337	EGFR	p.Leu858Arg	Missense	I	Oncogenic
339	TP53	p.His193Leu	Missense	II	Oncogenic
340	TP53	p.Ala138Val	Missense	II	Likely Oncogenic
341	KRAS	p.Gly13Asp	Missense	II	Oncogenic
	TP53	p.Glu298*	Nonsense	II	Oncogenic
342	BRAF	p.Val600Glu	Missense	I	Oncogenic
343	TP53	p.Gly266Val	Missense	II	Likely Oncogenic
	MET	Amplification	CNV	II	Oncogenic
344	KRAS	p.Gly12Ala	Missense	II	Oncogenic
	TP53	p.Gly266Arg	Missense	II	Oncogenic
346	KRAS	p.Gly12Asp	Missense	II	Oncogenic
348	TP53	p.Arg156Pro	Missense	II	Oncogenic
349	TP53	p.Arg248Gln	Missense	II	Oncogenic
	ALK	Unkown Partner	Fusion	I	Oncogenic
350	TP53	p.Arg267Trp	Missense	II	Likely Oncogenic
351	TP53	p.Tyr234Cys	Missense	II	Oncogenic
352	ERBB2	p.Gly776delinsLeuCys	ProteinIns	I	Likely Oncogenic
353	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Arg175His	Missense	II	Oncogenic
355	TP53	p.Arg196*	Nonsense	II	Oncogenic
357	TP53	p.Arg248Trp	Missense	II	Oncogenic
358	ALK	EML4:ALK	Fusion	I	Oncogenic
360	KRAS	p.Gly13Cys	Missense	II	Oncogenic
	TP53	p.Ala159Pro	Missense	II	Oncogenic
361	TP53	p.Phe134Cys	Missense	II	Likely Oncogenic
362	KRAS	p.Gly12Val	Missense	II	Oncogenic
	TP53	p.?	Splice_region	II	Oncogenic
363	TP53	p.Asp281His	Missense	II	Oncogenic
	ALK	Unkown Partner	Fusion	I	Oncogenic
364	TP53	p.Gly245Asp	Missense	II	Oncogenic
365	KRAS	p.Gly12Cys	Missense	I	Oncogenic
366	TP53	p.His168Leu	Missense	II	Likely Oncogenic

367	KRAS	p.Gln61His	Missense	II	Oncogenic
	TP53	p.Arg110Leu	Missense	II	Likely Oncogenic
368	TP53	p.Arg248Gln	Missense	II	Oncogenic
369	TP53	p.Arg337His	Missense	II	Oncogenic
370	TP53	p.Lys132Glu	Missense	II	Oncogenic
	PIK3CA	p.Pro539Arg	Missense	II	Likely Oncogenic
372	EGFR	p.Glu746_Ser752delinsVal	ProteinDel	I	Oncogenic
	TP53	p.Cys135Phe	Missense	II	Oncogenic
373	TP53	p.His214Arg	Missense	II	Oncogenic
	TP53	p.Lys351*	Nonsense	II	Oncogenic
374	KRAS	p.Gly12Cys	Missense	I	Oncogenic
375	TP53	p.Ser241Phe	Missense	II	Oncogenic
376	TP53	p.Gly199Glu	Missense	II	Likely Oncogenic
	TP53	p.Arg209*	Nonsense	II	Oncogenic
377	TP53	p.Met243Phefs*4	Frameshift	II	Oncogenic
	KRAS	p.Gly13Cys	Missense	II	Oncogenic
378	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Cys242Tyr	Missense	II	Oncogenic
379	KRAS	p.Gly12Asp	Missense	II	Oncogenic
	TP53	p.?	Splice_region	II	Oncogenic
380	TP53	p.Lys164_His168del	ProteinDel	II	Likely Oncogenic
382	TP53	p.Arg280Lys	Missense	II	Oncogenic
383	TP53	p.Arg213*	Nonsense	II	Oncogenic
384	KRAS	p.Gly13Cys	Missense	II	Oncogenic
	TP53	p.Val157Phe	Missense	II	Oncogenic
385	KRAS	p.Gly12Val	Missense	II	Oncogenic
	TP53	p.Tyr163Cys	Missense	II	Oncogenic
386	TP53	p.?	Splice_region	II	Oncogenic
387	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	PIK3CA	p.Glu542Lys	Missense	II	Oncogenic
	TP53	p.Glu258del	ProteinDel	II	Likely Oncogenic
388	TP53	p.Pro152Leu	Missense	II	Oncogenic
389	KRAS	p.Gln61Leu	Missense	II	Oncogenic
390	TP53	p.Leu252Ile254del	ProteinDel	II	Likely Oncogenic
391	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Cys124Trpfs*25	Frameshift	II	Oncogenic
394	TP53	p.Glu62*	Nonsense	II	Oncogenic
	EGFR	Amplification	CNV	II	Oncogenic
395	ERBB2	p.Tyr772_Ala775dup	ProteinIns	I	Oncogenic
	TP53	p.Gly105Asp	Missense	II	Likely Oncogenic
396	KRAS	p.Gly12Asp	Missense	II	Oncogenic
	TP53	p.Thr53*	Nonsense	II	Oncogenic
	MET	Amplification	CNV	II	Oncogenic
397	KRAS	p.Gly12Val	Missense	II	Oncogenic
	TP53	p.Glu298*	Nonsense	II	Oncogenic
398	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Val272Glyfs*73	Frameshift	II	Oncogenic
400	ROS1	Unkown Partner	Fusion	I	Oncogenic
402	KRAS	p.Gly12Ala	Missense	II	Oncogenic
403	TP53	p.Lys132Glu	Missense	II	Oncogenic
404	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.?	Splice_region	II	Oncogenic
406	KRAS	p.Gln61His	Missense	II	Oncogenic
	TP53	p.Ser241Phe	Missense	II	Oncogenic
407	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Thr91Cysfs*31	Frameshift	II	Oncogenic
408	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Leu194Arg	Missense	II	Likely Oncogenic
409	TP53	p.Arg175His	Missense	II	Oncogenic
413	KRAS	p.Gly12Val	Missense	II	Oncogenic
	TP53	p.Gly245Cys	Missense	II	Oncogenic
414	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	EGFR	p.Glu709Ala	Missense	I	Oncogenic

	EGFR	p.Gly719Ser	Missense	I	Oncogenic
415	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Val172Asp	Missense	II	Likely Oncogenic
416	EGFR	p.Ala767_Val769dup	Missense	I	Oncogenic
	PIK3CA	p.Glu545Lys	Missense	II	Oncogenic
	TP53	p.Cys135Tyr	Missense	II	Oncogenic
417	KRAS	p.Gly12Asp	Missense	II	Oncogenic
	TP53	p.Glu294*	Nonsense	II	Oncogenic
418	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Arg175His	Missense	II	Oncogenic
419	PIK3CA	p.Glu545Lys	Missense	II	Oncogenic
420	KRAS	p.Gly12Ala	Missense	II	Oncogenic
	TP53	p.?	Splice_region	II	Oncogenic
421	TP53	p.Arg249Ser	Missense	II	Oncogenic
422	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	EGFR	p.Leu747Ser	Missense	I	Oncogenic
423	TP53	p.Cys135Tyr	Missense	II	Oncogenic
424	KRAS	p.Gly12Val	Missense	II	Oncogenic
	TP53	p.Arg273Leu	Missense	II	Oncogenic
	TP53	p.Glu298*	Nonsense	II	Oncogenic
	EGFR	Amplification	CNV	II	Oncogenic
425	TP53	p.Lys320*	Nonsense	II	Oncogenic
426	NTRK1	PRKAR1A	Fusion	I	Oncogenic
427	TP53	p.Arg282Gln	Missense	II	Likely Oncogenic
	ALK	Unkown Partner	Fusion	I	Oncogenic
428	TP53	p.Cys275Arg	Missense	II	Likely Oncogenic
	ALK	Unkown Partner	Fusion	I	Oncogenic
430	KRAS	p.Gly12Val	Missense	II	Oncogenic
	TP53	p.Pro191del	ProteinDel	II	Likely Oncogenic
432	KRAS	p.Gly12Asp	Missense	I	Oncogenic
433	ALK	EML4:ALK	Fusion	I	Oncogenic
434	KRAS	p.Gly12Ser	Missense	II	Oncogenic
435	TP53	p.Arg249Ser	Missense	II	Oncogenic
436	TP53	p.Val272Leu	Missense	II	Likely Oncogenic
437	TP53	p.Pro316delinsLeu*	Nonsense	II	Oncogenic
438	EGFR	p.Glu746_Thr751delinsIle	ProteinDel	I	Oncogenic
	TP53	p.Asn131Tyr	Missense	II	Likely Oncogenic
440	ERBB2	p.Gly660Asp	Missense	I	Likely Oncogenic
	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
441	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Cys238Tyr	Missense	II	Oncogenic
442	KRAS	p.Gly12Cys	Missense	I	Oncogenic
445	KRAS	p.Gly12Cys	Missense	I	Oncogenic
446	TP53	p.Arg110Pro	Missense	II	Likely Oncogenic
	KRAS	p.Gly13dup	ProteinIns	II	Oncogenic
447	TP53	p.Thr123Ilefs*47	Frameshift	II	Oncogenic
	TP53	p.Arg273Cys	Missense	II	Oncogenic
449	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Glu294*	Nonsense	II	Oncogenic
450	EGFR	p.Gly719Cys	Missense	I	Oncogenic
	EGFR	p.Ser768Ile	Missense	I	Oncogenic
451	TP53	p.Met237Hisfs*3	Frameshift	II	Oncogenic
453	EGFR	p.Glu746_Ser752delinsVal	ProteinDel	I	Oncogenic
	TP53	p.Lys321Asnfs*24	Frameshift	II	Oncogenic
454	TP53	p.Glu180*	Nonsense	II	Oncogenic
455	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Arg249Ser	Missense	II	Oncogenic
456	ALK	Unkown Partner	Fusion	I	Oncogenic
457	ERBB2	p.Leu755Pro	Missense	I	Oncogenic
464	KRAS	p.Gly12Asp	Missense	II	Oncogenic
490	ALK	Unkown Partner	Fusion	I	Oncogenic
503	TP53	p.Pro177Alafs*70	Frameshift	II	Oncogenic
564	KRAS	p.Gly12Val	Missense	II	Oncogenic

566	EGFR	p.Leu858Arg	Missense	I	Oncogenic
590	KRAS	p.Gly12Cys	Missense	I	Oncogenic
594	TP53	p.His168Arg	Missense	II	Likely Oncogenic
596	KRAS	p.Gly12Asp	Missense	II	Oncogenic
	PIK3CA	p.Glu545Lys	Missense	II	Oncogenic
600	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Cys277Phe	Missense	II	Likely Oncogenic
602	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Ala161Asp	Missense	II	Likely Oncogenic
606	TP53	p.Arg175His	Missense	II	Oncogenic
	TP53	p.Gly105Cys	Missense	II	Likely Oncogenic
608	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Gln100*	Frameshift	II	Oncogenic
	PIK3CA	p.Glu545Lys	Missense	II	Oncogenic
	TP53	p.Glu294*	Nonsense	II	Oncogenic
610	EGFR	p.Ser720Phe	Missense	I	Oncogenic
	EGFR	p.Arg803Gln	Missense	I	Oncogenic
	ERBB2	p.Arg784His	Missense	I	Likely Oncogenic
	TP53	p.Val173Leu	Missense	II	Oncogenic
	TP53	p.Arg181His	Missense	II	Likely Oncogenic
611	EGFR	p.Arg776Cys	Missense	I	Oncogenic
	TP53	p.Gln104*	Nonsense	II	Oncogenic
612	TP53	p.Cys277Tyr	Missense	II	Likely Oncogenic
	TP53	p.Glu298*	Nonsense	II	Oncogenic
613	TP53	p.Gly262Val	Missense	II	Likely Oncogenic
	TP53	p.Pro190Leu	Missense	II	Likely Oncogenic
618	TP53	p.Cys242Tyr	Missense	II	Oncogenic
621	TP53	p.Arg248Gln	Missense	II	Oncogenic
	TP53	p.Cys238*	Nonsense	II	Oncogenic
	TP53	p.Pro191Leu	Missense	III	Likely Oncogenic
623	TP53	p.Thr118Glnfs*5	Frameshift	II	Oncogenic
	PIK3CA	p.Pro539Arg	Missense	II	Likely Oncogenic
632	PIK3CA	p.Glu542Lys	Missense	II	Oncogenic
633	ERBB2	p.Arg784Cys	Missense	I	Likely Oncogenic
	TP53	p.Ser215Asn	Missense	II	Oncogenic
	PIK3CA	p.Glu545Lys	Missense	II	Oncogenic
	PIK3CA	p.Glu542Lys	Missense	II	Oncogenic
	TP53	p.?	Splice_region	II	Oncogenic
634	PIK3CA	p.Glu542Lys	Missense	II	Oncogenic
	TP53	p.Lys319*	Nonsense	II	Likely Oncogenic
636	KRAS	p.Gly12Val	Missense	II	Oncogenic
	TP53	p.Tyr205Cys	Missense	II	Oncogenic
650	KRAS	p.Gly12Ala	Missense	II	Oncogenic
	TP53	p.Tyr126*	Nonsense	II	Oncogenic
651	KRAS	p.Gly12Phe	Missense	II	Oncogenic
	TP53	p.Arg337His	Missense	II	Oncogenic
	TP53	p.Lys351*	Nonsense	II	Oncogenic
653	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Gly105Cys	Missense	II	Likely Oncogenic
	TP53	p.Pro190Leu	Missense	II	Likely Oncogenic
655	KRAS	p.Gly12Cys	Missense	I	Oncogenic
658	ALK	Unkown Partner	Fusion	I	Oncogenic
659	ALK	Unkown Partner	Fusion	I	Oncogenic
660	TP53	p.Tyr205Phe	Missense	II	Likely Oncogenic
661	TP53	p.Cys135Trp	Missense	II	Likely Oncogenic
662	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Gly154Val	Missense	II	Oncogenic
664	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Glu298*	Nonsense	II	Oncogenic
665	KRAS	p.Gly12Val	Missense	II	Oncogenic
	BRAF	p.Asn581Ser	Missense	II	Likely Oncogenic
667	TP53	p.Arg175His	Missense	II	Oncogenic
	EGFR	Amplification	CNV	II	Oncogenic

668	TP53	p.Arg280*	Nonsense	II	Oncogenic
669	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	PIK3CA	p.Glu542Lys	Missense	II	Oncogenic
	TP53	p.Gly105Cys	Missense	II	Likely Oncogenic
670	TP53	p.Arg249Met	Missense	II	Oncogenic
	TP53	p.Arg273His	Missense	II	Oncogenic
671	EGFR	p.Ala767_Val769dup	ProteinIns	I	Oncogenic
	TP53	p.Val272Met	Missense	II	Oncogenic
675	KRAS	p.Gly12Ala	Missense	II	Oncogenic
	PIK3CA	p.His1047Arg	Missense	II	Likely Oncogenic
676	TP53	p.Arg273Leu	Missense	II	Oncogenic
678	TP53	p.Val157Phe	Missense	II	Oncogenic
679	RET	Unkown Partner	Fusion	I	Oncogenic
	MET	Amplification	CNV	II	Oncogenic
680	TP53	p.Gln192*	Nonsense	II	Oncogenic
681	PIK3CA	p.Glu542Lys	Missense	II	Oncogenic
	TP53	p.Arg248Gln	Missense	II	Oncogenic
	MET	Amplification	CNV	II	Oncogenic
684	TP53	p.Gly245Cys	Missense	II	Oncogenic
688	TP53	p.Cys238Leufs*9	Frameshift	II	Oncogenic
689	TP53	p.Gly154Val	Missense	II	Oncogenic
	TP53	p.Val272Leu	Missense	II	Likely Oncogenic
	KRAS	p.Gln61Leu	Missense	II	Oncogenic
690	TP53	p.Tyr220Cys	Missense	II	Oncogenic
	ALK	Unkown Partner	Fusion	I	Oncogenic
692	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
693	TP53	p.Arg337Gly	Missense	II	Likely Oncogenic
696	TP53	p.Val272Met	Missense	II	Oncogenic
698	TP53	p.Tyr220Alafs*2	Frameshift	II	Oncogenic
702	TP53	p.?	Splice_region	II	Oncogenic
704	TP53	p.Arg175His	Missense	II	Oncogenic
709	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Arg158Leu	Missense	II	Oncogenic
710	TP53	p.Cys141Trp	Missense	II	Oncogenic
712	TP53	p.Gly245Ser	Missense	II	Oncogenic
	EGFR	Amplification	CNV	II	Oncogenic
713	TP53	p.Pro301Glnfs*44	Frameshift	II	Oncogenic
715	TP53	p.Val274Phe	Missense	II	Oncogenic
775	TP53	p.Glu346*	Nonsense	II	Oncogenic
789	EGFR	p.Glu746_Thr751delinsAla	ProteinDel	I	Oncogenic
836	TP53	p.Gly266*	Nonsense	II	Oncogenic
838	TP53	p.Arg248Leu	Missense	II	Oncogenic
	ALK	Unkown Partner	Fusion	I	Oncogenic
840	TP53	p.Gly244Cys	Missense	II	Oncogenic
	TP53	p.Glu258Lys	Missense	II	Likely Oncogenic
842	TP53	p.Tyr220Cys	Missense	II	Oncogenic
916	TP53	p.His193Arg	Missense	II	Oncogenic
941	TP53	p.Glu180*	Nonsense	II	Oncogenic
958	KRAS	p.Gly12Val	Missense	II	Oncogenic
	TP53	p.Ile162Phe	Missense	II	Likely Oncogenic
961	EGFR	p.Leu858Arg	Missense	I	Oncogenic
968	TP53	p.Met246_Leu252delinsIleArg	ProteinDel	III	Likely Oncogenic
971	ALK	EML4:ALK	Fusion	I	Oncogenic
1128	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Arg110Leu	Missense	II	Likely Oncogenic
1153	TP53	p.Pro151His	Missense	II	Oncogenic
1262	ERBB2	p.Leu726Phe	Missense	I	Likely Oncogenic
	TP53	p.Ala138Val	Missense	II	Likely Oncogenic
1501	ALK	EML4:ALK	Fusion	I	Oncogenic
1572	EGFR	p.Asn756Lys	Missense	I	Oncogenic
1580	TP53	p.Arg248Gln	Missense	II	Oncogenic
1587	KRAS	p.Gly12Val	Missense	II	Oncogenic
	TP53	p.Gly245Val	Missense	II	Oncogenic

1596	PIK3CA	p.Glu542Lys	Missense	II	Oncogenic
1660	EGFR	p.Leu747_Pro753delinsSer	ProteinDel	I	Oncogenic
1668	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Arg337His	Missense	II	Oncogenic
1682	KRAS	p.Gln61His	Missense	II	Oncogenic
	TP53	p.Glu298*	Nonsense	II	Oncogenic
1698	KRAS	p.Gly12Phe	Missense	II	Oncogenic
	TP53	p.Arg337Leu	Missense	II	Oncogenic
1727	TP53	p.Glu346*	Nonsense	II	Oncogenic
1773	EGFR	p.Glu746_Alal750del	ProteinDel	I	Oncogenic
	TP53	p.Asn345Metfs*25	Frameshift	II	Oncogenic
1774	EGFR	p.Lys714Asn	Missense	I	Oncogenic
	TP53	p.Gly334Val	Missense	II	Likely Oncogenic
1782	EGFR	p.Gly779Phe	Missense	I	Oncogenic
	TP53	p.Cys135Phe	Missense	II	Oncogenic
1784	EGFR	p.Ala767_Val769dup	ProteinIns	I	Oncogenic
1785	KRAS	p.Gly12Cys	Missense	II	Oncogenic
	PIK3CA	p.Glu545Lys	Missense	II	Oncogenic
1786	KRAS	p.Gly12Cys	Missense	II	Oncogenic
1787	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Arg175Leu	Missense	II	Likely Oncogenic
1788	TP53	p.?	Splice_region	II	Oncogenic
1789	TP53	p.Arg248Gly	Missense	II	Oncogenic
1790	EGFR	p.Glu746_Alal750del	ProteinDel	I	Oncogenic
1791	KRAS	p.Gly12Ala	Missense	I	Oncogenic
1795	TP53	p.Arg337His	Missense	II	Oncogenic
	TP53	p.Cys176Tyr	Missense	II	Oncogenic
1796	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Ser127Phe	Missense	II	Oncogenic
1798	KRAS	p.Gly12Cys	Missense	I	Oncogenic
1800	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Leu145Pro	Missense	II	Likely Oncogenic
1801	TP53	p.Glu298*	Nonsense	II	Oncogenic
1802	KRAS	p.Gly12Asp	Missense	II	Oncogenic
1803	EGFR	p.Ser768_Val769delinslleLeu	Missense	I	Oncogenic
	TP53	p.Val216Leu	Missense	II	Likely Oncogenic
1804	TP53	p.Met237Lys	Missense	II	Likely Oncogenic
1805	TP53	p.Glu339*	Nonsense	II	Oncogenic
	TP53	p.?	Splice_region	II	Oncogenic
1806	TP53	p.Asn310*	Nonsense	II	Oncogenic
1810	TP53	p.Tyr220Cys	Missense	II	Oncogenic
1812	ERBB2	p.Tyr772_Alal775dup	ProteinIns	I	Oncogenic
1815	TP53	p.Val272Met	Missense	II	Oncogenic
1817	KRAS	p.Gly12Asp	Missense	II	Oncogenic
1848	TP53	p.Arg248Gln	Missense	II	Oncogenic
1855	TP53	p.Arg110Leu	Missense	II	Likely Oncogenic
1864	TP53	p.Gln167*	Nonsense	II	Oncogenic
1865	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Glu286*	Nonsense	II	Oncogenic
1866	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Arg337His	Missense	II	Oncogenic
1867	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Pro177_Cys182del	ProteinDel	II	Likely Oncogenic
1868	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Asp259Tyr	Missense	II	Likely Oncogenic
1869	EGFR	p.Glu746_Thr751delinsAla	ProteinDel	I	Oncogenic
	TP53	p.His193Arg	Missense	II	Oncogenic
1871	ALK	Unkown Partner	Fusion	I	Oncogenic
1872	TP53	p.Val274Asp	Missense	II	Likely Oncogenic
	TP53	p.Met237Ile	Missense	II	Oncogenic
1873	KRAS	p.Gly12Val	Missense	II	Oncogenic
	TP53	p.Arg248Leu	Missense	II	Oncogenic
1874	EGFR	p.Pro848Leu	Missense	I	Oncogenic

1875	KRAS	p.Gly12Asp	Missense	II	Oncogenic
	PIK3CA	p.Glu545Lys	Missense	II	Oncogenic
	TP53	p.Arg282Trp	Missense	II	Oncogenic
1876	KRAS	p.Gly12Ala	Missense	II	Oncogenic
	TP53	p.Val157Phe	Missense	II	Oncogenic
1878	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
1880	ALK	Unkown Partner	Fusion	I	Oncogenic
1881	TP53	p.Arg273His	Missense	II	Oncogenic
1882	TP53	p.Gly199*	Nonsense	II	Oncogenic
1887	KRAS	p.Gly12Cys	Missense	I	Oncogenic
1888	KRAS	p.Gly12Cys	Missense	I	Oncogenic
1891	KRAS	p.Gly12Asp	Missense	II	Oncogenic
	TP53	p.Cys238Phe	Missense	II	Oncogenic
1892	EGFR	p.Asn771delinsGlyPhe	ProteinIns	I	Oncogenic
1893	TP53	p.Cys176Phe	Missense	II	Oncogenic
1894	BRAF	p.Val600Glu	Missense	I	Oncogenic
1895	TP53	p.Thr140Profs*5	Frameshift	II	Oncogenic
	ALK	Unkown Partner	Fusion	I	Oncogenic
1896	TP53	p.Arg273Leu	Missense	II	Oncogenic
1897	EGFR	p.Glu758Lys	Missense	I	Oncogenic
	TP53	p.Gln167*	Nonsense	II	Oncogenic
1898	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Leu111Gln	Missense	II	Likely Oncogenic
1899	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Tyr220Cys	Missense	II	Oncogenic
1900	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Arg273His	Missense	II	Oncogenic
	EGFR	Amplification	CNV	II	Oncogenic
1901	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Asn288Argfs*13	Frameshift	II	Oncogenic
1902	TP53	p.Ala276Asp	Missense	II	Likely Oncogenic
1903	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.His214Arg	Missense	II	Oncogenic
1904	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Ser127Phe	Missense	II	Oncogenic
1905	TP53	p.Glu180*	Nonsense	II	Oncogenic
1906	KRAS	p.Gly12Asp	Missense	II	Oncogenic
	TP53	p.Gln331*	Nonsense	II	Oncogenic
1907	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Arg273Cys	Missense	II	Oncogenic
1908	TP53	p.Arg280*	Nonsense	II	Oncogenic
1909	EGFR	p.Leu858Arg	Missense	I	Oncogenic
1911	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Arg209*	Nonsense	II	Oncogenic
1912	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Thr118Glnfs*5	Frameshift	II	Oncogenic
1914	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	PIK3CA	p.Glu545Lys	Missense	II	Oncogenic
	TP53	p.Arg280Thr	Missense	II	Oncogenic
1917	KRAS	p.Asp57Asn	Missense	II	Likely Oncogenic
1921	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Arg248Trp	Missense	II	Oncogenic
1933	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
1934	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Arg249Ser	Missense	II	Oncogenic
1935	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Arg273Cys	Missense	II	Oncogenic
1936	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
1937	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Gly245Val	Missense	II	Oncogenic
1938	EGFR	p.Leu747_Thr751del	ProteinDel	I	Oncogenic
	TP53	p.?	Splice_region	II	Oncogenic
1939	PIK3CA	p.Glu542Lys	Missense	II	Oncogenic

	TP53	p.Arg158Leu	Missense	II	Oncogenic
1941	KRAS	p.Gln61His	Missense	II	Oncogenic
1942	TP53	p.Ser241Phe	Missense	II	Oncogenic
	ALK	Unkown Partner	Fusion	I	Oncogenic
1945	ALK	Unkown Partner	Fusion	I	Oncogenic
1947	ERBB2	p.Tyr772_Ala775dup	ProteinIns	I	Oncogenic
	TP53	p.?	Splice_region	II	Oncogenic
1948	TP53	p.Arg273Leu	Missense	II	Oncogenic
1949	TP53	p.?	Splice_region	II	Oncogenic
1950	EGFR	p.Glu746_Thr751delinsIlePro	ProteinDel	I	Oncogenic
	TP53	p.Val216Leu	Missense	II	Likely Oncogenic
	KIT	p.Arg634Trp	Missense	III	Likely Oncogenic
1951	TP53	p.Arg249Gly	Missense	II	Likely Oncogenic
1952	TP53	p.Arg248Pro	Missense	II	Likely Oncogenic
1953	TP53	p.Thr125Lys	Missense	II	Likely Oncogenic
1956	KRAS	p.Gln61His	Missense	II	Oncogenic
1959	KRAS	p.Gln61His	Missense	II	Oncogenic
	TP53	p.Arg273His	Missense	II	Oncogenic
	EGFR	Amplification	CNV	II	Oncogenic
	TP53	p.Ala159Pro	Missense	II	Oncogenic
1968	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Arg337His	Missense	II	Oncogenic
1969	TP53	p.Pro278Ser	Missense	II	Oncogenic
	TP53	p.Asp259Tyr	Missense	II	Likely Oncogenic
1975	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.?	Splice_region	II	Oncogenic
1980	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	PIK3CA	p.Glu545Lys	Missense	II	Oncogenic
	TP53	p.?	Splice_region	II	Oncogenic
1983	EGFR	p.Leu858Arg	Missense	I	Oncogenic
1986	TP53	p.Pro177Leu	Missense	II	Likely Oncogenic
	TP53	p.Arg110Leu	Missense	II	Likely Oncogenic
	TP53	p.Ser127Phe	Missense	II	Oncogenic
1990	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Arg337His	Missense	II	Oncogenic
1991	EGFR	p.Asp770_Asn771insSerValGlu	ProteinIns	I	Oncogenic
1992	TP53	p.Pro87Glnfs*36	Frameshift	II	Likely Oncogenic
1994	TP53	p.Gln104*	Nonsense	II	Oncogenic
	MET	METΔex14	Exon Skipping	I	Oncogenic
1995	ALK	Unkown Partner	Fusion	I	Oncogenic
1996	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Arg249Met	Missense	II	Oncogenic
1997	KRAS	p.Gly12Asp	Missense	II	Oncogenic
2000	TP53	p.Asn268Ile	Missense	II	Likely Oncogenic
2006	TP53	p.Ser241Phe	Missense	II	Oncogenic
2013	PIK3CA	p.His1047Arg	Missense	II	Likely Oncogenic
	TP53	p.Gln331*	Nonsense	II	Oncogenic
2014	TP53	p.Ala347Asp	Missense	II	Likely Oncogenic
2015	KRAS	p.Gly12Phe	Missense	II	Oncogenic
2017	KRAS	p.Gly12Cys	Missense	I	Oncogenic
2018	EGFR	p.Ala767_Val769dup	ProteinIns	I	Oncogenic
	TP53	p.Leu130Val	Missense	II	Likely Oncogenic
2019	MET	METΔex14	Exon Skipping	I	Oncogenic
2020	KRAS	p.Gly13Asp	Missense	II	Oncogenic
2021	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Val73Trpfs*50	Frameshift	II	Oncogenic
2022	TP53	p.Arg213*	Nonsense	II	Oncogenic
2023	TP53	p.Cys277Phe	Missense	II	Likely Oncogenic
2024	TP53	p.Arg158Gly	Missense	II	Oncogenic
2026	KRAS	p.Gly12Asp	Missense	II	Oncogenic
2027	TP53	p.Gln136*	Nonsense	II	Oncogenic
2029	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
2031	EGFR	p.Leu858Arg	Missense	I	Oncogenic

	TP53	p.Gly245Cys	Missense	II	Oncogenic
2032	TP53	p.Arg337His	Missense	II	Oncogenic
2034	EGFR	p.Leu858Arg	Missense	I	Oncogenic
2036	TP53	p.Met246Val	Missense	II	Likely Oncogenic
	ALK	EML4:ALK	Fusion	I	Oncogenic
2037	TP53	p.Tyr163Cys	Missense	II	Oncogenic
2038	TP53	p.Arg337Cys	Missense	II	Oncogenic
	ALK	Unkown Partner	Fusion	I	Oncogenic
2041	ERBB2	p.Gly776delinsValCys	ProteinIns	I	Likely Oncogenic
2044	TP53	p.Val157Phe	Missense	II	Oncogenic
2045	NRAS	p.Gln61Leu	Missense	II	Likely Oncogenic
	TP53	p.Arg337His	Missense	II	Oncogenic
	TP53	p.His179Arg	Missense	II	Oncogenic
2050	ALK	Unkown Partner	Fusion	I	Oncogenic
2053	TP53	p.Gly245Val	Missense	II	Oncogenic
2054	TP53	p.Cys242Phe	Missense	II	Oncogenic
2055	TP53	p.Arg248Pro	Missense	II	Likely Oncogenic
	MET	Amplification	CNV	II	Oncogenic
2056	ERBB2	p.Tyr742_Ala745dup	ProteinIns	I	Likely Oncogenic
2057	TP53	p.Met237Ile	Missense	II	Oncogenic
	MET	Amplification	CNV	II	Oncogenic
2059	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.His214Arg	Missense	II	Oncogenic
2060	TP53	p.Cys141Trp	Missense	II	Oncogenic
2062	TP53	p.Cys141Trp	Missense	II	Oncogenic
2064	KRAS	p.Gly13Cys	Missense	II	Oncogenic
2065	TP53	p.Ser90Profs*33	Frameshift	II	Oncogenic
2067	ERBB2	p.Tyr772_Ala775dup	ProteinIns	I	Oncogenic
	TP53	p.Met237Ile	Missense	II	Oncogenic
2068	NTRK3	EML4:NTRK3	Fusion	I	Oncogenic
2070	TP53	p.Val274Phe	Missense	II	Oncogenic
2072	TP53	p.Gly245Val	Missense	II	Oncogenic
	PIK3CA	p.Gly1049Arg	Missense	II	Likely Oncogenic
2075	KRAS	p.Gly12Val	Missense	II	Oncogenic
2079	ALK	Unkown Partner	Fusion	I	Oncogenic
2080	KRAS	p.Gly13Asp	Missense	II	Oncogenic
	TP53	p.Cys242Phe	Missense	II	Oncogenic
2081	KRAS	p.Gly12Ala	Missense	II	Oncogenic
	TP53	p.Glu224*	Nonsense	II	Oncogenic
2082	KRAS	p.Gly12Asp	Missense	II	Oncogenic
2083	TP53	p.Asp208Val	Missense	II	Likely Oncogenic
2084	KRAS	p.Gly12Cys	Missense	I	Oncogenic
2086	KRAS	p.Gly12Asp	Missense	II	Oncogenic
	TP53	p.Arg337His	Missense	II	Oncogenic
2087	EGFR	p.Leu747_Pro753delinsSer	ProteinDel	I	Oncogenic
	PIK3CA	p.His1047Arg	Missense	II	Likely Oncogenic
2088	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
2089	TP53	p.Ser15Phefs*25	Frameshift	II	Oncogenic
	KRAS	p.Gly12Asp	Missense	II	Oncogenic
2090	BRAF	p.Asp594Gly	Missense	I	Oncogenic
	TP53	p.Pro301Glnfs*44	Frameshift	II	Oncogenic
2091	KRAS	p.Gly12Val	Missense	II	Oncogenic
2092	ALK	Unkown Partner	Fusion	I	Oncogenic
2093	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Ser90Profs*33	Frameshift	II	Oncogenic
2094	TP53	p.?	Splice_region	II	Oncogenic
2096	TP53	p.His168Leu	Missense	II	Likely Oncogenic
2097	EGFR	p.Ser768_Asp770dup	ProteinIns	I	Oncogenic
	TP53	p.Gly105Val	Missense	II	Oncogenic
2098	EGFR	p.Glu746_Ser752delinsVal	ProteinDel	I	Oncogenic
2100	TP53	p.Asp352Metfs*18	Frameshift	II	Oncogenic
	KRAS	p.Gly13Cys	Missense	II	Oncogenic
2101	KRAS	p.Gly12Cys	Missense	I	Oncogenic

	TP53	p.Glu204*	Nonsense	II	Oncogenic
2102	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
2103	KRAS	p.Gly12Tyr	Missense	II	Oncogenic
	TP53	p.Arg273Leu	Missense	II	Oncogenic
	TP53	p.Glu68*	Nonsense	II	Oncogenic
2104	KRAS	p.Gly12Val	Missense	II	Oncogenic
2105	ERBB2	p.Val777Leu	Missense	I	Oncogenic
2106	BRAF	p.Val600Lys	Missense	I	Likely Oncogenic
	TP53	p.Pro177Leu	Missense	II	Likely Oncogenic
2107	KRAS	p.Gly12Ala	Missense	II	Oncogenic
	TP53	p.Arg248Leu	Missense	II	Oncogenic
2109	EGFR	p.Glu746_Ser752delinsVal	ProteinDel	I	Oncogenic
2110	EGFR	p.Leu747_Thr751del	ProteinDel	I	Oncogenic
2111	KRAS	p.Gly12Val	Missense	II	Oncogenic
	TP53	p.Arg248Leu	Missense	II	Oncogenic
2113	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Cys176Tyr	Missense	II	Oncogenic
2114	KRAS	p.Gly12Cys	Missense	I	Oncogenic
2115	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
2116	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Cys176Arg	Missense	II	Likely Oncogenic
2117	ALK	Unkown Partner	Fusion	I	Oncogenic
2118	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Cys242Tyr	Missense	II	Oncogenic
2119	KRAS	p.Gly12Asp	Missense	II	Oncogenic
2120	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Arg175His	Missense	II	Oncogenic
2121	KRAS	p.Gly12Val	Missense	II	Oncogenic
	TP53	p.Arg337His	Missense	II	Oncogenic
2122	KRAS	p.Gly12Cys	Missense	I	Oncogenic
2123	EGFR	p.Leu747_Pro753delinsSer	ProteinDel	I	Oncogenic
	TP53	p.Arg248Trp	Missense	II	Oncogenic
2124	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Arg273Leu	Missense	II	Oncogenic
2125	EGFR	p.Glu746_Thr751delinsAla	ProteinDel	I	Oncogenic
	TP53	p.Arg337His	Missense	II	Oncogenic
2128	KRAS	p.Gly12Val	Missense	II	Oncogenic
	TP53	p.Cys277Phe	Missense	II	Likely Oncogenic
2130	ERBB2	p.Val659Glu	Missense	I	Likely Oncogenic
2132	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	EGFR	p.Thr790Met	Missense	I	Oncogenic
2133	EGFR	p.Ser768Alafs*50	Frameshift	I	Oncogenic
	TP53	p.Leu188Glnfs*59	Frameshift	II	Oncogenic
2134	TP53	p.Leu35Cysfs*9	Frameshift	II	Oncogenic
	KRAS	p.Gly12Asp	Missense	II	Oncogenic
2136	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Arg337His	Missense	II	Oncogenic
2137	EGFR	p.Leu858Arg	Missense	I	Oncogenic
2139	TP53	p.Ser90Profs*33	Frameshift	II	Oncogenic
2140	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Gly154Val	Missense	II	Oncogenic
2144	ALK	Unkown Partner	Fusion	I	Oncogenic
2145	ALK	Unkown Partner	Fusion	I	Oncogenic
2146	KRAS	p.Gly12Ser	Missense	II	Oncogenic
	TP53	p.Glu294*	Nonsense	II	Oncogenic
2147	KRAS	p.Gly12Cys	Missense	I	Oncogenic
2148	BRAF	p.Val600Glu	Missense	I	Oncogenic
2149	BRAF	p.Val600Glu	Missense	I	Oncogenic
	TP53	p.Arg273His	Missense	II	Oncogenic
	TP53	p.Ser241Thr	Missense	II	Likely Oncogenic
	TP53	p.Arg249Ser	Missense	II	Oncogenic
2151	KRAS	p.Gly12Cys	Missense	I	Oncogenic
2153	EGFR	p.Ala767_Val769dup	ProteinIns	I	Oncogenic

2154	TP53	p.Glu298*	Nonsense	II	Oncogenic
2155	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Glu271Val	Missense	II	Likely Oncogenic
2156	BRAF	p.Val600Glu	Missense	I	Oncogenic
	PIK3CA	p.Glu545Lys	Missense	II	Oncogenic
	TP53	p.Glu285Lys	Missense	II	Oncogenic
2157	EGFR	p.Asn771_His773dup	ProteinIns	I	Oncogenic
2158	KRAS	p.Gly12Asp	Missense	II	Oncogenic
2159	ALK	Unkown Partner	Fusion	I	Oncogenic
2160	NRAS	p.Gly12Cys	Missense	II	Likely Oncogenic
	TP53	p.Val157_Arg158del	ProteinDel	II	Likely Oncogenic
2162	MET	METΔex14	Exon Skipping	I	Oncogenic
2164	TP53	p.Tyr220Cys	Missense	II	Oncogenic
2165	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Cys176Tyr	Missense	II	Oncogenic
2166	KRAS	p.Gly12Cys	Missense	I	Oncogenic
2167	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Arg273Leu	Missense	II	Oncogenic
2168	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
2170	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	EGFR	p.Arg776His	Missense	I	Oncogenic
2171	BRAF	p.Val600Glu	Missense	I	Oncogenic
	EGFR	p.Glu866Asp	Missense	I	Oncogenic
	TP53	p.Arg273Cys	Missense	II	Oncogenic
2172	EGFR	p.Leu861Gln	Missense	I	Oncogenic
	TP53	p.Arg249Glyfs*96	Frameshift	II	Oncogenic
2174	ERBB2	p.Gly776delinsLeuCys	ProteinIns	I	Likely Oncogenic
	TP53	p.Arg175His	Missense	II	Oncogenic
2175	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Cys275Phe	Missense	II	Likely Oncogenic
	TP53	p.Arg282Gln	Missense	II	Likely Oncogenic
2178	TP53	p.Pro85Leufs*38	Frameshift	II	Oncogenic
2179	TP53	p.Arg158Leu	Missense	II	Oncogenic
2181	TP53	p.Arg282Trp	Missense	II	Oncogenic
2182	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	EGFR	p.Ser768Ile	Missense	I	Oncogenic
2183	TP53	p.?	Splice_region	II	Oncogenic
2184	TP53	p.Gly245Cys	Missense	II	Oncogenic
2186	TP53	p.Ser127Tyr	Missense	II	Likely Oncogenic
2188	KRAS	p.Gly12Val	Missense	II	Oncogenic
2189	EGFR	p.Glu746_Ser752delinsVal	ProteinDel	I	Oncogenic
	TP53	p.Gln104*	Nonsense	II	Oncogenic
2190	PIK3CA	p.Glu545Lys	Missense	II	Oncogenic
2191	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Met246Val	Missense	II	Likely Oncogenic
2192	KRAS	p.Gly12Cys	Missense	I	Oncogenic
2193	EGFR	p.His773delinsSerAsnProTyr	ProteinIns	I	Oncogenic
	TP53	p.Arg273Leu	Missense	II	Oncogenic
2194	TP53	p.Arg248Leu	Missense	II	Oncogenic
2195	KRAS	p.Gly12Cys	Missense	I	Oncogenic
2196	TP53	p.Met237Ile	Missense	II	Oncogenic
	ROS1	Unkown Partner	Fusion	I	Oncogenic
2197	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Arg248Leu	Missense	II	Likely Oncogenic
2198	ERBB2	p.Tyr772_Ala775dup	ProteinIns	I	Oncogenic
2199	ERBB2	p.Tyr772_Ala775dup	ProteinIns	I	Oncogenic
	TP53	p.Arg280Lys	Missense	II	Oncogenic
2200	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.His214Arg	Missense	II	Oncogenic
2202	KRAS	p.Gly12Ser	Missense	II	Oncogenic
	TP53	p.Glu271Lys	Missense	II	Likely Oncogenic
2203	TP53	p.Arg337His	Missense	II	Oncogenic
	TP53	p.Glu198*	Nonsense	II	Oncogenic

2204	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.His178Serfs*69	Frameshift	II	Oncogenic
2205	BRAF	p.Val600Glu	Missense	I	Oncogenic
2206	EGFR	p.Glu709Lys	Missense	I	Oncogenic
	EGFR	p.Gly719Ser	Missense	I	Oncogenic
2207	EGFR	p.?	Splice_region	I	Oncogenic
	KRAS	p.Gly12Asp	Missense	II	Oncogenic
2209	BRAF	p.Val600Glu	Missense	I	Oncogenic
	NRAS	p.Gly12Asp	Missense	II	Oncogenic
2212	EGFR	p.Leu747_Thr751del	ProteinDel	I	Oncogenic
	TP53	p.?	Splice_region	II	Oncogenic
2213	KRAS	p.Gly12Val	Missense	II	Oncogenic
2214	TP53	p.Val122Aspfs*26	Frameshift	II	Oncogenic
2217	TP53	p.Arg273Leu	Missense	II	Oncogenic
2219	KRAS	p.Gly12Ile	Missense	II	Oncogenic
	TP53	p.Arg273Cys	Missense	II	Oncogenic
2220	TP53	p.Pro301Glnfs*44	Frameshift	II	Oncogenic
2221	TP53	p.Cys242Arg	Missense	II	Likely Oncogenic
2222	KRAS	p.Gly12Val	Missense	II	Oncogenic
	TP53	p.Val157Phe	Missense	II	Oncogenic
	TP53	p.Arg273Leu	Missense	II	Oncogenic
2224	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Arg158Leu	Missense	II	Oncogenic
2225	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	EGFR	p.His870Arg	Missense	I	Oncogenic
	TP53	p.?	Splice_region	II	Oncogenic
2226	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Gly245Ser	Missense	II	Oncogenic
2227	KRAS	p.Gly12Ser	Missense	II	Oncogenic
	TP53	p.Arg158Leu	Missense	II	Oncogenic
2228	KRAS	p.Gly12Cys	Missense	I	Oncogenic
2230	BRAF	p.Val600Glu	Missense	I	Oncogenic
2231	TP53	p.Asp259Glufs*85	Frameshift	II	Oncogenic
2232	KRAS	p.Gly12Val	Missense	II	Oncogenic
2233	ALK	Unkown Partner	Fusion	I	Oncogenic
2234	TP53	p.?	Splice_region	II	Oncogenic
2235	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	PIK3CA	p.His1047Arg	Missense	II	Likely Oncogenic
2236	KRAS	p.Gly12Val	Missense	II	Oncogenic
	TP53	p.Gly245Cys	Missense	II	Oncogenic
2237	KRAS	p.Gly12Cys	Missense	I	Oncogenic
2240	ALK	Unkown Partner	Fusion	I	Oncogenic
2241	EGFR	p.Leu858Arg	Missense	I	Oncogenic
2242	EGFR	p.Lys754Glu	Missense	I	Oncogenic
	EGFR	p.Leu747_Glu749del	ProteinDel	I	Oncogenic
	TP53	p.Ser215Gly	Missense	II	Likely Oncogenic
2243	TP53	p.Leu35Cysfs*9	Frameshift	II	Oncogenic
	ALK	Unkown Partner	Fusion	I	Oncogenic
2244	KRAS	p.Gly12Cys	Missense	I	Oncogenic
2245	BRAF	p.Val600Glu	Missense	I	Oncogenic
2246	TP53	p.Ser215Ile	Missense	II	Oncogenic
2247	MET	METΔex14	Exon Skipping	I	Oncogenic
2249	KRAS	p.Gly12Asp	Missense	II	Oncogenic
	TP53	p.Arg280Ile	Missense	II	Likely Oncogenic
2252	KRAS	p.Gly13Asp	Missense	II	Oncogenic
	TP53	p.Cys176Ser	Missense	II	Oncogenic
2253	ALK	Unkown Partner	Fusion	I	Oncogenic
2257	BRAF	p.Asp594Gly	Missense	I	Oncogenic
	TP53	p.His179Arg	Missense	II	Oncogenic
2258	EGFR	p.Glu746_Thr751delinsVal	ProteinDel	I	Oncogenic
	TP53	p.Gly245Val	Missense	II	Oncogenic
2259	ALK	Unkown Partner	Fusion	I	Oncogenic
2262	TP53	p.Arg273Leufs*72	Frameshift	II	Oncogenic

2263	KRAS	p.Gly12Asp	Missense	II	Oncogenic
2265	TP53	p.Gly244Val	Missense	II	Likely Oncogenic
2266	TP53	p.Asp281Glu	Missense	II	Oncogenic
2267	EGFR	p.Ser768Ile	Missense	I	Oncogenic
	EGFR	p.Gly719Cys	Missense	I	Oncogenic
	TP53	p.Pro177Arg	Missense	II	Likely Oncogenic
2268	TP53	p.Gly245Asp	Missense	II	Oncogenic
2269	KRAS	p.Gly12Cys	Missense	I	Oncogenic
2270	EGFR	p.Leu858Arg	Missense	I	Oncogenic
2272	TP53	p.Gly154Alafs*16	Frameshift	II	Oncogenic
	KRAS	p.Gly12Ala	Missense	II	Oncogenic
2277	TP53	p.Leu194Arg	Missense	II	Likely Oncogenic
2278	KRAS	p.Gly12Ser	Missense	II	Oncogenic
	TP53	p.Phe109Ile	Missense	II	Likely Oncogenic
2279	TP53	p.Gly266Val	Missense	II	Likely Oncogenic
2281	TP53	p.His179Tyr	Missense	II	Oncogenic
2282	KRAS	p.Gly12Val	Missense	II	Oncogenic
	TP53	p.His179Arg	Missense	II	Oncogenic
2309	EGFR	p.Lys754Glu	Missense	I	Oncogenic
	EGFR	p.Leu747_Thr751del	ProteinDel	I	Oncogenic
2311	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Arg273His	Missense	II	Oncogenic
2313	KRAS	p.Gly12Cys	Missense	I	Oncogenic
2314	KRAS	p.Gly12Asp	Missense	II	Oncogenic
2315	TP53	p.Ser127Phe	Missense	II	Oncogenic
2323	KRAS	p.Gly12Cys	Missense	I	Oncogenic
2330	TP53	p.Glu298*	Nonsense	II	Oncogenic
2331	TP53	p.Arg158Leu	Missense	II	Oncogenic
2333	TP53	p.Cys242Phe	Missense	II	Oncogenic
2334	KRAS	p.Gly13Cys	Missense	II	Oncogenic
	TP53	p.Arg249Ser	Missense	II	Oncogenic
	TP53	p.Val274Ala	Missense	II	Likely Oncogenic
2335	KRAS	p.Gly13Cys	Missense	II	Oncogenic
2336	KRAS	p.Gly12Cys	Missense	I	Oncogenic
2337	KRAS	p.Gly12Asp	Missense	II	Oncogenic
2340	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Arg337His	Missense	II	Oncogenic
	EGFR	Amplification	CNV	II	Oncogenic
2342	KRAS	p.Gly12Val	Missense	II	Oncogenic
2343	TP53	p.Ala69GluFs*54	Frameshift	II	Oncogenic
2344	EGFR	p.Leu858Arg	Missense	I	Oncogenic
2345	ROS1	Unkown Partner	Fusion	I	Oncogenic
2346	EGFR	p.Leu858Arg	Missense	I	Oncogenic
2348	EGFR	p.Leu747_Ala750delinsPro	ProteinDel	I	Oncogenic
2349	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Ile251Phe	Missense	II	Likely Oncogenic
2350	KRAS	p.Gly12Val	Missense	II	Oncogenic
2351	KRAS	p.Gly12Ala	Missense	II	Oncogenic
2355	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.His179Gln	Missense	II	Oncogenic
2356	KRAS	p.Gly12Cys	Missense	I	Oncogenic
2358	KRAS	p.Gly12Cys	Missense	I	Oncogenic
2359	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
2360	TP53	p.Val157Phe	Missense	II	Oncogenic
2362	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Arg213Gln	Missense	II	Oncogenic
	TP53	p.?	Splice_region	II	Oncogenic
2363	KRAS	p.Gly12Asp	Missense	II	Oncogenic
	TP53	p.Tyr163Cys	Missense	II	Oncogenic
2364	EGFR	p.Leu747Pro	Missense	I	Oncogenic
	KRAS	p.Ala146Glu	Missense	II	Likely Oncogenic
2366	ERBB2	p.Tyr742_Ala745dup	ProteinIns	I	Likely Oncogenic
2367	EGFR	p.Leu858Arg	Missense	I	Oncogenic

	TP53	p.Glu68*	Nonsense	II	Oncogenic
2368	KRAS	p.Gly12Cys	Missense	I	Oncogenic
2369	TP53	p.Met160Asn	Missense	II	Likely Oncogenic
2370	TP53	p.Asp228Glyfs*9	Frameshift	II	Oncogenic
	ALK	Unkown Partner	Fusion	I	Oncogenic
2372	TP53	p.Trp146*	Nonsense	II	Oncogenic
2374	TP53	p.?	Splice_region	II	Oncogenic
2375	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
2376	TP53	p.Asn311Lysfs*26	Frameshift	II	Oncogenic
2379	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	EGFR	Amplification	CNV	II	Oncogenic
2380	TP53	p.Phe328Serfs*17	Frameshift	II	Oncogenic
	KRAS	p.Gly12Val	Missense	II	Oncogenic
2381	EGFR	p.Ser752_Ile759del	ProteinDel	I	Oncogenic
	EGFR	Amplification	CNV	II	Oncogenic
2382	TP53	p.Asn239Ser	Missense	II	Likely Oncogenic
	ROS1	Unkown Partner	Fusion	I	Oncogenic
2383	TP53	p.Arg158Leu	Missense	II	Oncogenic
2385	TP53	p.?	Splice_region	II	Oncogenic
2386	BRAF	p.Val600Glu	Missense	I	Oncogenic
	TP53	p.Arg248Leu	Missense	II	Oncogenic
2387	TP53	p.Arg196*	Nonsense	II	Oncogenic
	MET	METΔex14	Exon Skipping	I	Oncogenic
2388	ERBB2	Amplification	CNV	II	Oncogenic
2389	MET	METΔex14	Exon Skipping	I	Oncogenic
2390	TP53	p.Val274Phe	Missense	II	Oncogenic
2391	KRAS	p.Gly12Cys	Missense	I	Oncogenic
2392	TP53	p.Ser241Phe	Missense	II	Oncogenic
2393	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Arg337His	Missense	II	Oncogenic
2394	TP53	p.Cys242Tyr	Missense	II	Oncogenic
2395	TP53	p.Gly105Val	Missense	II	Oncogenic
	TP53	p.?	Splice_region	II	Oncogenic
2396	KRAS	p.Gly12Asp	Missense	II	Oncogenic
	TP53	p.Arg306*	Nonsense	II	Oncogenic
2397	TP53	p.Gly154Val	Missense	II	Oncogenic
2398	KRAS	p.Gly13Cys	Missense	II	Oncogenic
2399	TP53	p.Tyr234Cys	Missense	II	Oncogenic
2401	TP53	p.Gly245Arg	Missense	II	Likely Oncogenic
2402	ALK	EML4:ALK	Fusion	I	Oncogenic
2403	TP53	p.Arg273Leu	Missense	II	Oncogenic
2404	TP53	p.?	Splice_region	II	Oncogenic
2405	EGFR	p.Ala767_Val769dup	ProteinIns	I	Oncogenic
	TP53	p.His179Arg	Missense	II	Oncogenic
2406	KRAS	p.Gly12Cys	Missense	I	Oncogenic
2407	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Gln136*	Nonsense	II	Oncogenic
2408	TP53	p.Lys291*	Nonsense	II	Oncogenic
	MET	Amplification	CNV	II	Oncogenic
2409	TP53	p.Gly266Arg	Missense	II	Oncogenic
2411	NRAS	p.Gln61Leu	Missense	II	Likely Oncogenic
2414	EGFR	p.Leu747_Thr751del	ProteinDel	I	Oncogenic
	TP53	p.His214Arg	Missense	II	Oncogenic
	EGFR	Amplification	CNV	II	Oncogenic
2415	KRAS	p.Gly12Phe	Missense	II	Oncogenic
	TP53	p.Arg158Leu	Missense	II	Oncogenic
2416	KRAS	p.Gly12Cys	Missense	I	Oncogenic
2417	BRAF	p.Val600Glu	Missense	I	Oncogenic
2418	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Ala138Thr	Missense	II	Likely Oncogenic
2419	TP53	p.Ala84Profs*39	Frameshift	II	Oncogenic
2420	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Cys135Phe	Missense	II	Oncogenic

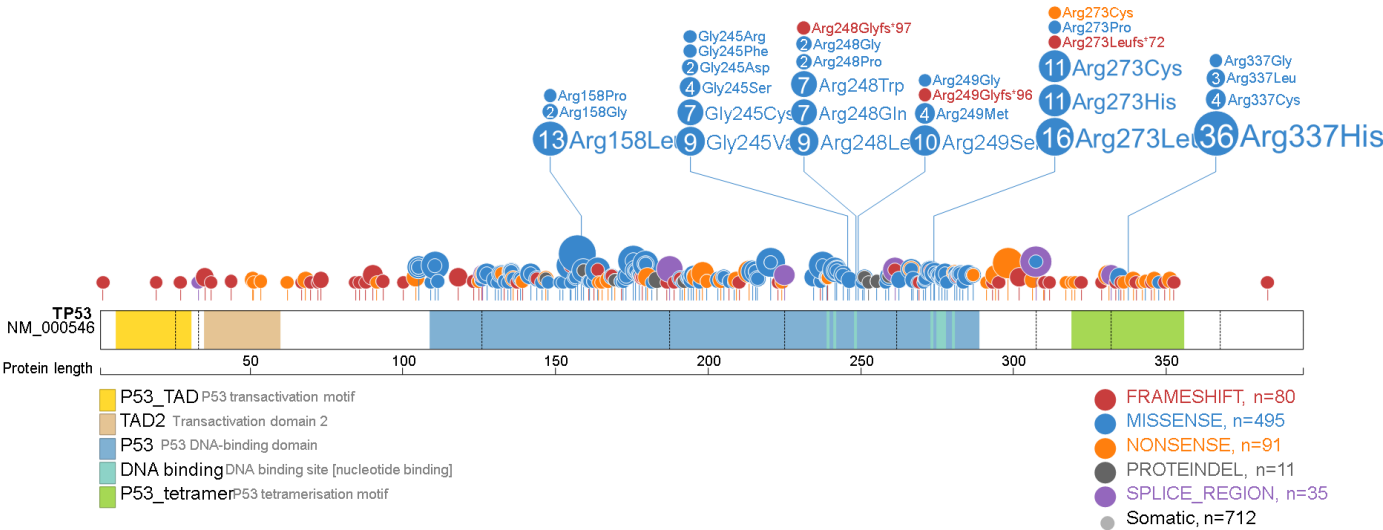
	MET	METΔex14	Exon Skipping	I	Oncogenic
2421	KRAS	p.Gly12Cys	Missense	I	Oncogenic
2423	TP53	p.His178Profs*3	Frameshift	II	Oncogenic
	TP53	p.Tyr220Cys	Missense	II	Oncogenic
	TP53	p.Gly154Val	Missense	II	Oncogenic
2427	KRAS	p.Gly12Ser	Missense	II	Oncogenic
	TP53	p.Leu145del	ProteinDel	II	Likely Oncogenic
2430	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Lys139Argfs*31	Frameshift	II	Likely Oncogenic
2431	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	EGFR	p.Ser811Phe	Missense	I	Oncogenic
	TP53	p.Arg306*	Nonsense	II	Likely Oncogenic
2432	TP53	p.Pro27Thrfs*2	Frameshift	II	Likely Oncogenic
	KRAS	p.Gly12Asp	Missense	II	Oncogenic
2433	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Cys275Phe	Missense	II	Likely Oncogenic
2435	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.His178Thrfs*69	Frameshift	II	Oncogenic
2437	TP53	p.Thr118Glnfs*5	Frameshift	II	Likely Oncogenic
2439	TP53	p.Ile195Phe	Missense	II	Oncogenic
	ERBB2	Amplification	CNV	II	Oncogenic
2440	KRAS	p.Gly13Cys	Missense	II	Oncogenic
2441	TP53	p.?	Splice_region	II	Oncogenic
	MET	Amplification	CNV	II	Oncogenic
2443	TP53	p.His178Profs*3	Frameshift	II	Oncogenic
	TP53	p.Gly154Val	Missense	II	Oncogenic
	chr7	Amplification	CNV	II	Oncogenic
2444	KRAS	p.Gly12Ala	Missense	II	Oncogenic
	TP53	p.Arg342*	Nonsense	II	Likely Oncogenic
2445	EGFR	p.Ser768_Asp770dup	ProteinIns	I	Oncogenic
	TP53	p.Cys141Trp	Missense	II	Oncogenic
2446	ALK	EML4:ALK	Fusion	I	Oncogenic
2447	EGFR	p.Glu746_Ser752delinsVal	ProteinDel	I	Oncogenic
2449	ERBB2	p.Val777Leu	Missense	I	Oncogenic
2451	BRAF	p.Val600Glu	Missense	I	Oncogenic
2452	TP53	p.Trp91*	Nonsense	II	Likely Oncogenic
	ERBB2	Amplification	CNV	II	Oncogenic
2489	ALK	Unkown Partner	Fusion	I	Oncogenic
2583	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
2601	KRAS	p.Gly12Val	Missense	II	Oncogenic
2633	KRAS	p.Gly12Asp	Missense	II	Oncogenic
	TP53	p.Ser215Ile	Missense	II	Oncogenic
2642	KRAS	p.Gly12Val	Missense	II	Oncogenic
2644	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Arg337His	Missense	II	Oncogenic
2648	ERBB2	p.Tyr772_Ala775dup	ProteinIns	I	Oncogenic
	TP53	p.Arg337Cys	Missense	II	Oncogenic
2695	KRAS	p.Gly12Arg	Missense	II	Oncogenic
	TP53	p.Arg273Cys	Missense	II	Oncogenic
2701	TP53	p.Val203Leu	Missense	II	Likely Oncogenic
	ALK	EML4:ALK	Fusion	I	Oncogenic
2744	TP53	p.Met133Thr	Missense	II	Likely Oncogenic
2745	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Arg273Cys	Nonsense	II	Oncogenic
2751	EGFR	p.Leu747_Pro753delinsSer	ProteinDel	I	Oncogenic
2759	BRAF	p.Val600Glu	Missense	I	Oncogenic
2762	MET	METΔex14	Exon Skipping	I	Oncogenic
2763	TP53	p.Thr125Met	Missense	II	Likely Oncogenic
	MET	METΔex14	Exon Skipping	I	Oncogenic
2764	TP53	p.Gly154Val	Missense	II	Oncogenic
2765	ERBB2	p.Leu755Pro	Missense	I	Oncogenic
	TP53	p.Arg267Trp	Missense	II	Likely Oncogenic
2767	TP53	p.Ile255Phe	Missense	II	Likely Oncogenic

2768	KRAS	p.Gly12Cys	Missense	I	Oncogenic
2769	TP53	p.Pro151Argfs*10	Frameshift	II	Likely Oncogenic
2812	TP53	p.Glu286Lys	Missense	II	Oncogenic
2815	KRAS	p.Gly12Cys	Missense	I	Oncogenic
2817	TP53	p.Gly245Val	Missense	II	Oncogenic
2818	KRAS	p.Gly13Val	Missense	II	Oncogenic
2819	TP53	p.?	Splice_region	II	Likely Oncogenic
2820	BRAF	p.Val600Glu	Missense	I	Oncogenic
2821	TP53	p.?	Missense	II	Likely Oncogenic
2822	ERBB2	Amplification	CNV	II	Oncogenic
2827	TP53	p.Cys176Tyr	Missense	II	Oncogenic
2828	KRAS	p.Gly12Asp	Missense	II	Oncogenic
	TP53	p.Arg110Leu	Missense	II	Likely Oncogenic
	TP53	p.Val157Phe	Missense	II	Oncogenic
2829	TP53	p.Leu93Cysfs*30	Frameshift	II	Likely Oncogenic
2830	ALK	EML4:ALK	Fusion	I	Oncogenic
2831	KRAS	p.Gly12Ala	Missense	II	Oncogenic
	TP53	p.Tyr234Cys	Missense	II	Oncogenic
2832	TP53	p.Arg110Leu	Missense	II	Likely Oncogenic
2833	TP53	p.Glu198*	Nonsense	II	Oncogenic
2834	MET	METΔex14	Exon Skipping	I	Oncogenic
2836	TP53	p.Ser215Ile	Missense	II	Oncogenic
2837	TP53	p.Cys141Tyr	Missense	II	Oncogenic
2839	TP53	p.Tyr220His	Missense	II	Likely Oncogenic
2840	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
2841	ALK	EML4:ALK	Fusion	I	Oncogenic
2844	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Glu343*	Missense	II	Likely Oncogenic
	EGFR	Amplification	CNV	II	Oncogenic
2846	KRAS	p.Gly12Cys	Missense	I	Oncogenic
2848	TP53	p.Leu43*	Frameshift	II	Likely Oncogenic
2849	TP53	p.Leu111Pro	Missense	II	Likely Oncogenic
2850	TP53	p.Cys275Tyr	Missense	II	Likely Oncogenic
	ALK	EML4:ALK	Fusion	I	Oncogenic
2855	EGFR	p.Ser768_Asp770dup	ProteinIns	I	Oncogenic
2858	TP53	p.Pro177_Cys182del	ProteinDel	II	Likely Oncogenic
2859	TP53	p.Glu294Glyfs*51	Frameshift	II	Likely Oncogenic
2861	TP53	p.?	Splice_region	II	Likely Oncogenic
2862	TP53	p.Gly154Val	Missense	II	Oncogenic
2864	PIK3CA	p.Glu542Lys	Missense	II	Oncogenic
	TP53	p.Arg249Ser	Missense	II	Oncogenic
	Chr17	Amplification	CNV	II	Oncogenic
2865	ALK	EML4:ALK	Fusion	I	Oncogenic
2866	KRAS	p.Gly12Asp	Missense	II	Oncogenic
2867	KRAS	p.Gly12Cys	Missense	I	Oncogenic
2869	TP53	p.Val272Leu	Missense	II	Likely Oncogenic
2870	TP53	p.Ser37Profs*7	Frameshift	II	Likely Oncogenic
2871	TP53	p.Lys132Asn	Missense	II	Oncogenic
2872	TP53	p.Gly245Val	Missense	II	Oncogenic
2873	TP53	p.Arg158Leu	Missense	II	Oncogenic
	ERBB2	Amplification	CNV	II	Oncogenic
2874	TP53	p.Ser215Ile	Missense	II	Oncogenic
	ERBB2	Amplification	CNV	II	Oncogenic
2876	TP53	p.Met237Ile	Missense	II	Likely Oncogenic
2877	TP53	p.Arg337His	Missense	II	Oncogenic
	TP53	p.Gly266Val	Missense	II	Likely Oncogenic
2880	KRAS	p.Gly12Ala	Missense	II	Oncogenic
2881	ALK	EML4:ALK	Fusion	I	Oncogenic
2884	TP53	p.Val203Leu	Missense	II	Likely Oncogenic
	TP53	p.Trp146*	Nonsense	II	Oncogenic
2885	EGFR	p.Asn771delinsGlyPhe	ProteinIns	I	Oncogenic
	TP53	p.Arg337His	Missense	II	Oncogenic
2886	TP53	p.Tyr220Cys	Missense	II	Oncogenic

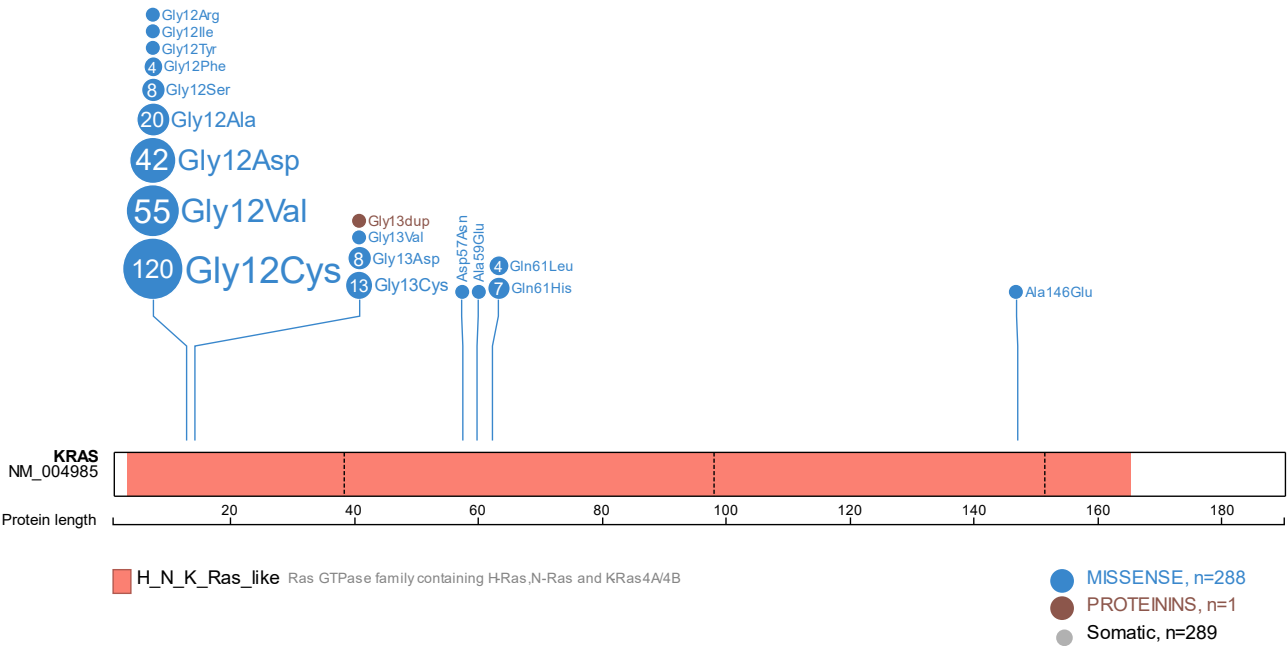
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2888	TP53	p.Arg110Leu	Missense	II	Likely Oncogenic
2889	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
2890	TP53	p.Arg280Ser	Missense	II	Likely Oncogenic
	MET	METΔex14	Exon Skipping	I	Oncogenic
	MET	Amplification	CNV	II	Oncogenic
2892	TP53	p.Gly105Cys	Missense	II	Likely Oncogenic
	KRAS	p.Gly12Ala	Missense	II	Oncogenic
	ALK	Unkown Partner	Fusion	I	Oncogenic
2908	EGFR	p.Leu858Arg	Missense	I	Oncogenic
2919	EGFR	p.Leu858Arg	Missense	I	Oncogenic
2920	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Glu285Lys	Missense	II	Oncogenic
2921	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
2922	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	PIK3CA	p.Glu542Lys	Missense	II	Oncogenic
	TP53	p.Arg248Trp	Missense	II	Oncogenic
	EGFR	Amplification	CNV	II	Oncogenic
2923	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
2924	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.?	Splice_region	II	Likely Oncogenic
2925	EGFR	p.Leu747_Ala750delinsPro	ProteinDel	I	Oncogenic
	TP53	p.Arg337Cys	Missense	II	Oncogenic
	TP53	p.Arg337His	Missense	II	Oncogenic
2930	TP53	p.His214Pro	Missense	II	Likely Oncogenic
	KRAS	p.Gly13Cys	Missense	II	Oncogenic
2931	ALK	EML4:ALK	Fusion	I	Oncogenic
2932	BRAF	p.Asp594Gly	Missense	I	Oncogenic
	TP53	p.Glu204*	Nonsense	II	Oncogenic
2933	KRAS	p.Gln61His	Missense	II	Oncogenic
2934	KRAS	p.Gly12Asp	Missense	II	Oncogenic
2937	ALK	Unkown Partner	Fusion	I	Oncogenic
2938	ALK	EML4:ALK	Fusion	I	Oncogenic
2940	BRAF	p.Asn581Ser	Missense	II	Likely Oncogenic
2942	RET	Unkown Partner	Fusion	I	Oncogenic
2944	KRAS	p.Gly12Val	Missense	II	Oncogenic
2945	TP53	p.Arg158Pro	Missense	II	Likely Oncogenic
	KRAS	p.Gly13Asp	Missense	II	Oncogenic
2946	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Ala159Hisfs*21	Missense	II	Likely Oncogenic
2947	TP53	p.Arg337His	Missense	II	Oncogenic
2948	TP53	p.Lys132Asn	Missense	II	Oncogenic
2949	KRAS	p.Gly12Cys	Missense	I	Oncogenic
	TP53	p.Lys382Asnfs*40	Frameshift	II	Likely Oncogenic
2951	NRAS	p.Gly12Cys	Missense	II	Likely Oncogenic
	PIK3CA	p.Glu545Lys	Missense	II	Oncogenic
	TP53	p.Val157Phe	Missense	II	Oncogenic
2952	TP53	p.Tyr163Cys	Missense	II	Oncogenic
2955	TP53	p.Val147Asp	Missense	II	Likely Oncogenic
2956	TP53	p.Arg175His	Missense	II	Oncogenic
2957	TP53	p.Leu206Trpfs*41	Frameshift	II	Likely Oncogenic
	RET	Unkown Partner	Fusion	I	Oncogenic
2958	NRAS	p.Gly12Cys	Missense	II	Likely Oncogenic
	TP53	p.Arg248Trp	Missense	II	Oncogenic
	MET	Amplification	CNV	II	Oncogenic
2960	TP53	p.Ile195Phe	Missense	II	Oncogenic
2961	KRAS	p.Gln61Leu	Missense	II	Oncogenic
2963	KRAS	p.Gly12Ser	Missense	II	Oncogenic
	TP53	p.Glu298*	Nonsense	II	Likely Oncogenic
2964	KRAS	p.Gly12Val	Missense	II	Oncogenic
2965	KRAS	p.Gly12Cys	Missense	I	Oncogenic
2966	TP53	p.Val197Met	Missense	II	Likely Oncogenic

2967	ALK	EML4:ALK	Fusion	I	Oncogenic
2968	MET	METΔex14	Exon Skipping	I	Oncogenic
2969	TP53	p.Ile195Tyrfs*14	Missense	II	Oncogenic
2972	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	TP53	p.Asn239*	Nonsense	II	Likely Oncogenic
	EGFR	Amplification	CNV	II	Oncogenic
2986	EGFR	p.Glu746_Ala750del	ProteinDel	I	Oncogenic
	BRAF	p.Leu584Ile	Missense	II	Likely Oncogenic
2987	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Arg337His	Missense	II	Oncogenic
2998	TP53	p.Ser241Phe	Missense	II	Oncogenic
	MET	METΔex14	Exon Skipping	I	Oncogenic
2999	TP53	p.Pro250Leu	Missense	II	Likely Oncogenic
3000	KRAS	p.Gly12Cys	Missense	I	Oncogenic
3001	KRAS	p.Gly12Asp	Missense	II	Oncogenic
3003	TP53	p.Cys135Ser	Missense	II	Likely Oncogenic
	MET	METΔex14	Exon Skipping	I	Oncogenic
3006	TP53	p.Glu285Lys	Missense	II	Oncogenic
	TP53	p.Arg213*	Nonsense	II	Oncogenic
3008	TP53	p.Arg273Pro	Missense	II	Oncogenic
	PIK3CA	p.Tyr1021Cys	Missense	II	Likely Oncogenic
3009	KRAS	p.Gly12Asp	Missense	II	Oncogenic
3010	TP53	p.Thr155Profs*26	Missense	II	Likely Oncogenic
3016	EGFR	p.Val769Met	Missense	I	Oncogenic
3017	EGFR	p.Thr790Met	Missense	I	Oncogenic
	TP53	p.Arg337His	Missense	II	Oncogenic
3019	ALK	EML4:ALK	Fusion	I	Oncogenic
3020	TP53	p.Val173Leu	Missense	II	Oncogenic
3021	KRAS	p.Gly12Cys	Missense	I	Oncogenic
3022	TP53	p.Val73Trpfs*50	Frameshift	II	Oncogenic
3023	TP53	p.His179Leu	Missense	II	Oncogenic
3025	BRAF	p.Gly596Arg	Missense	I	Likely Oncogenic
	PIK3CA	p.Glu542Lys	Missense	II	Oncogenic
3049	EGFR	p.Gly719Ala	Missense	I	Oncogenic
	EGFR	p.Ser768Ile	Missense	I	Oncogenic
	TP53	p.Arg337His	Missense	II	Oncogenic
3050	EGFR	p.Leu858Arg	Missense	I	Oncogenic
	TP53	p.Arg337His	Missense	II	Oncogenic
3057	KRAS	p.Gly12Val	Missense	II	Oncogenic

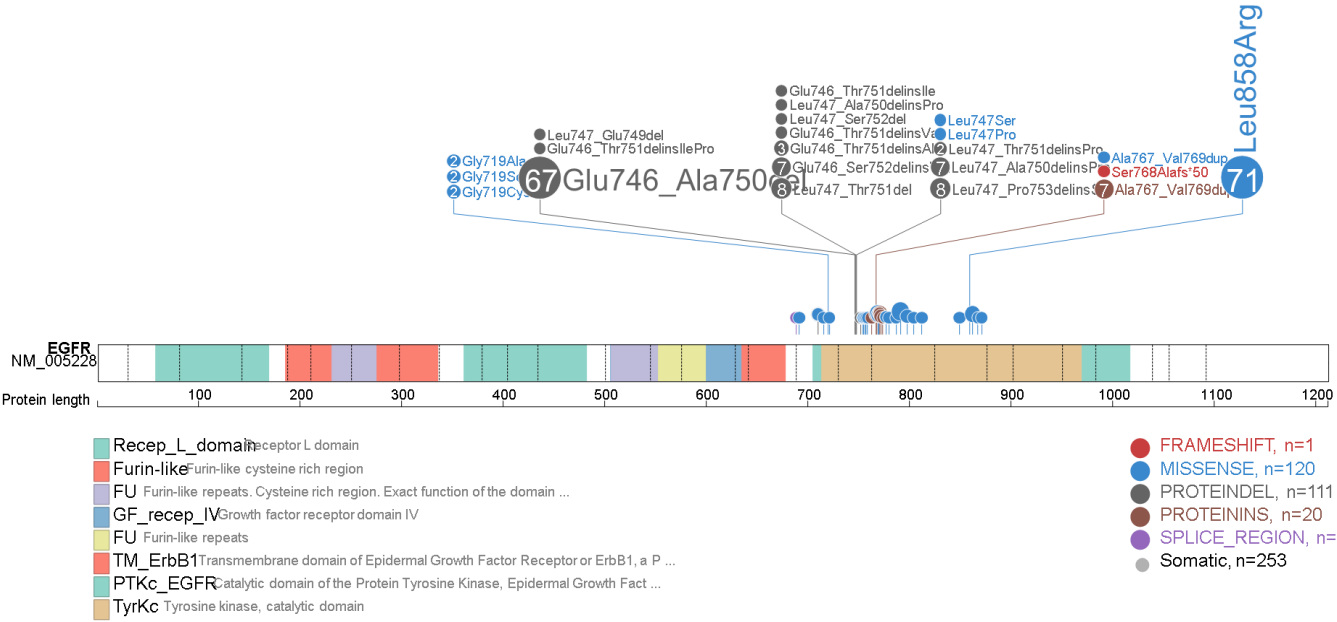
Supplementary Figures



Supplementary Figure 1- Lollipop representing the *TP53* mutations identified in the Brazilian patients diagnosed with NSCLC.



Supplementary Figure 2- Lollipop representing the *KRAS* mutations identified in the Brazilian patients diagnosed with NSCLC.



Supplementary Figure 3- Lollipop representing the *EGFR* mutations identified in the Brazilian patients diagnosed with NSCLC.

5.9 Análise funcional da sobre-expressão dos genes NTRK em câncer de pulmão e seu impacto a resposta a terapia *in vitro*.

Este artigo descreve a frequência da sobreexpressão nos genes *NTRKs* avaliado por nCounter e seu impacto a resposta a terapia alvo *in vitro*.

***NTRK* mRNA overexpression is common in human tumors and is associated with sensitivity to entrectinib in cell line models.**

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Abstract

Background: *NTRK1*, *NTRK2*, and *NTRK3* gene fusions occur in less than 1% of cancers globally. Patients with lung cancer harboring these fusions benefit from anti-TRK therapies such as entrectinib and larotrectinib. However, the prevalence and clinical implications of *NTRK* overexpression without fusions remain unclear. This study aimed to evaluate the frequency of *NTRK* overexpression in tumors lacking fusions and assess the in vitro response of *NTRK*-overexpressing cell lines to entrectinib. **Methods:** Tumor samples from 278 patients at Barretos Cancer Hospital (Brazil) and 60 from Pangaea Oncology Laboratory (Spain) were analyzed using a nCounter® panel to detect *NTRK* fusions and overexpression. Overexpression was defined as expression levels exceeding the mean+4*SD of all samples. Survival analysis was performed for patients without actionable mutations. Seven lung cancer cell lines (four with *NTRK* overexpression) and 10 from other tumor types were treated with entrectinib, with inhibitory effects assessed in 2D and 3D cultures. **Results:** *NTRK* fusions were detected in 0.9% of Brazilian and 3.3% of Spanish cases, all associated with mRNA overexpression. Notably, 10.8% of Brazilian and 6.6% of Spanish cases exhibited wild-type (wt)-*NTRK* overexpression without fusions.

Patients with NTRK overexpression had poorer survival outcomes. In vitro, cell lines with high wt-NTRK levels (>30) had significantly lower IC50 values for entrectinib (~1 μ M) in 2D models compared to those with low expression ($p=0.02$). In 3D models, high wt-NTRK lines showed IC50 values of 0.4–1 μ M, while low-expression lines ranged from 3–25 μ M ($p=0.04$). **Conclusions:** NTRK overexpression is more frequent than fusions in human tumors and it may predict sensitivity to entrectinib. Further research is needed to establish wt-NTRK overexpression as a biomarker for NTRK inhibitor response.

Keywords: *NTRK* overexpression, Cell Lines, Entrectinib, Lung Cancer

Introduction

Lung cancer remains the leading cause of cancer-related mortality both globally and in Brazil[1,2]. Non-small cell lung cancer (NSCLC), the most prevalent histological subtype, accounts for approximately 85% of all lung cancer cases. NSCLC is a highly heterogeneous disease, with molecular profiling revealing various alterations in key oncogenes that can be targeted therapeutically[3,4]. These discoveries have revolutionized the treatment landscape for NSCLC patients in recent years, offering new hope and improved outcomes[5].

Translocations in the three genes of the NTRK family (*NTRK1/2/3*) are implicated in various types of solid tumors, including pediatric glioblastoma, colorectal cancer, and non-small cell lung cancer. These are considered critical alterations in tumor development[6]. The use of inhibitory drugs such as entrectinib and larotrectinib in clinical trials has demonstrated satisfactory response rates and improved patient survival, highlighting the potential of these drugs for patients harboring NTRK gene translocations[7,8].

The frequency of *NTRK* fusions varies by tumor type, occurring in approximately 2–17% of thyroid cancers, 5–15% of salivary gland tumors, and around 1% of non-small cell lung cancers (NSCLC)[9–13]. Due to the rarity and the high number of different partners, the RNA-based nCounter assays, is capable of identifying *NTRK* fusions in Formalin-Fixed Paraffin-Embedded (FFPE) tissues using the imbalance method, where the ratio between probes designed for the 5' and 3' from *NTRK* genes indicates the presence of *NTRK* fusions[13]. We hypothesized that *NTRK* expression may be calculated using the probes counting for *NTRK* fusion detection using the nCounter approach.

Nevertheless, comprehensive data regarding *NTRK* overexpression frequency and clinical implications, particularly in lung cancer, remain scarce. We used the RNA-based nCounter assay to evaluate the frequency and clinical impact of *NTRK* overexpression in lung tumors.

Materials and Methods

Patients

The study population consisted of 278 patients diagnosed with non-small cell lung cancer at the Barretos Cancer Hospital who were referred for *NTRK1,2,3* testing by nCounter, and 60 patients diagnosed at the Pangaea Laboratory (Barcelona, Spain) were included in the studies on NTRK overexpression. Samples from Brazilian patients were analyzed in the routine workflow of the Molecular Diagnostics Laboratory at the Barretos Cancer Hospital, while samples from Spanish patients were processed in the routine workflow of the Pangaea Laboratory (Barcelona, Spain). The clinicopathological and molecular data were collected from the patient's medical records. The institutional review board, Barretos Cancer Hospital IRB, approved the study protocol (CAAE 05744712.3.0000.5437) and waived written informed consent due to the study's retrospective nature.

Analysis of *NTRK1*, *NTRK2*, and *NTRK3* Gene Overexpression

As previously published, the expression levels of the *NTRK1*, *NTRK2*, and *NTRK3* genes were calculated using data from the nCounter panel employed for detecting translocations [13]. The score for each sample was calculated based on probe count data from the 3' region of the gene, normalized using the equation: $\frac{S - (PN + 2 * SDPN)}{MGE - (PN + 2 * SDPN)} * 100$, where S is the probe count, PN is the mean of negative controls, SDPN is the standard deviation of negative controls, and MGE is the geometric mean of endogenous genes. After normalization, the counts from the 5' probes were summed to generate an expression score for each *NTRK* gene per sample. Samples were classified as overexpressed if their expression score exceeded the cutoff, calculated as the mean score of all non-overexpressed samples plus four times the standard deviation. This calculation principle was adapted from a previously published study[14].

Selection of Cell Lines with NTRK Overexpression *In Silico*

Cell lines overexpressing *NTRK1*, *NTRK2*, or *NTRK3* were selected for cell culture experiments. Cell lines with available NTRK expression data in the COSMIC database (https://cancer.sanger.ac.uk/cell_lines) and the DEPMAP database (<https://depmap.org/portal/>) were analyzed, and those with an expression score greater than two were selected. After evaluating the available cell lines in both databases, the following cell lines accessible at the Pangaea Laboratory were chosen: H82, HEL, and SET-2 for *NTRK1*; H358 for *NTRK2*; and A549, H522, SW1353, TT, and SK-N-FI for *NTRK3*. Additionally, cell lines without expression of any *NTRK* genes, including PC9, H1819, MDAMB-468, Mia-Paca-2, CALU-6, WM-763, NOMO-1, and HL-60, were included as negative controls (**Table 1**). *NTRK1*, *NTRK2*, and *NTRK3* expression scores for all selected cell lines were confirmed using the nCounter platform.

Table 1 - Cell lines used in *in vitro* experiments for *NTRK* overexpression.

Cell Line	Overexpressed Gene	Histology	Cell Culture Type		
			Adherent	Suspension	Matrigel
H358	NTRK2	Lung Adenocarcinoma	Yes	No	Yes
A549	NTRK3	Lung Adenocarcinoma	Yes	Yes	N.A.
H522	NTRK3	Lung Adenocarcinoma	Yes	Yes	N.A.
H3122	Positive Control	Lung Adenocarcinoma	Yes	N.A.	N.A.
PC9	Negative Control	Lung Adenocarcinoma	Yes	Yes	Yes
H1819	Negative Control	Lung Adenocarcinoma	Yes	No	N.A.
SW1353	NTRK3	Chondrosarcoma	Yes	No	Yes
MDAMB-468	Negative Control	Breast Tumor	Yes	N.A.	N.A.
Mia-Paca-2	Negative Control	Pancreatic Tumor	Yes	N.A.	N.A.
TT	NTRK3	Thyroid Tumor	Yes	N.A.	Yes
CALU 6	Negative Control	Lung Adenocarcinoma	Yes	N.A.	N.A.
SK-N-FI	NTRK3	Neuroblastoma	Yes	No	Yes
WM-763	Negative Control	Melanoma	Yes	N.A.	N.A.

H82	NTRK1	Neuroendocrine SCLC	No	Yes	N.A.
HEL	NTRK1	Acute Myeloid Leukemia	No	Yes	Yes
SET-2	NTRK1	Acute Myeloid Leukemia	No	Yes	Yes
NOMO1	Negative Control	Acute Myeloid Leukemia	No	Yes	N.A.
HL-60	Negative Control	Acute Myeloid Leukemia	No	Yes	N.A.

SCLC, Small Cell Lung Cancer, N.A., Not Available

Overexpression Analysis by Western Blotting

The cultures will be rinsed twice with cold PBS and scraped with RIPA buffer (Cell Signaling Technology, Danvers, MO, USA), containing 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na₂EDTA, 1 mM EGTA, 1% NP-40, 1% sodium deoxycholate, 2.5 mM sodium pyrophosphate, 1 mM β -glycerophosphate, 1 mM Na₃VO₄, 1 μ g/ml leupeptin, 2 mM PMSF, and a protease inhibitor cocktail (Roche Diagnostics, Basel, Switzerland). The mixture will be passed through an insulin syringe to further break up the cells. Cell extracts will be transferred to a microcentrifuge tube, incubated on ice for 15 minutes, centrifuged for 10 minutes at 18,500 $\times$ g, and either immediately analyzed or stored at –80°C for later use. Protein samples (25 μ g) will be heated in Laemmli buffer (NuPAGE-LDS sample buffer 4 $\times$; Invitrogen, Carlsbad, CA), separated using SDS-polyacrylamide gels, and transferred to a polyvinylidene fluoride (PVDF) membrane (Bio-Rad, Hercules, CA). Membranes will then be blocked with Odyssey blocking solution (Li-Cor Biosciences, Lincoln, NE) for 1 hour. After blocking, membranes will be cut, incubated with primary antibodies at 4°C overnight, washed three times for 5 minutes in PBS-Tween 0.1%, and incubated for 2 hours with secondary antibodies (anti-rabbit or anti-mouse IgG horseradish peroxidase-conjugated secondary antibody; GE Healthcare, New York, NY). Finally, after three additional washes, membranes will be incubated with SuperSignal Chemiluminescent Substrate (Thermo Fisher Scientific, Waltham, MA, USA) and analyzed using a Bio-Rad ChemiDoc MP Imaging System.

***In Vitro* Evaluation of 2D Cell Viability**

The cell lines included, which had adhesion growth characteristics, were cultured in a humidified atmosphere with 5% CO₂ at 37°C, using RPMI medium supplemented with 10% fetal bovine serum (FBS), 50 µg/mL of penicillin-streptomycin, and 2 mM of L-glutamine in T-75 flasks, allowing their attachment to the surface. The cell lines were routinely tested for mycoplasma contamination. For the 2D proliferation assay (adherent cells), the cells were seeded at a density of 2,000 to 4,000 cells per well (following the specifications of each cell line) in 96-well plates, allowing attachment for 24 hours. The cell lines were then treated with Entrectinib, using serial doses ranging from 0 to 25 µM (0 µM - 0.01 µM - 0.05 µM - 0.1 µM - 0.5 µM - 1 µM - 2.5 µM - 5 µM - 10 µM - 25 µM) for at least 72 hours. After this period, the cells were incubated with culture medium containing 0.75 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT) for 1 to 2 hours at 37°C. The medium was then removed, and the formed formazan crystals were dissolved in 100 µL of DMSO.

***In Vitro* Evaluation of 3D Cell Viability**

The cultures were rinsed twice with cold PBS and scraped with RIPA buffer (Cell Signaling Technology, Danvers, MO, USA), containing 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na₂EDTA, 1 mM EGTA, 1% NP-40, 1% sodium deoxycholate, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na₃VO₄, 1 µg/ml leupeptin, 2 mM PMSF, and a mixture of protease inhibitors (Roche Diagnostics, Basel, Switzerland). The mixture was passed through an insulin syringe to aid cell disruption. The cell extracts were transferred to a microcentrifuge tube, incubated on ice for 15 minutes, centrifuged for 10 minutes at 18,500 × g, and either immediately analysed or stored at –80°C for later use. Protein samples (25 µg) were heated in Laemmli buffer (NuPAGE-LDS sample buffer 4×; Invitrogen, Carlsbad, CA), separated on SDS polyacrylamide gels, and transferred to a polyvinylidene fluoride (PVDF) membrane (Bio-Rad, Hercules, CA). The membranes were then blocked with Odyssey blocking solution (Li-Cor Biosciences, Lincoln, NE) for 1 hour. After blocking, the membranes were cut, incubated with primary antibodies at 4°C overnight, washed three times for 5 minutes in PBS-Tween 0.1%, and incubated for 2

hours with secondary antibodies (anti-rabbit or anti-mouse IgG horseradish peroxidase-conjugated secondary antibody; GE Healthcare, New York, NY). Finally, after three additional washes, the membranes were incubated with SuperSignal Chemiluminescence substrate (Thermo Fisher Scientific, Waltham, MA, USA) and analyzed using a Bio-Rad ChemiDoc MP Imaging System. Moreover, proliferation was tested in 3D culture using Matrigel (Corning® Matrigel® Matrix) at a concentration of 5 mg/mL. Fifty microliters of Matrigel matrix solution were pipetted into each well of a 96-well plate and incubated for 30 minutes at 37°C to allow the gel to form. Subsequently, cells were resuspended in RPMI + 10% FBS, and 7.5 µL of the cell suspension was mixed with 67.5 µL of Matrigel matrix solution and pipetted into each well (with a density of 5×10^5 cells/mL). The mixture was then incubated for 30 minutes at 37°C to form the gel. After gel formation, 100 µL of RPMI + 10% FBS was added. Treatment with Entrectinib and Larotrectinib was performed, and the culture was maintained for 8 days, with medium changes every 3 days. CCK8 assay kit or MTT assay was conducted according to the type of cells used.

Statistical Analysis

Categorical variables were presented as absolute frequencies and percentages, while continuous variables were expressed as means. The Mann-Whitney U test was used to compare the mean IC50 values from cell line proliferation assays. The Kaplan-Meier method and Log-rank test were employed to estimate patient survival. All statistical analyses were conducted using IBM SPSS Statistics, version 25.0 (IBM Corp, Armonk, NY, USA) and Prism GraphPad Software, version 6 (GraphPad Software, Boston, MA, USA), with a significance level set at 0.05.

Results

NTRK overexpression analysis for patients

The expression score for *wt-NTRK1*, *wt-NTRK2*, and *wt-NTRK3* genes was calculated for each sample based on the sum of probes derived from the 5' region of the gene. Overexpression was defined as an expression score greater than the mean plus four times the standard deviation of samples considered non-overexpressed. Samples were considered overexpressed when the expression score exceeded 10.62 for *NTRK1*, 16.56 for *NTRK2*, and 19.76 for *NTRK3* in the cohort from the Barretos Cancer Hospital, while for the Pangaea Laboratory cohort, values greater than 4.30 for *NTRK1*, 6.32 for *NTRK2*, and 9.22 for *NTRK3* were used (**Table 2**). Among patients from the Barretos Cancer Hospital, 0.9% (n=2/277) exhibited fusion in *NTRK1* and *NTRK3* genes, while 3.3% (n=2/60) of patients from the Pangaea Laboratory displayed fusion in these genes. Regarding overexpression in the absence of gene fusions, 10.8% (n=30/277) of patients from the Barretos Cancer Hospital and 6.6% (n=4/58) of patients from the Pangaea Laboratory exhibited overexpression.

Table 2 – Description of the calculated cut-offs for the mRNA levels for *wt-NTRK* genes for the Brazilian and Spanish patient cohorts.

Barretos Cancer Hospital (n=278)		Pangaea Laboratory (n=60)	
Gene	Cutt-off (Mean+4*SD)	Gene	Cutt-off (Mean+4*SD)
<i>NTRK1</i>	10,62	<i>NTRK1</i>	4,30
<i>NTRK2</i>	16,56	<i>NTRK2</i>	6,32
<i>NTRK3</i>	19,76	<i>NTRK3</i>	9,22

SD, Standar Deviation

Histologically, we observed no difference in frequency though different histologies for *wt-NTRK1* and *wt-NTRK3* overexpression (**Table 3**). However, a high percentage (30.4%) of samples were observed with *wt-NTRK2* overexpression.

Table 3 – Frequency of wt-*NTRK* overexpression according to histology

	Adenocarcinoma (n=248)		Squamous (n=23)		Others (n=57)	
<i>NTRK1</i>						
No overexpressed	236	95.2%	21	91.3%	53	93.0%
Overexpressed	12	4.8%	2	8.7%	4	7.0%
<i>NTRK2</i>						
No overexpressed	239	96.4%	16	69.6%	53	93.0%
Overexpressed	9	3.6%	7	30.4%	4	7.0%
<i>NTRK3</i>						
No overexpressed	238	96.0%	22	95.7%	54	94.7%
Overexpressed	10	4.0%	1	4.3%	3	5.3%

Functional Impact of *NTRK* overexpression

Next, to analyze the functional impact of wt-*NTRK* gene overexpression, cell lines (Table 3) were treated with the drug Entrectinib (Rozlytrek), a selective inhibitor of the NTRK family.

Table 3 – Molecular characteristics of cell lines treated with Entrectinib.

Cell Line	Gene Overexpressed	Histology	Molecular	mRNA Overexpression Score by nCounter					
				<i>NTRK1</i>	<i>NTRK2</i>	<i>NTRK3</i>	<i>ROS1</i>	<i>RET</i>	<i>ALK</i>
H358	<i>NTRK2</i>	Lung Adenocarcinoma	<i>KRAS p.G12C</i>	1,37	15,03	1,96	1,03	1,07	1,27
A549	<i>NTRK3</i>	Lung Adenocarcinoma	<i>KRAS p.G12S</i>	0,06	0,19	6,31	0,27	0,47	0,3
H522	<i>NTRK3</i>	Lung Adenocarcinoma	<i>TP53 p.P191LfsTer56</i>	2,91	1,85	7,63	0,79	1,44	1,25
H3122	Positive Control	Lung Adenocarcinoma	<i>ALK-fusion</i>	0	0,22	0,45	0,16	0,90	55,16
PC9	Negative Control	Lung Adenocarcinoma	<i>EGFR deletion 19</i>	0	0,04	0,22	0,24	0,74	0,04
H1819	Negative Control	Lung Adenocarcinoma	<i>No driver</i>	0	0,08	0,09	0,43	0,38	0,49
SW1353	<i>NTRK3</i>	Chondrosarcoma	<i>TP53 V203L</i>	0	0,08	11,16	68,54	0,55	0,36
MDAMB-468	Negative Control	Breast Tumor	<i>TP53 R273H; BRCA2 p.M965I</i>	0	0,11	0,17	1,01	0,48	0,08
Mia-Paca-2	Negative Control	Pancreatic Tumor	<i>KRAS p.G12C</i>	0	0,03	0,08	0,1	3,15	0,09
TT	<i>NTRK3</i>	Thyroid Tumor	<i>RET p.C634W</i>	8,88	2,04	75,61	2,88	1,38	3,64
CALU 6	Negative Control	Lung Adenocarcinoma	<i>KRAS Q61K</i>	0	1,62	0,26	1,94	1,27	0,51
SK-N-FI	<i>NTRK3</i>	Neuroblastoma	<i>TP53 M246R</i>	1,37	15,03	1,96	1,03	1,07	1,27
WM-763	Negative Control	Melanoma	<i>BRAF V600E</i>	0,04	0,16	0,86	1,01	0,48	0,08
H82	<i>NTRK1</i>	Neuroendocrine SCLC	<i>TP53 p.T125=</i>	10,17	0,39	0,53	0,35	0,47	8,81
HEL	<i>NTRK1</i>	Acute Myeloid Leukemia	<i>TP53 M133K; JAK2 V617F</i>	29,19	0,64	1,05	0,3	0,7	0,6
SET-2	<i>NTRK1</i>	Acute Myeloid Leukemia	<i>TP53 R248W; JAK2 V617F</i>	55,72	0,79	2,02	0,79	1,27	0,93
NOMO1	Negative Control	Acute Myeloid Leukemia	<i>KRAS G13D</i>	0,01	0,13	0,39	322,3	0,35	0
HL-60	Negative Control	Acute Myeloid Leukemia	<i>NRAS Q61L</i>	0	0,29	0,27	33,58	0,36	0,07

SCLC, Small Cell Lung Cancer

Initially, five lung cancer cell lines with NTRK overexpression (H358, A549, H522, H3122, H82) and two (PC9, H1819) without NTRK expression, as determined by the COSMIC and DEPMAP databases, were selected and their expression scores confirmed by nCounter. Additionally, six cell lines from other histologies (SW1353, TT, SK-N-FI, HEL, SET-2) with overexpression and six other lines (MDAMB-468, Mia-Paca-2, CALU-6, WM-763, NOMO-1, HL-60) without NTRK expression were also selected and their scores confirmed by nCounter. The overexpression in four lines without expression and nine lines with overexpression was further confirmed by Western Blotting (**Figure 1**).

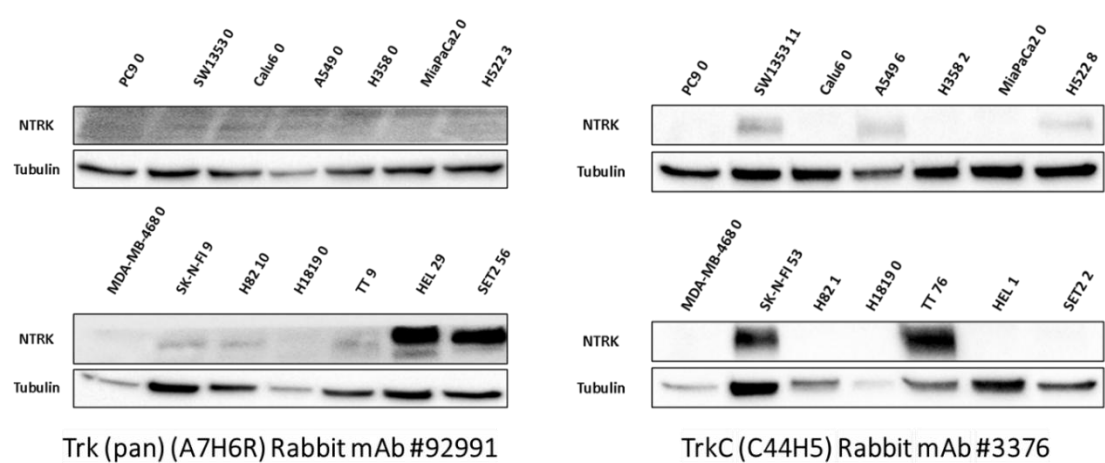


Figure 1 - Western Blotting was performed to confirm the overexpression and absence of expression of NTRK genes in the cell lines. Two antibodies were used: a pan-Trk antibody (A7H6R) for the detection of TrkA, B, and C proteins, and a TrkC antibody (C44H5) for the specific detection of TrkC proteins.

The IC50 of each cell line was calculated using the average of triplicates (**Table 4**). In both adherent (2D) and floating (3D) cell assays, all cell lines exhibited an IC50 higher than that observed in the Entrectinib-sensitive H3158 cell line. Most of the NTRK-overexpressing cell lines showed IC50 values similar to those of the *NTRK*-negative cell lines (**Table 4**; **Figure 2**).

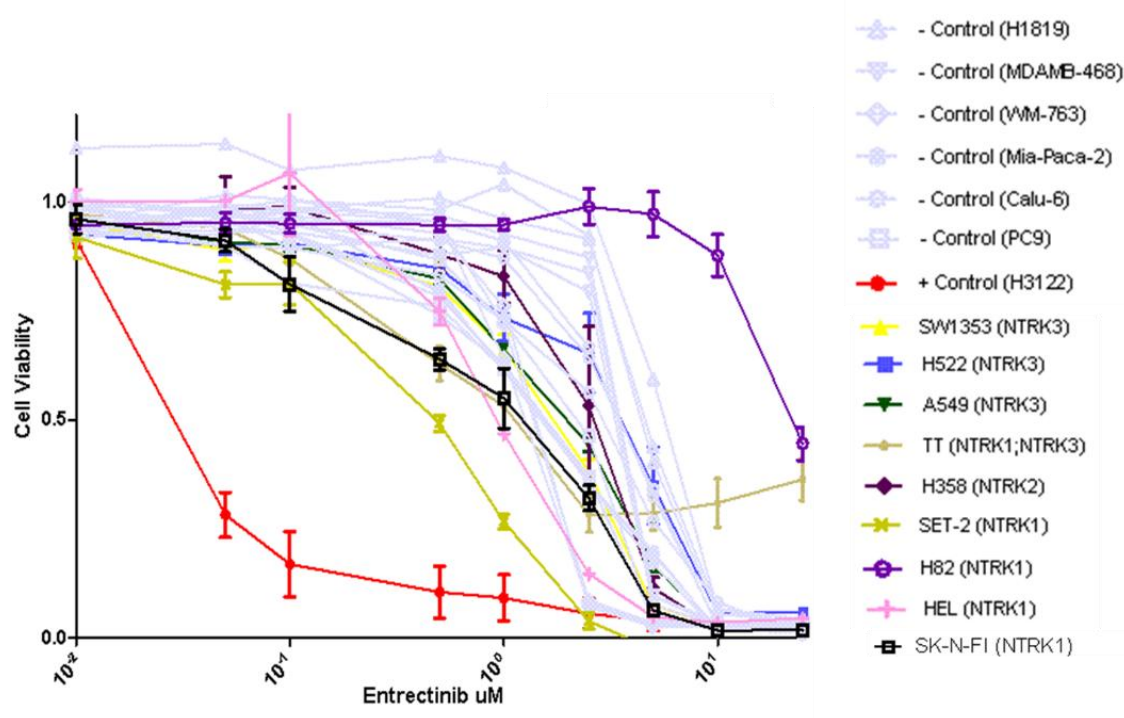


Figure 2 - Representative graph of cell viability following treatment with different concentrations of the drug Entrectinib.

Table 4 – IC50 values for different cell culture types treated with Entrectinib.

Cell Line	IC50 μ M (Entrectinib)		
	2D	3D	Matrigel
H358	2,2	N.A.	>1
A549	1,7	>25	N.A.
H522	2,6	>25	N.A.
H3122	0.036	N.A.	N.A.
PC9	1,5	5,4	>1
H1819	5,2	N.A.	N.A.
SW1353	1,6	N.A.	0,1
MDAMB-468	2,8	N.A.	N.A.
Mia-Paca-2	1,5	N.A.	N.A.
TT	1,0	N.A.	0,01
CALU 6	3,3	N.A.	N.A.
SK-N-FI	1,1	N.A.	>1.
WM-763	1,3	N.A.	N.A.
H82	N.A.	>25	N.A.
HEL	N.A.	1,0	>1
SET-2	N.A.	0,4	>1
NOMO1	N.A.	3,1	N.A.
HL-60	N.A.	4,0	N.A.

N.A., Not available

In the 2D in vitro models (adherence), the two cell lines with the highest expression levels (>30) exhibited an IC₅₀ for Entrectinib around 1 μ M, significantly lower than the 11 cell lines with NTRK expression <30 ($p=0.02$ in the Mann-Whitney U test) (Figure 3). In the 3D models (flotation/spheroids), the six cell lines with NTRK expression <30 showed IC₅₀ values for Entrectinib ranging from 3 to 25 μ M, compared to 0.4 to 1 μ M in the two cell lines with NTRK expression >30 ($p=0.04$ in the Mann-Whitney U test) (Figure 3).

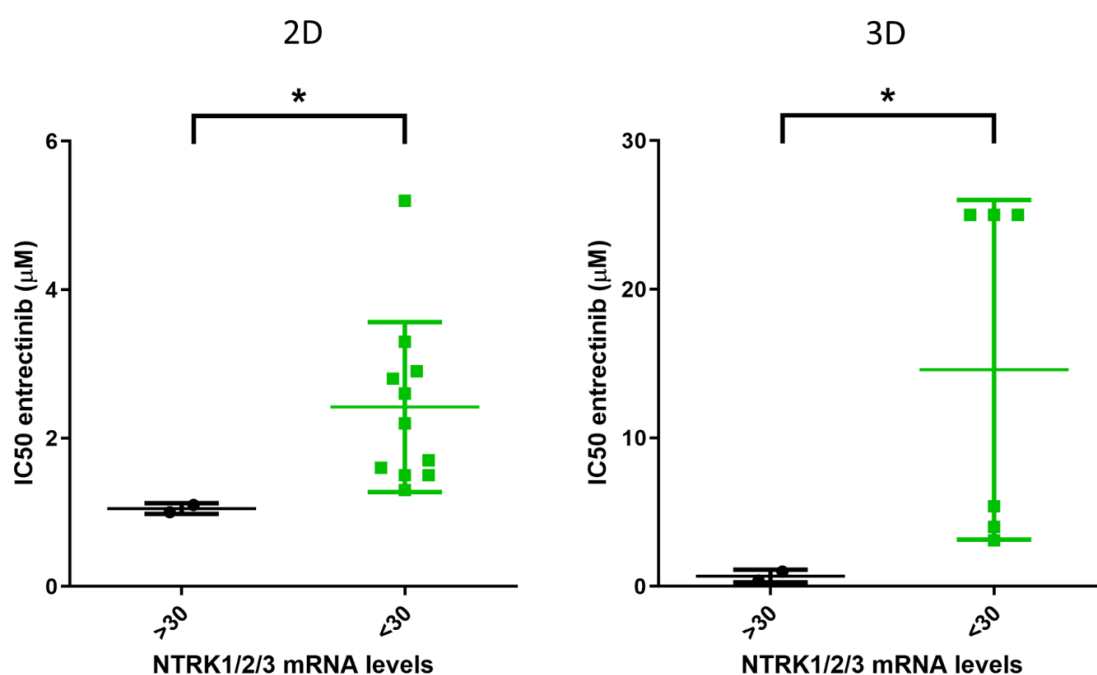


Figure 3 - A significant difference (U-Mann-Whitney) in the IC₅₀ was observed between the cells with an NTRK expression score >30 and <30 treated with Entrectinib. The 2D model includes cell lines that grew in adherence, while the 3D model includes cell lines that grew in flotation.

Finally, for the 3D model with Matrigel, a negative control (PC9) and four cell lines with varying levels of over-expression scores were selected. After treatment with Entrectinib, one cell line with NTRK expression >30 (TT) showed high sensitivity to Entrectinib (IC₅₀ ~ 10 nM), in contrast to the complete resistance observed in cells with low NTRK levels (PC9) (Figure 4).

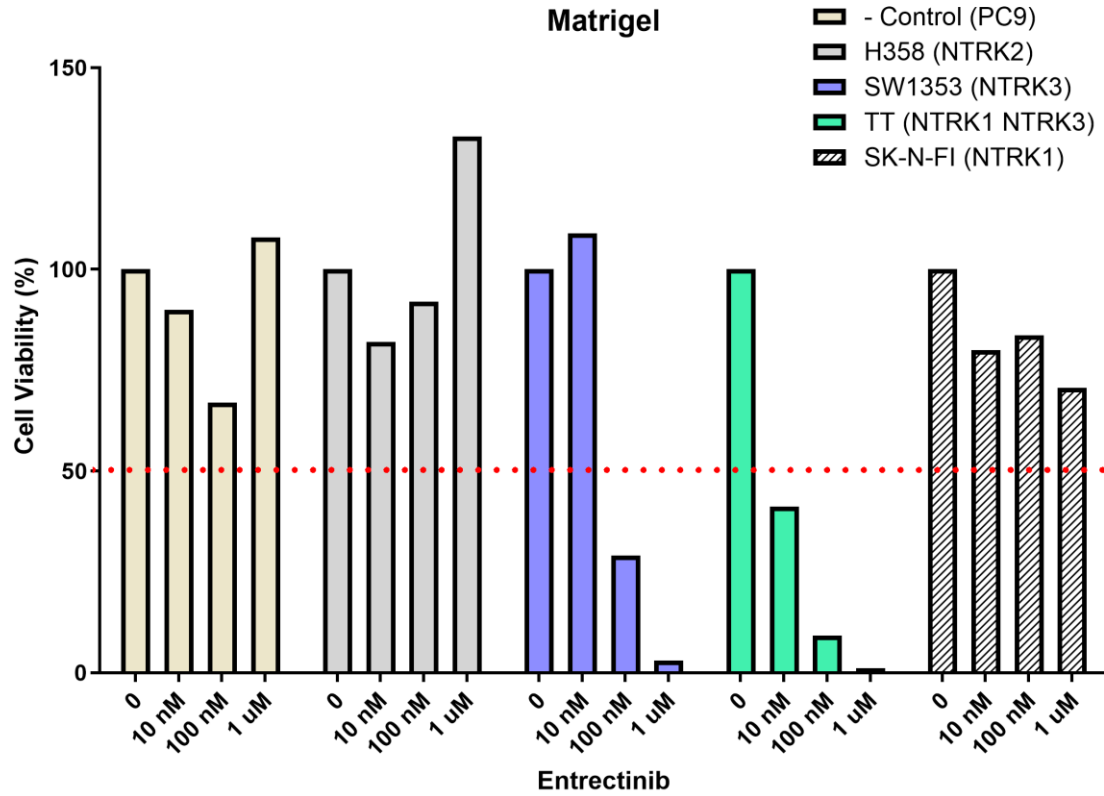


Figure 4 - Graphical representation of cell viability of the cell lines tested in Matrigel. SW1353 and TT exhibited an IC50 below 100 nM, while the other cell lines showed resistance to Entrectinib.

Overall Survival of *NTRK* overexpression

Finally, we employed Kaplan-Meier and Log-rank test to observe if patients with wt-*NTRK* overexpression show better or worse overall survival. Because clinical data availability, only the Brazilian patients were used for overall survival analysis. Moreover, to avoid bias of other driver genes, only patients with wt-*NTRK* overexpression and patients harboring no other driver alterations were included (**Figure 5**). Despite the non-significant difference ($p=0.85$), patients with wt-*NTRK* overexpressed had shorter overall survival than patients with no *NTRK* overexpressed (6.5 months and 9.0 months, respectively).

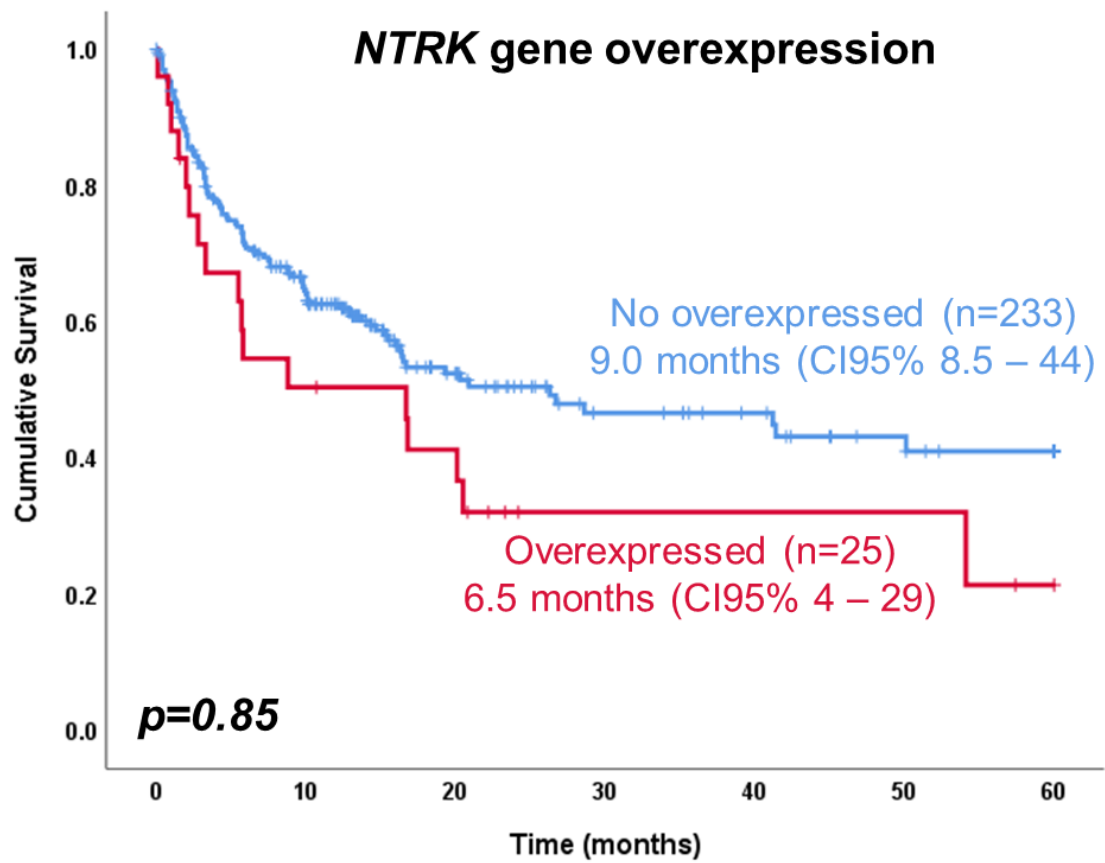


Figure 5 - Overall survival for Brazilian patients with wt-*NTRK* overexpression.

Discussion

In this study, we observed a higher frequency of *NTRK* overexpression compared to the frequency of *NTRK* fusions in lung tumors. Overall, 10.8% of Brazilian patients from and 6.6% of Spanish patients presented overexpression in any *NTRK* gene. A recent study using the pan-Trk immunohistochemistry (IHC) evaluated the expression of *NTRK* genes, but [15]. In contrast to our results, they observed 4.6% (n=43/940), lower than frequency we observed in our cohorts, . However, the authors reported high frequency of positive pan-Trk IHC (69.8%; n=30/43) in the subset of lung squamous cell carcinomas[15]. Yet, another study have reported association between *NTRK1* and *NTRK2* expression by IHC with lung squamous cell carcinoma[16]. Similarly, we observed higher frequency of wt-*NTRK2* overexpression (30.4%; n=7/23) in lung squamous cell carcinomas. The difference in frequencies may be due the different populations, number of patients evaluated, and the different methodologies. While pan-Trk IHC consider the protein expression for all three *NTRK* genes, our nCounter methodology considers the level of mRNAs for each *NTRK* gene separately[13].

We evaluated the efficacy of exposure of Entrectinib in cell lines overexpressing wt-*NTRK* genes. We observed that cell lines with higher levels of wt-*NTRK* overexpression showed lower IC50s for Entrectinib, suggesting that patients with high levels of wt-*NTRK* overexpression could be benefited with treatments using Entrectinib. However, only non-lung cell lines (TT, SET-2, HEL, and SK-N-FI) showed significant effect after treatment with low IC50 (<1nm) of Entrectinib. We hypothesize Entrectinib had no significant effect in lung cancer cell lines due the lower levels of wt-*NTRK* over expression compared to the other cell line histology. Yet, to our knowledge, there is no data evaluating the efficacy of Entrectinib or other *NTRK* inhibitors in lung tumors or cell lines overexpressing wt-*NTRK* genes. However, previous study observed that Entrectinib could induce apoptosis and inhibits the Epithelial-Mesenchymal transition in gastric cancer cell lines with *NTRK* overexpression[17]. Despite these findings, a recent phase II trial of Larotrectinib, another *NTRK*-inhibitor, reported that patients with high extreme mRNA levels of wt-*NTRK* genes have no benefit from treatments using the Larotrectinib, unlike its efficacy in patients harboring *NTRK*-fusions[18].

Moreover, we reported here patients with wt-*NTRK* overexpression shown shorter overall survival than patients with no *NTRK* overexpression, yet not statistically significant. Therefore, further study with higher number of cases and in different tumor types, are need to fully identify possible prognostic impact of wt-*NTRK* overexpression.

Our study is limited due the low number of lung tumor cell lines with higher mRNA levels of wt-*NTRK* and the lack of a cell line harboring a *NTRK*-fusion to be a positive control in the experiments. Yet, the retrospective nature of the study and the populational difference between our patients is a challenge. However, we demonstrated Entrectinib may have some efficacy in cell lines with higher mRNA levels of wt-*NTRK* genes. Future studies are needed focusing in the *NTRK* overexpression for better comprehension of its biological and clinical impact.

Conclusion

Overexpression of wt-*NTRK1-3* genes is more frequent than fusions in human tumors. Further preclinical research is warranted to determine if high wt-*NTRK* expression could be a marker of sensitivity to Entrectinib and other NTRK inhibitors. Additional studies are needed to establish wt-NTRK overexpression as a biomarker for NTRK inhibitor response.

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6 DISCUSSÃO GERAL E PERSPECTIVAS FUTURAS

O câncer de pulmão de não pequenas células apresenta um perfil molecular heterogêneo, podendo apresentar diversos tipos de alterações moleculares em múltiplos diferentes genes. Tais alterações se mostram cruciais para a determinação do diagnóstico, prognóstico e tratamento da doença. Nesta tese, buscou-se elucidar o perfil molecular do câncer de pulmão em pacientes brasileiros diagnosticados no Hospital de Amor de Barretos. Os mais de 1000 pacientes estudados são provenientes das 5 regiões do Brasil, permitindo uma avaliação nacional do problema. Os resultados de ancestralidade genética obtidos neste estudo demonstraram diferenças nas proporções de ancestralidade entre as regiões. Como esperado, a ancestralidade europeia foi predominante, com média de 71,1%, seguido pela africana com 14,6%, nativo-americana com 7,4% e asiática com 6,1%, similar a estudos prévios^{95–100}. Em acordo com a literatura, maior proporção de ancestralidade europeia foi descrita na região sul (81%) e sudeste (73%)^{95,96,98,100}. Neste estudo, foi observado uma maior proporção de ancestralidade ameríndia na região norte (20%) e maior ancestralidade africana na região nordeste (21%), similar ao reportado na literatura, onde estudos reportaram ancestralidade ameríndia entre 18-47% para região norte e africana entre 17-29%^{100–102}. Entretanto, um estudo prévio que analisou 1127 mulheres reportou maior ancestralidade africana na região norte (21,4%) e menor ancestralidade ameríndia (9,7%), comparado com os resultados deste estudo¹⁰⁰. A proporção de ancestralidade asiática foi similar entre as 5 regiões do Brasil (5-9%), estando de acordo com estudos prévios que avaliaram a ancestralidade asiática na população brasileira^{95–97,100}.

O protocolo de rotina dos pacientes com CPNPC na instituição envolve o estudo molecular de genes alvos (*EGFR*, *ALK*, *KRAS*, *ERBB2* e *BRAF*), que são laudados devido a importância na conduta do oncologista, utilizando painel “*Targeted TruSight Tumor 15*”, que avalia mutações em regiões *drivers* de 15 genes comumente mutados em tumores sólidos para identificação de mutações oncogênicas acionáveis. Adicionalmente, é utilizado um painel customizado pela instituição para a plataforma nCounter para detecção das principais fusões gênicas para câncer de pulmão^{8,47,48,103–107}. Todos estes dados moleculares foram reavaliados na presente tese, e o seu perfil foi associado com as características clínicas e de ancestralidade genética dos pacientes.

De forma geral, avaliou-se molecularmente 1133 pacientes, sendo o gene *TP53* o mais frequentemente mutado (57,9%; n=657/1133) na nossa casuística. Em um *subset* de 446 CPNPC, reportamos uma frequência de 60% apenas na histologia de adenocarcinoma⁸, estando de acordo com frequências descritas por outros estudos mundialmente^{71,108}. No Brasil, o estudo de Mascarenhas *et al.* reportou o gene *TP53* mutado em cerca de 53% dos pacientes com CPNPC, similar com nossa casuística¹⁰⁹. Ademais, reportamos a alteração R337H do gene *TP53*, conhecida pelo seu efeito fundador na população brasileira, como a mais frequente, estando em cerca de 3% dos paciente com CPNPC⁸. Estudos prévios reportaram a mutação R337H do gene *TP53* como frequente na população brasileira, com frequências que variam de 3-9%, também sendo associada com a presença de mutações no gene *EGFR*^{107,110–112}. Recentemente, a coocorrência entre a alteração R337H do gene *TP53* e o polimorfismo E134* do gene *XAF1* foi associada ao maior risco de câncer^{113,114}. Apesar da alteração R337H do *TP53* ser considerada uma variante germinativa, nesta tese não foi possível determinar se as variantes identificadas eram germinativas ou somáticas. Contudo, os pacientes identificados com a alteração R337H do gene *TP53* foram encaminhados para o Departamento de Oncogenética da instituição para possível confirmação de sua natureza germinativa e acompanhamento se necessário. Estudos avaliando o impacto da R337H nos pacientes brasileiros e suas famílias com CPNPC são necessários para melhor compreensão do risco associado a esta variante.

Considerando características clinicopatológicas da casuística, as mutações no gene *TP53* foram associadas a pacientes do sexo masculino, fumantes e ex-fumantes, histologia escamosa, metástases no sistema nervoso central e baixa ancestralidade europeia⁸. Mutações em *TP53* já foram previamente associadas a pacientes do sexo masculino, presença de metástases no SNC (Sistema Nervoso Central), consumo de tabaco e histologia escamosa^{8,115–118}. É conhecida a dicotomia e relação entre ancestralidade europeia e africana, na população brasileira, estando estes dados em linha com análises em um *subset* de casos⁸, e associações entre mutações no *TP53* e a população de maior ascendência africana^{99,111}. Finalmente, as análises do *subset* de casos⁸, evidenciou que pacientes com mutações no gene *TP53*, particularmente mutações disruptivas, associaram-se a pior prognóstico para os pacientes com adenocarcinoma de pulmão⁸, em linha com a literatura^{70,71,120–123}.

Mutações no gene *KRAS* foram identificadas em cerca de 25% (n=287/1133) dos pacientes com CPNPC, estando em sintonia com estudos prévios^{63,101,116–118}. A mutação G12C

foi identificada em cerca de 10% (n=120/1133) da casuística, similar com o descrito anteriormente considerando uma casuística menor de pacientes e estudos previamente publicados na população brasileira⁴⁸. Observou-se que as mutações no gene *KRAS* foram associadas com pacientes fumantes e ex-fumantes, sendo mais frequentes em pacientes diagnosticados com adenocarcinoma de pulmão, estando de acordo com estudos previamente publicados^{48,108,97}. Não foi observado associação das mutações no gene *KRAS* com a ancestralidade genética dos pacientes. Contudo, essas alterações são menos frequentes em populações asiáticas (cerca de 11%) e mais frequentes em populações europeias^{30,63,125,126}. Ademais, mutações no gene *KRAS* são associadas com pior prognóstico para os pacientes^{30,48,127}. Dentre todas as mutações identificadas no gene *KRAS* em pacientes com CPNPC, a variante G12C é considerada uma alteração acionável, com o desenvolvimento de fármacos desenhados especificamente para bloquear esta variante, o adagrasib e o sotorasib^{58,59}. Em perspectiva, faz-se necessário a ampliação das análises do gene *KRAS* em pacientes com CPNPC considerando mutações no gene *TP53* em coocorrência. Um estudo de revisão reportou que pacientes de CPNPC com mutações em coocorrência nos genes *KRAS* e *TP53* tem melhor resposta ao tratamento com imunoterapia¹²⁸. Análises futuras focando no impacto clínico dos tratamentos envolvendo tumores com mutações nestes dois genes, *KRAS* e *TP53*, serão necessários em pacientes Brasileiros para melhor entendimento do perfil molecular e melhor manejo clínico dos pacientes com CPNPC.

Tal como seria esperado, o gene *EGFR* foi um dos mais frequentemente mutados, estando presente em cerca de 20% (n=232/1133) dos pacientes. Esta frequência está de acordo com estudos prévios que avaliaram a presença de mutações no gene *EGFR* em pacientes brasileiros com câncer de pulmão^{109,97,129,130}. Considerando outras populações, pacientes com CPNPC em populações asiáticas possuem uma maior frequência de mutações no gene *EGFR* do que em pacientes europeus, sendo, inclusive, as mutações no gene *EGFR* associadas com alta ancestralidade asiática em pacientes brasileiros com adenocarcinoma de pulmão^{30,125,131}. Além disso, mutações do gene *EGFR* são mais frequentes e associadas à pacientes que nunca fumaram^{106,97,132}. Quando avaliamos um subconjunto de 119 pacientes com adenocarcinoma que nunca fumaram, mutações do gene *EGFR* foram identificadas em 49% dos pacientes¹⁰⁷. Nesse contexto, pacientes brasileiros com adenocarcinoma de pulmão demonstraram frequência de mutações no *EGFR* maior comparado a outras populações, com exceção da

população chinesa^{107,133–137}. Esta frequência mais alta pode estar relacionado ao fato dos pacientes brasileiros serem extremamente miscigenados, e estar associado as proporções de ancestralidade asiática presentes na população. Ademais, um estudo demonstrou maior frequência de mutações no gene *EGFR* em populações com ancestralidade ameríndia, o que também pode contribuir para a maior frequência na população brasileira¹³³. Além dos pacientes que nunca fumaram, as mutações do gene *EGFR* foram significativamente associadas com pacientes mulheres e presença de metástase cerebral ao diagnóstico, estando em acordo com estudos prévios na literatura^{30,106,107,131,138–140}. Estudos prévios demonstraram os benefícios do tratamento com TKIs em pacientes com mutações no gene *EGFR* em pacientes brasileiros com CPNPC, principalmente adenocarcinoma^{30,106}. O tratamento de mutações no gene *EGFR* para pacientes com CPNPC e seus benefícios são consolidados na literatura^{46,141,142}. Contudo, tem sido descrito que a co-ocorrência de mutações do gene *TP53* reduzem a eficácia em tratamentos com TKIs¹⁴¹. Assim, estudos com pacientes brasileiros com CPNPC, principalmente adenocarcinoma, focando no impacto das mutações do gene *TP53* são necessários para melhorias no manejo clínico dos pacientes.

Além dos genes mais frequentemente mutados (*TP53*, *KRAS* e *EGFR*), identificamos em menor frequência mutações nos genes *PIK3CA* (3,3%), *ERBB2* (2,9%), *BRAF* (2,7%) e *NRAS* (0,6%). Estas frequências são de maneira geral similares com o reportado na literatura para outras populações^{47,63,143}. A única exceção, é no gene *PIK3CA* que segundo os dados do TCGA, é de 6%¹⁰⁸, e outro estudo brasileiro, 8,8%¹⁰⁹. Esta diferença pode estar relacionada a diferença nas técnicas utilizadas, onde as mutações no gene *PIK3CA* na casuística desta tese foi avaliado em um painel “*hotspot*” que não analisa o gene completo. Com relação ao gene *ERBB2*, inserções no exon 20 do gene foram as alterações mais comuns, além de serem consideradas acionáveis. Estudo prévio mostrou que essas inserções estão presentes em cerca de 1% dos pacientes brasileiros com CPNPC, além de mais frequentes em pacientes que nunca fumaram⁴⁷. Clinicamente, as inserções e do exon 20 no gene *ERBB2* e as mutações presentes no gene *BRAF*, principalmente as variantes V600E, são consideradas mutações acionáveis, podendo os pacientes com CPNPC carreadores dessas alterações se beneficiarem de tratamentos com drogas alvos^{144,145}. A baixa frequência de alterações nestes genes, impede análises mais amplas tanto de associações clinicopatológicas. Assim, estudos futuros abrangendo um maior número

de pacientes serão necessários para expandir o entendimento dos impactos que essas alterações possam ter nos pacientes brasileiros com CPNPC.

Além das mutações identificadas, também identificamos que cerca de 7% dos nossos pacientes são portadores de fusões oncogênicas nos genes *ALK*, *RET*, *ROS1* e *NTRK1-2-3*, além de cerca de 2% dos pacientes com alterações de *METΔex14*. Devido aos principais genes envolvidos com fusões em CPNPC e a grande diversidade de genes parceiros que são identificados, o uso de técnicas *multiplex* que sejam capazes de identificar o grande número de alterações *drivers* e acionáveis em quantidades escassas de tecido tumoral são essenciais para melhorar a detecção e eficiência dessas alterações. A plataforma nCounter é uma técnica robusta e semi-automatizada, principalmente para materiais biológicos degradados, como no casos dos materiais parafinados, e que oferece um custo-benefício melhor que outras técnicas, além de possuir alta sensibilidade e baixa taxa de resultados falsos negativos e positivos quando utilizando um painel customizado^{103,146,147}.

Pacientes com estas alterações, respondem de forma muito satisfatória a terapias alvos específicas^{141,148}. Fusões no gene *ALK* foram identificadas em cerca de 6% dos pacientes, estando em acordo com estudos prévios com pacientes da população brasileira e ao redor do mundo com CPNPC^{109,95,149–151}. Clinicamente, fusões no gene *ALK* foram associados com pacientes mais jovens e que nunca fumaram, e com baixa expressão de PD-L1, tal como descrito na literatura^{107,140,141,95,150}. Fusões do gene *ALK* foram associadas com presença de metástase cerebral em pacientes brasileiros com CPNPC¹⁰³. Considerando as outras fusões nos genes *RET* (0,5%), *ROS1* (0,3%) e *NTRK1-2-3* (<1%) obtivemos frequências similares com demais estudos^{30,109,130,95,152}. Apesar do número reduzido, constatamos também na nossa série, que pacientes carreadores de fusões tratados com terapias alvos específicas demonstraram uma sobrevida significativamente maior que os pacientes com alterações que não receberam tratamento, em linha com os relatos dos benefícios causados pelos TKIs em pacientes com CPNPC carreadores de fusões envolvendo os genes *ALK*, *RET*, *ROS1* e *NTRKs*^{85,87,104,141,153–156}.

Avaliamos também o papel da sobreexpressão dos genes *NTRKs* sem a presença de fusões. Utilizando linhagens celulares com sobreexpressão de *NTRK* identificamos uma maior sensibilidade das células ao entrectinib. Contudo, estudos futuros *in vivo*, e ensaios clínicos

serão necessários para avaliar a resposta de pacientes com sobreexpressão destes oncogenes, sem presença de fusão, à fármacos anti-NTRK.

Finalmente, as alterações de *METΔex14* foram identificadas em cerca de 1,4% dos pacientes, considerando a casuística total, ou em cerca de 4% considerando apenas os pacientes que foram avaliados pelo painel nCounter para detecção dessa alteração. Na literatura, a alteração em *METΔex14* foi reportada em cerca de 3-4% dos pacientes com câncer de pulmão^{140,157,158}. No Brasil, a frequência reportada foi de cerca de 4,7%¹⁰⁹. Observamos que alterações de *METΔex14* foram associadas com pacientes que nunca fumaram e com alta expressão de PD-L1, estando de acordo com estudos prévios na literatura^{158,159}. Ademais, pacientes neste estudo que carregavam alterações *METΔex14* tiveram um pior prognóstico comparado a outros pacientes, o que pode ser explicado pela maioria dos pacientes carregadores dessa alteração (n=17) não terem recebido nenhum tratamento com TKIs. Estudos futuros com um maior número de pacientes com CPNPC carregadores de *METΔex14* e que tenham recebido tratamentos utilizando TKIs serão essenciais para melhor entendimento e melhor do manejo clínico dos pacientes brasileiros diagnosticados com CPNPC e que tenham a alteração *METΔex14* identificada.

Apesar dos importantes achados, esta tese apresenta algumas limitações, como a sua natureza retrospectiva e nem todos os pacientes terem sido avaliados para todas as alterações, uma vez que seguem a rotina da Instituição que preconiza a realização do painel de fusões apenas quando não há mutações nos *EGFR* e *KRAS* identificadas. Outra limitação é o tamanho do painel *TruSight Tumor 15* que analisa apenas regiões *hotspot* dos genes mais comumente mutados em tumores sólidos, deixando de avaliar mutações em genes como o *STK11*, que em coocorrência com mutações no gene *KRAS* conferem resistência a tratamentos com imunoterapia¹⁴¹. Ademais, consideramos apenas as alterações classificadas como oncogênicas ou provavelmente oncogênicas, deixando de fora as alterações classificadas como variantes de significado incerto.

Neste estudo, o perfil molecular dos pacientes brasileiros com câncer de pulmão foi descrito em cerca de 22 genes avaliados rotineiramente em uma única instituição, e associou com os dados clinicopatológicos dos pacientes, sendo provavelmente uma das maiores séries de pacientes brasileiros. Os dados de mundo real gerados deste estudo poderam ser aplicados

em estudos futuros que foquem no impacto clínico das mutações do gene *TP53* quando estas ocorrem simultaneamente com mutações no gene *EGFR* em pacientes tratados com TKIs, além de possíveis impactos e características das alterações classificadas com significado incerto, sendo necessário, para isso, estudos futuros com números maiores que permitam análises mais exatas. Uma visão mais detalhada separando o perfil molecular pelas diferentes regiões do Brasil da qual os pacientes se originam e procedem também será importante para uma melhor compreensão, considerando as diferenças de ancestralidade entre as regiões demonstradas neste estudo e em estudo anterior¹⁰³.

7 CONCLUSÃO

O perfil molecular do câncer de pulmão em pacientes brasileiros é extremamente heterogêneo. Aproximadamente 87% dos pacientes brasileiros possuem pelo menos uma alteração oncogênica. O gene *TP53* é o mais frequentemente mutado e demonstra valor prognóstico para os pacientes, além de estar significativamente associado com mutações no gene *EGFR* e com pacientes com alta ancestralidade africana. Mutações do gene *EGFR* estão presentes em cerca de 20% dos pacientes, estando associadas a pacientes que nunca fumaram. No grupo de não fumadores, a sua frequência foi de 50% dos casos. O gene *KRAS* se encontra mutado em cerca de 25% dos pacientes brasileiros com câncer de pulmão, sendo a mutação G12C presente em cerca de 10% dos casos. Fusões dos genes *ALK*, *RET*, *ROS1* e *NTRKs*, assim como alterações do *METΔex14* estão presentes em cerca de 10% dos casos. O perfil molecular dos pacientes com adenocarcinoma que nunca fumaram demonstram uma maior quantidade de alterações acionáveis do que aqueles que já fumaram. Além disso, o nosso estudo *in vitro*, levanta a possibilidade de resposta favorável a terapias alvos, em casos com sobre expressão de *NTRKs*, mesmo na ausência de fusões nesses genes. Em suma, a descrição do perfil molecular, permitiu elucidar o impacto clinicopatológico em pacientes brasileiros com câncer de pulmão, fornecendo dados importantes para futuras estratégias em saúde pública.

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ANEXO A – Parecer de aprovação no CEP (Comitê e Ética em Pesquisa)



HOSPITAL DO CÂNCER DE BARRETOS / FUNDAÇÃO PIO XII



PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: Estudo de Biomarcadores Terapêuticos em pacientes com Câncer de Pulmão do Hospital de Câncer de Barretos

Pesquisador: Rui Manuel Vieira Reis

Área Temática:

Versão: 10

CAAE: 05744712.3.0000.5437

Instituição Proponente: Fundação Pio XII

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 4.676.973

Apresentação do Projeto:

As informações elencadas nos campos denominados "apresentação do projeto", "objetivos" e "avaliação dos riscos e benefícios" foram retiradas do documento intitulado "PB_INFORMAÇÕES_BÁSICAS_1722508_E7.pdf" (submetido na Plataforma Brasil em 28/04/2021).

Resumo:

O câncer de pulmão é a principal causa de morte por câncer no mundo, contabilizando quase um terço de todas as mortes por câncer. O tratamento do câncer de pulmão baseado na quimioterapia é frequentemente muito tóxica e com um aumento modesto de sobrevida. Estudos observaram que em 40-80% dos pacientes com NSCLC (Non-small-cell lung carcinoma) havia uma superexpressão da proteína EGFR (epidermal growth factor receptor) associada com a agressividade clínica e pobre prognóstico. O EGFR é uma glicoproteína transmembrana e o seu ligante ativa vias de transdução de sinal (via RAF / MAPK / ERK e PI3K/AKT/mTOR). A desregulação desta via pode resultar na oncogênese e na progressão do câncer. Algumas estratégias terapêuticas têm sido desenvolvidas para inibem o domínio tirosina quinase de EGFR como o medicamento Erlotinib, que induz uma dramática resposta clínica, com aumento da sobrevida e reduzida toxicidade. No entanto, observou-se diferenças de respostas ao tratamento em certos grupos. Mutações encontradas no gene EGFR em NSCLC, a maioria localizadas nos éxons 18-21, explicam parte destas diferenças. E estas mutações tem sido correlacionadas com a sensibilidade a

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Continuação do Parecer: 4.676.973

EGFR- TKIs. Apesar da resposta favorável ao tratamento com TKIs, praticamente metade dos pacientes NSCLC, apresentam recaída com aquisição de resistência, que tem sido caracterizada como eventos moleculares secundários responsáveis pela recidiva do tumor. Variações genéticas em genes envolvido na via de sinalização de EGFR (via / RAF / MAPK / ERK e PI3K/AKT/mTOR), também podem modular a sinalização através destas vias e resultar na variação do desenvolvimento de toxicidade ou na evolução clínica do paciente pós-terapia. Recentemente, rearranjos cromossômicos envolvendo os oncogenes ALK, RET e ROS, foram identificadas como importantes alterações na tumorigênese pulmonar. Além disto, recentemente vem sendo observado também uma clara variação étnica com a relação a frequência das mutações EGFR e nos genes envolvido em sua via de sinalização, mostrando a preocupação que se deve ter com as terapias alvo contra NSCLC em diferentes regiões do mundo, considerando os diferentes subconjuntos clínicos. Portanto o presente projeto tem como objetivo caracterizar a frequência de mutações nos oncogenes EGFR e BRAF e em genes relacionados em sua via de sinalização, correlacionando-os com o impacto de sobrevida ao Erlotinib em 2 mil pacientes com câncer de pulmão atendidos pelo H.C, validar a análise do status de metilação do resíduo CpG cg05575921 como marcador biológico sanguíneo associado à exposição ao tabaco, analisar o status de metilação do gene HOXA9 e seu papel como fator prognóstico e, por último, comparar métodos de detecção de mutações mais sensíveis, conciliando ao custo benefício para a prática clínica de diagnóstico.

Hipótese:

Se a frequência das mutações em EGFR e em genes de sua via de sinalização na população brasileira for diferenciada das demais regiões do mundo, e se cada etnia pode também influenciar estas frequências e a resposta a terapia com inibidores de tirosina quinase. Desta forma nossos pacientes devem ser tratados com base em uma terapia personalizada, aumentando a sua sobrevida.

Metodologia Proposta:

Estudo retrospectivo, serão utilizadas 2000 amostras de um banco de DNA pré-existente extraído de tecido incluídos em blocos de parafina, seguindo protocolo do kit QIAamp DNA Micro kit (QIAGEN). Análise das mutações hotspots nos oncogenes EGFR e BRAF por Sequenciamento direto. Análise Quantitativa Estudo da amplificação dos genes MET e PTEN. Validação das mutações encontradas no gene EGFR por Imunohistoquímica A super-expressão de EGFR será analisada

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Continuação do Parecer: 4.676.973

utilizando os anticorpos monoclonais específicos para receptor EGFR (E746- A750del) (6B6) e (L858R Mutante) (43B2). As espécimes cirúrgicas são fixadas em formalina e embebidas em parafina e posteriormente utilizada para a marcação imunohistoquímica. Análise em multiplex de diversas mutações relevantes no tratamento específico para câncer de pulmão por Minisequenciamento (SNaPshot) Como efeito de comparação de metodologias e poder de detecção em multiplex, será avaliada a frequência mutacional nos oncogenes no gene EGFR , KRAS, BRAF, PI3KCA, NRAS, MEK1, AKT1 e PTEN, por meio do sistema comercial SNaPshot™ Multiplex System , acessando simultaneamente 38 mutações somáticas pontuais em 8 genes com relevância no tratamento específico para câncer de pulmão. Análise das mutações hotspots em 15 genes relevantes para o contexto oncológico (TruSight Tumor 15 - Illumina), em amostras tumorais de blocos de parafina, Determinar o perfil de rearranjos de ALK, RET, ROS1, NTRK1/2 pela metodologia nCounter® / NanoString. Determinação de ancestralidade utilizando um painel de 46 marcadores de ancestralidade, constituídos por polimorfismos de inserção e deleção, de acordo com o trabalho recente (Pereira et al., 2012). Análise de PD-L1 por IHQ. As análises do status de metilação do resíduo CpG cg05575921 como marcador biológico sanguíneo associado à exposição ao tabaco bem como o status de metilação do gene HOXA9 como marcador prognóstico serão realizadas por PCR digital (Droplet digital PCR - BioRad). Análise dos exames diagnósticos pela técnica PET/CT e Radiomics e posterior associação com o perfil mutacional dos pacientes. Todas as metodologias estão detalhadas no projeto.

Critério de Inclusão:

Existência de bloco de parafina representativa do tumor, de pacientes com Câncer de Pulmão do Hospital de Câncer de Barretos (Unidades Barretos e Porto Velho) coletados até Julho de 2020.

Critério de Exclusão:

Qualidade insuficiente de DNA obtido do bloco de parafina.

Objetivo da Pesquisa:

Objetivo Primário:

Estudar biomarcadores terapêuticos em 2 mil pacientes com câncer de pulmão do Hospital de Câncer de Barretos (HCB).

Objetivo Secundário:

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Continuação do Parecer: 4.676.973

1. Detecção da frequência das mutações nos genes EGFR, KRAS e BRAF (Rotina Padrão Mundial/ Câncer de Pulmão) em pacientes com câncer de pulmão do HCB;
2. Detecção da frequência de alterações moleculares em genes da via de sinalização de EGFR;
3. Comparação de diferentes metodologias de detecção de mutações no gene EGFR;
4. Detectar a frequência de variantes encontradas pelo painel TruSight Tumor 15
5. Determinar o perfil de rearranjos de ALK, RET, ROS1 e NTRK1/2/3 pela metodologia nCounter® / NanoString
6. Avaliar o status de metilacao do gene HOXA9 como biomarcador para risco de recorrência;
7. Avaliar o status de metilação do gene RKIP como biomarcador preditivo para tratamento com inibidor de tirosina quinase;
8. Avaliar a expressão de PD-L1
9. Associar o perfil mutacional dos pacientes com parâmetros clínicos, patológicos e metabólicos pela PET/CT com FDG.
10. Determinação de Ancestralidade e seu impacto na frequência de alterações dos genes anteriormente analisados;
11. Validar a análise do status de metilação do resíduo CpG cg05575921 como marcador biológico sanguíneo associado à exposição ao tabaco nas amostras e associar os resultados obtidos com o status de mutação do gene KRAS.
12. Avaliação do impacto das mutações na sobrevida global dos pacientes e ao tratamento com TKIs.

Avaliação dos Riscos e Benefícios:

De acordo com os pesquisadores:

Riscos:

O presente estudo é retrospectivo, não oferecendo qualquer risco aos pacientes envolvidos, salvaguardando o sigilo dos pacientes em todas as etapas do trabalho e o esgotamento do material biológico. No entanto, os pesquisadores se comprometem a tomar as medidas necessárias para manter o sigilo dos dados paciente e também a não esgotar o material biológico.

Benefícios:

A partir do conhecimento da característica de nossa população podemos indicar um tratamento personalizado, com provável interferência no prognóstico e/ou resposta à terapia dependendo do genótipo do paciente.

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Comentários e Considerações sobre a Pesquisa:

RESPOSTAS REFERENTES ÀS PENDÊNCIAS EMITIDAS NO PARECER N° 4.637.536 submetidos pelo CEP em 08/04/2021.

Ofício nº 50-2021 - Parecer Consubstanciado nº 4.637.536 emitido de forma errônea (Emenda7, versão 9).

Vimos por meio do presente instrumento informar que o Comitê de Ética em Pesquisa do Hospital de Amor de Barretos/SP emitiu o Parecer Consubstanciado nº 4.637.536 em 08/04/2021, referente a Emenda 7 versão 9 do projeto intitulado “Estudo de Biomarcadores Terapêuticos em pacientes com Câncer de Pulmão do Hospital de Câncer de Barretos”, CAAE 05744712.3.0000.5437, que tem como pesquisador principal deste Centro Coordenador o pesquisador Dr. Rui Manuel Vieira Reis, com o status “Pendente”, contudo não foram encontrados óbices éticos nesta emenda, devendo ser considerada como “Aprovada”, tal status “Pendente” foi emitido erroneamente. Portanto, solicitamos que seja considerada como “Aprovada” tal Emenda.

Solicitamos que o pesquisador submeta a Resposta de Pendência da Emenda via Plataforma Brasil adicionando este documento, para que este CEP possa emitir o Parecer Consubstanciado de Aprovação da Emenda, haja vista que não é possível alterar o status de um Parecer já emitido.

Considerações sobre os Termos de apresentação obrigatória:

Todos os termos foram adequadamente apresentados.

Recomendações:

Sem recomendações.

Conclusões ou Pendências e Lista de Inadequações:

Sem óbices éticos.

Considerações Finais a critério do CEP:

O Comitê de Ética em Pesquisa da Fundação Pio XII - Hospital de Câncer de Barretos analisou o(s) seguinte(s) documento(s) do projeto 630/2012, e:

- Aprovou a emenda ao estudo, submetida em 28/04/2021;

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Após análise do(s) documento(s) supracitado(s), o Comitê faz a seguinte recomendação:

- (x) O Estudo deve Continuar;
- () O Estudo dever ser Interrompido;
- () O Estudo está Finalizado;
- () Solicita-se Esclarecimento.

Ressalta-se que cabe ao pesquisador responsável encaminhar os relatórios parciais e final da pesquisa, por meio da Plataforma Brasil, via notificação do tipo “relatório” para que sejam devidamente apreciados no CEP, conforme Norma Operacional nº001/13, item XI.2.d.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_1722508_E7.pdf	28/04/2021 08:21:39		Aceito
Outros	Oficio_n50_2021_Parecer_4637536emiti doerroneamento E7.pdf	28/04/2021 08:20:50	Letícia Ferro Leal	Aceito
Outros	PROTOCOL_ESR_20_20857_Portuguese.pdf	22/03/2021 19:48:31	RODRIGO DE OLIVEIRA	Aceito
Outros	Emenda_March2020_AZ_Sig.pdf	22/03/2021 19:41:50	RODRIGO DE OLIVEIRA	Aceito
Outros	Emenda_March2020_AZ.docx	22/03/2021 19:12:48	Letícia Ferro Leal	Aceito
Outros	carta_reposta_pendenciasassinada.pdf	17/12/2020 21:05:29	RODRIGO DE OLIVEIRA	Aceito
Outros	carta_de_resposta_pendencia.docx	17/12/2020 16:39:19	RODRIGO DE OLIVEIRA	Aceito
Outros	declaracao_pesquisador_externo.pdf	17/12/2020 16:29:09	RODRIGO DE OLIVEIRA	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_Resp_Pendencia_DrRui_DiagnosticoMolecular_Emenda_outubro2020.docx	17/12/2020 15:31:48	RODRIGO DE OLIVEIRA CAVAGNA	Aceito
Outros	emenda_outubro2020.docx	01/10/2020 14:25:45	RODRIGO DE OLIVEIRA	Aceito
Solicitação Assinada pelo Pesquisador Responsável	emendaassinada_out2020.pdf	01/10/2020 14:24:44	RODRIGO DE OLIVEIRA CAVAGNA	Aceito
Projeto Detalhado / Brochura	Projeto_DrRui_DiagnosticoMolecular_Emenda_outubro2020.docx	01/10/2020 14:21:16	RODRIGO DE OLIVEIRA	Aceito

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HOSPITAL DO CÂNCER DE BARRETOS / FUNDAÇÃO PIO XII



Continuação do Parecer: 4.676.973

Investigador	Projeto_DrRui_DiagnosticoMolecular_E menda_outubro2020.docx	01/10/2020 14:21:16	RODRIGO DE OLIVEIRA	Aceito
Outros	Emenda_Agosto2020.docx	06/08/2020 17:29:52	Letícia Ferro Leal	Aceito
Outros	Emenda_Agosto2020.pdf	06/08/2020 17:28:41	Letícia Ferro Leal	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_DrRui_DiagnosticoMolecular_E menda_Julho2020.docx	27/07/2020 11:18:41	Letícia Ferro Leal	Aceito
Outros	Formulario_EMENDA_OUT2019.docx	29/10/2019 15:14:36	Letícia Ferro Leal	Aceito
Outros	Emenda_Out2019.jpg	29/10/2019 15:14:02	Letícia Ferro Leal	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_DrRui_DiagnosticoMolecular_E menda_Out2019.pdf	29/10/2019 15:13:14	Letícia Ferro Leal	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_DrRui_DiagnosticoMolecular_E menda_2000Casos.pdf	24/10/2017 11:57:26	Letícia Ferro Leal	Aceito
Outros	Formulario_EMENDA_OUT2017.docx	24/10/2017 11:56:07	Letícia Ferro Leal	Aceito
Recurso Anexado pelo Pesquisador	Formulario_EMENDA_OUT2017_Signed .pdf	24/10/2017 11:55:41	Letícia Ferro Leal	Aceito
Folha de Rosto	Folha_de_rosto_Signed.pdf	24/10/2017 11:53:26	Letícia Ferro Leal	Aceito
Outros	CEP_DOUTORADO_Carolina_Dias[1].d oc	15/07/2012 12:08:33		Aceito
Outros	Declaração de ciência e autorização de estudo.pdf	13/07/2012 14:31:47		Aceito
Outros	Declaração de ciência e autorização de estudo 6.pdf	13/07/2012 14:31:32		Aceito
Outros	Declaração de ciência e autorização de estudo 5.pdf	13/07/2012 14:31:04		Aceito
Outros	Declaração de ciência e autorização de estudo 4.pdf	13/07/2012 14:30:43		Aceito
Outros	Declaração de ciência e autorização de estudo 3.pdf	13/07/2012 14:30:27		Aceito
Outros	Declaração de ciência e autorização de estudo 2.pdf	13/07/2012 14:30:12		Aceito
Outros	Declaração de ciência e autorização de estudo 1.pdf	13/07/2012 14:29:57		Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Endereço: Rua Antenor Duarte Vilela, 1331

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HOSPITAL DO CÂNCER DE
BARRETOS / FUNDAÇÃO PIO
XII



Continuação do Parecer: 4.676.973

Não

BARRETOS, 28 de Abril de 2021

Assinado por:
Maicon Fernando Zanon da Silva
(Coordenador(a))

Endereço: Rua Antenor Duarte Vilela, 1331

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