

DISTRIBUTION OF MOLECULAR SUBTYPES OF ADVANCED LUNG ADENOCARCINOMA AND CLINICAL OUTCOMES IN A CENTER OF ARGENTINA

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Abstract The prevalence of relevant oncogenic drivers in lung adenocarcinoma varies in our region and data on clinical outcomes is scarce. The objective of the study was to describe the prevalence of *KRAS*, *BRAF* and *EGFR* mutations and *ALK* translocations in patients with advanced lung adenocarcinoma, and to depict the clinical outcome according to treatment strategies. Patients with adequate tumor biopsy sampling were included. *KRAS*, *BRAF* and *EGFR* mutations were studied by Sanger sequencing. *ALK* translocations were studied by fluorescent in situ hybridization (FISH) and immunohistochemistry (IH) with antibodies against *ALK* with clones D5F3 and 5A4. Informed consent was signed by 118 patients and 84 (72%) with complete molecular analysis were included. *KRAS* mutations were detected in 16 samples (19%), *EGFR* in 11 (13%), 9 of them conferring sensitivity to *EGFR* inhibitors, and *BRAF* mutations in 1 (1%). *ALK* translocations were detected in 3 samples (4%). Median follow-up was 42.4 [interquartile range (IQR): 27.0-64.2] months. Globally, median overall survival was 10.3 [IQR: 5.6-20.2] months. Median survival was 10.8 [IQR: 6.0-20.3] months in the group of patients without detectable molecular alteration, 9.6 [IQR: 3.7-16.1] months in *KRAS* mutant population (HR: 1.08; $p = 0.82$) and 32.5 [IQR: 19.6-38.4] months in patients with *ALK* translocations or sensitizing *EGFR* mutated tumors treated with tyrosine kinase inhibitors (HR: 0.27; $p = 0.03$). In conclusion, the prevalence of molecular alterations and outcomes in our population is similar to that reported in other studies in Western countries.

Key words: lung cancer, molecular subtypes, survival

Resumen *Distribución de subtipos moleculares de adenocarcinoma de pulmón y resultados clínicos en un centro de Argentina.* La prevalencia de alteraciones en oncogenes en adenocarcinoma de pulmón varía en nuestra región. El objetivo fue describir la prevalencia de mutaciones en *KRAS*, *BRAF* y *EGFR* y las translocaciones de *ALK* en pacientes con adenocarcinoma de pulmón y estudiar la supervivencia de acuerdo a subtipos moleculares. Se incluyeron pacientes con biopsias adecuadas para el estudio. Se evaluó el estado mutacional de *KRAS*, *BRAF* y *EGFR* por secuenciación con la técnica de Sanger. Las translocaciones de *ALK* se estudiaron por hibridación in situ por fluorescencia (FISH) e inmunohistoquímica (IHQ) contra *ALK* (clones D5F3 y 5A4). De 118 pacientes evaluados, se incluyeron 84 (72%) con análisis molecular completo. Se detectaron mutaciones de *KRAS* en 16 muestras (19%), *EGFR* en 11 (13%), y *BRAF* en 1 muestra (1%). Se detectaron rearrreglos de *ALK* en 3 muestras (4%). La mediana de seguimiento de los pacientes fue de 42.4 [rango intercuartil (RIC): 27.0-64.2] meses. Globalmente, la mediana de supervivencia en la población fue 10.3 [RIC: 5.6-20.2] meses y fue de 10.8 [RIC: 6.0-20.3] meses en pacientes sin alteraciones moleculares detectables. La mediana de supervivencia de los pacientes con mutación en *KRAS* fue de 9.6 [RIC: 3.7-16.1] meses (HR: 1.08; $p = 0.82$) y 32.5 [RIC: 19.6-38.4] meses en el grupo con rearrreglos de *ALK* o mutaciones en *EGFR* tratados con inhibidores de tirosina quinasa (HR: 0.27; $p = 0.03$). En conclusión, la prevalencia de alteraciones moleculares en nuestra población fue similar a otros países occidentales.

Palabras clave: cáncer de pulmón, subtipos moleculares, supervivencia

Lung cancer is the first cause of cancer-related deaths in the world. In Argentina, by the year 2018, lung cancer accounted for 10 662 estimated deaths, being the first in cancer mortality among men¹. Mortality has been increasing among females and decreasing among males in the last decade. Worldwide, only 15% of patients remain alive at 5 years, mainly because around 70% of lung cancers present at advanced stages. Non-small cell lung cancer (NSCLC) accounts for 85% of cases, with lung adenocarcinoma being the most common histology².

For decades, chemotherapy was the sole treatment for metastatic lung adenocarcinoma until the development of targeted therapies against driver oncogenes and the emerging role of immunotherapy. Over 60% of lung adenocarcinomas harbor a genomic mutation, amplification or translocation in key cell signaling pathways³⁻⁷. Many of them can be targeted with actionable drugs^{8, 9}. The V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*) mutations accounted for 32% of cases in The Cancer Genome Atlas (TCGA), with no effective therapy against this mutation⁵. The epidermal growth factor receptor mutations (*EGFR*) occur in 11% to 15% in Western countries, and sensitizing mutations predict responses with EGFR inhibitors in around 70% of patients¹⁰⁻¹⁵. Erlotinib, gefitinib and afatinib are approved EGFR tyrosine kinase inhibitors (TKI) currently available for first line therapy in the metastatic setting. Osimertinib is a third generation EGFR inhibitor with proven efficacy in reverting treatment resistance when the T790M secondary mutation is present¹⁶.

Anaplastic lymphoma kinase (*ALK*) rearrangements occur in 3% to 5% of lung adenocarcinomas and confer sensitivity to *ALK* inhibitors like crizotinib, ceritinib, alectinib, brigatinib and lorlatinib^{5, 9, 17-21}. As for *EGFR* inhibitors, second and third generation *ALK* inhibitors can potentially revert acquired resistance and have enhanced central nervous system penetration^{22, 23}. Dabrafenib and trametinib, *BRAF* and *MEK* inhibitors respectively, have shown activity in patients with *BRAF* V600E mutant lung cancers²⁴.

The distributions of these molecular alterations vary according to race and, consequently, geographical localization. Approximately 30-50% of Asian patients with lung adenocarcinoma harbor *EGFR* mutations compared to 11% in the Caucasian population²⁵. There are no significant differences in *ALK* rearrangements between Asian and Caucasian populations²⁶.

In Latin America, the largest analysis of 5738 lung cancer patients reported variable results in the prevalence of *EGFR* mutations ranging from 14% in Argentina, 25% in Colombia, 27% in Panama, 31% in Costa Rica, 34% in Mexico to 51%

in Peru²⁷. The largest series in Brazil shows a 25% to 30% prevalence of *EGFR* mutations^{28, 29}. The frequency of *KRAS* mutations in Latin-American population is around 14%, as evidenced by data from México, Colombia and Peru, and Brazil^{27, 29}. There are no data available on the prevalence of *KRAS* and *BRAF* mutations in lung cancer in Argentina.

This study reports the prevalence of somatic mutations in *EGFR*, *KRAS*, *BRAF* and *ALK* translocation in consecutive patients with advanced stage lung adenocarcinoma in a prospective population at a single institution in Argentina. The treatments and survival of the patients in this cohort are also described.

Materials and methods

Consecutive patients with newly diagnosed stage IIIB or IV lung adenocarcinoma or disease relapse after surgery were eligible to participate in the study. Eligible patients were over 18 years of age, had an Eastern Cooperative Group (ECOG) performance status ranging from 0 to 2, and tissue sample available for complete molecular analysis. Patients were excluded from the study in the case of inadequate tissue sample for complete analysis or different histopathological diagnosis on biopsy revision.

This is a descriptive prospective study carried out at a single academic institution in the city of Buenos Aires. Patients were enrolled from March 2012 to December 2014 and were followed until their death, withdrawal of informed consent or study cut-off on February 20th, 2016. The primary endpoint of the study was to determine the prevalence of mutations in *EGFR*, *KRAS* and *BRAF*, as well as *ALK* translocations. The secondary endpoints were to describe patient's characteristics, treatment strategies and clinical outcomes according to the tumor molecular subtype and compare the yield of surgical and non-surgical biopsies (endoscopic or percutaneous image-guided) for complete molecular analysis. The attending physician selected patient treatments based on molecular biology status and the standard treatments approved in Argentina.

The protocol was reviewed and approved by the institution ethics committee and was conducted in accordance with the Declaration of Helsinki and the Good Clinical Practice protocol requirements. All patients signed an informed consent form to participate in the study.

Histopathology diagnosis: formalin-fixed paraffin-embedded tissue (FFPET) samples were cut in 3-4µm sections and stained with hematoxylin and eosin for morphological analysis. Additional complementary immunohistochemistry assays were performed when necessary for diagnostic purposes, and tumor histology was defined by the World Health Organization (WHO) classification of lung cancer³⁰. All samples were reviewed to confirm adenocarcinoma histology.

Molecular biology: DNA was purified from FFPET samples with a minimum of 70% of tumor cells selected by tissue microdissection. Paraffin was removed with xilol-ethanol 100% and DNA was purified with QIAamp[®] DNA FFPET (Qiagen, Germany). Purity and yield were measured with spectrophotometry. PCR amplification was performed with intronic primers

TABLE 1.– Primer sequences, annealing temperature (°C), amplicon size (bp) for hotspot sequencing of candidate genes

Gene	Exon	Primer	5'-3'	°C	Bp	Hot Spot
KRAS	2	Forward	GGCCTGCTGAAAATGACTGAA	60	163	G12V/D/S/A/C/R - G13D - V14X - G15X
		Reverse	GGTCCTGCACCAGTAATATGC			
EGFR	18	Forward	CAGCATGGTGAGGGCTGAGGT	66	186	G719C/S/A - V869M - N700D - E709K/Q - S720P (#)
		Reverse	GGCCTGTGCCAGGGACCTTAC			
	19	Forward	GCACCATCTCACAATTGCCAGTTA	63	207	Deletion (E746-P753 region) - D761Y (#)
		Reverse	AAAAGGTGGGCTGAGTTCA			
	20	Forward	CGAAGCCACACTGACGTGCCT	66	201	T790M - insertion (D770- N771 region) - V769L - S768I - V765A - T783A (#)
		Reverse	AGTTGAGCAGGTAAGTGGAGCCA			
21	Forward	CCTCACAGCAGGGTCTTCTCTGT	66	222	L858R - N826S - A839T - K846R - L861Q - G863D (#)	
	Reverse	TCAGGAAAATGCTGGCTGACCTA				
BRAF	11	Forward	TCCCTCTCAGGCATAAGGTAA	58	313	G466E/V - G469A
		Reverse	CGAACAGTGAATATTTCTTTGAT			
	15	Forward	TCATAATGCTTGCTCTGATAGGA	58	224	V600E/D/K/R - D594V/G - K601E
		Reverse	GGCCAAAATTTAATCAGTGGA			

for *KRAS* exon 2, *EGFR* exons 18 to 21 and *BRAF* exons 11 and 15 (Table 1). All PCR products were evaluated by electrophoresis with agarose gel 2% in TBE 1X solution with ethidium bromide, and were analyzed under UV light. Sanger sequencing was done with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). The sequences were separated by capillary electrophoresis in ABI PRISM 310 Genetic analyzer (Applied Biosystem, USA) and analyzed with Sequencing Analysis Software v5.2 (Applied Biosystem, Foster City, CA, USA).

ALK determination was done by immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH): *ALK* fusions were tested by FISH with Vysis *ALK* Break Apart Probe kit (2p23/*ALK* translocation detection, Abbott, USA), IHC with *ALK* Testing (clone D5F3, Ventana-Roche, CE-IVD) and manual IHC with monoclonal antibody 5A4 (ab17127, ABCAM-Inc. Cambridge, MA, USA).

FFPE FISH tissue samples were processed in 4-5 micron sections. Enzymatic digestion was performed with Vysis Paraffin Pretreatment IV. The Food and Drug Administration (FDA) approved Vysis *ALK* Break Apart Probe kit (2p23/*ALK* translocation detection, Abbott, USA) was used. A positive test was considered when green and red signals were separated by >2 signal diameters in over 15% of cells.

ALK testing (Ventana-Roche, CE-IVD): FDA approved IHC kit with rabbit monoclonal antibody (clone D5F3) in combination with OptiView DAB IHC Detection kit and OptiView Amplification Kit. This IHC was done with BenchMark® GX-Ventana-Roche platform. The high sensitivity and specificity of this method admits a binary interpretation, being positive with an intense cytoplasmic granular stain.

Manual IHC (clone 5A4): FFPE 3-4µm sections underwent dehydration and endogenous peroxidase blocking, and were incubated for 2 hours in an IHC-humidity-chamber with primary

ALK monoclonal antibody clone 5A4 (ab17127, ABCAM-Inc. Cambridge, MA, USA). The revealing process was made with 3,3'-diaminobenzidine (DAB) chromogen. A semi-quantitative score was established to assess this method: 0 score was absence of staining, 1+ weak cytoplasm staining < 10%, 2+ moderate staining, and 3+ intense and granular staining, the later considered as positive.

For descriptive statistics, variables were grouped and categorized as continued or categorical. Continuous variables were summarized with median (p50) and interquartile range (p25-p75). Categorical variables were presented as proportions. Overall survival was calculated from the time of diagnosis of stage IIIB or metastatic disease until death or study termination. Survival analyses were estimated with Kaplan-Meier, and the comparison between groups was evaluated with Wilcoxon rank-sum (Mann-Whitney) test. Median follow-up time was estimated with the reverse Kaplan-Meier method. The yield of surgical and non-surgical (endoscopic or percutaneous) biopsies for molecular analysis was assessed by comparing the proportion of complete, incomplete and absence of molecular analysis between procedure types using the Fisher exact test.

Results

From March 2012 to December 2014, 118 consecutive patients signed the informed consent. Two individuals with an alternative diagnosis on pathology revision were excluded from the study, one with diagnosis of pleural mesothelioma and one with squamous NSCLC. From 116 individuals with lung adenocarcinoma histology, 32 (28%) had tumor biopsies with inadequate or insufficient tissue for complete analysis: 21 had incomplete molecular/

IHC profiling and 11 samples were not suitable for any molecular/IHC technique. Therefore, 84 individuals (72%) with complete molecular profiling in the tumor sample were included in the analysis. (Fig. 1)

Lung biopsies were the most frequently studied (n = 63), followed by lymph nodes (n = 25), bone (n = 13), brain (n = 7), liver (n = 4), adrenal gland (n = 2), kidney (n = 1) and soft tissue metastasis (n = 1). From 116 biopsies with diagnosis of lung adenocarcinomas evaluated for IHC/molecular analysis, 71 (61.2%) were surgical biopsies, 34 (29.3%) were percutaneous image-guided biopsies and 11 (9.5%) were endoscopic biopsies. The yield of surgical biopsies for complete molecular analysis was significantly higher compared to non-surgical biopsies, 87% versus 49% (p = 0.0001). Around 20% of non-surgical biopsies were not suitable for molecular analysis compared to 3% of surgical biopsies (Fig. 2).

The median age for patients with complete molecular analysis was 64 years [IQR: 58-71] and 55% were men. A total of 54 patients (64%) presented with upfront metastatic disease and 30 (36%) had disease relapse after initial curative intent: stage I (n = 15), stage II (n = 8), and stage III disease (n = 7). With regard to smoking habits, 40 patients (48%) were former smokers, 28 (33%) were current smokers and 16 (19%) were never smokers. ECOG performance status score was 0 or 1 in 84% of individuals and 18% had

experienced greater than 10% of weight loss at diagnosis. The most common sites of metastasis were the bones, lymph nodes, lungs and central nervous system (Table 2).

A genetic alteration was reported in 37% of analyzed tumor samples: *KRAS* mutations were detected in 16 samples (19%), *EGFR* mutations in 11 samples (13%), *ALK* rearrangements in 3 samples (4%) and a *BRAF* mutation in 1 sample (1%). No molecular alterations were detected in 52 samples (53%) (Fig. 3).

The following mutations were detected in *KRAS*: G12C (n = 5), G12D (n = 3), G12V (n = 3), G13S (n = 3), G13B (n = 2) and G13C (n = 1). One sample harbored a *KRAS* G12C and G13S co-mutation. In samples with *EGFR* mutations, 9 had mutations predicting benefit to treatment with *EGFR* TKI: exon 19 deletions (n = 5), L858R in exon 21 (n = 3), E709K in exon 18 (n = 1). Two samples harbored exon 20 insertions (D770_N771 and V774_C775), associated with primary resistance to treatment with *EGFR* TKI.

ALK rearrangement were detected by FISH and *ALK* IHC with clone D5F3 in 3 samples (4%). We observed an IHC false negative result in one case with clone 5A4 (score 1+). One sample (1%) had a *BRAF* G469A mutation in exon 11. This mutation is associated with lack of response to *BRAF* inhibitors. We did not detect *BRAF* V600E mutations.

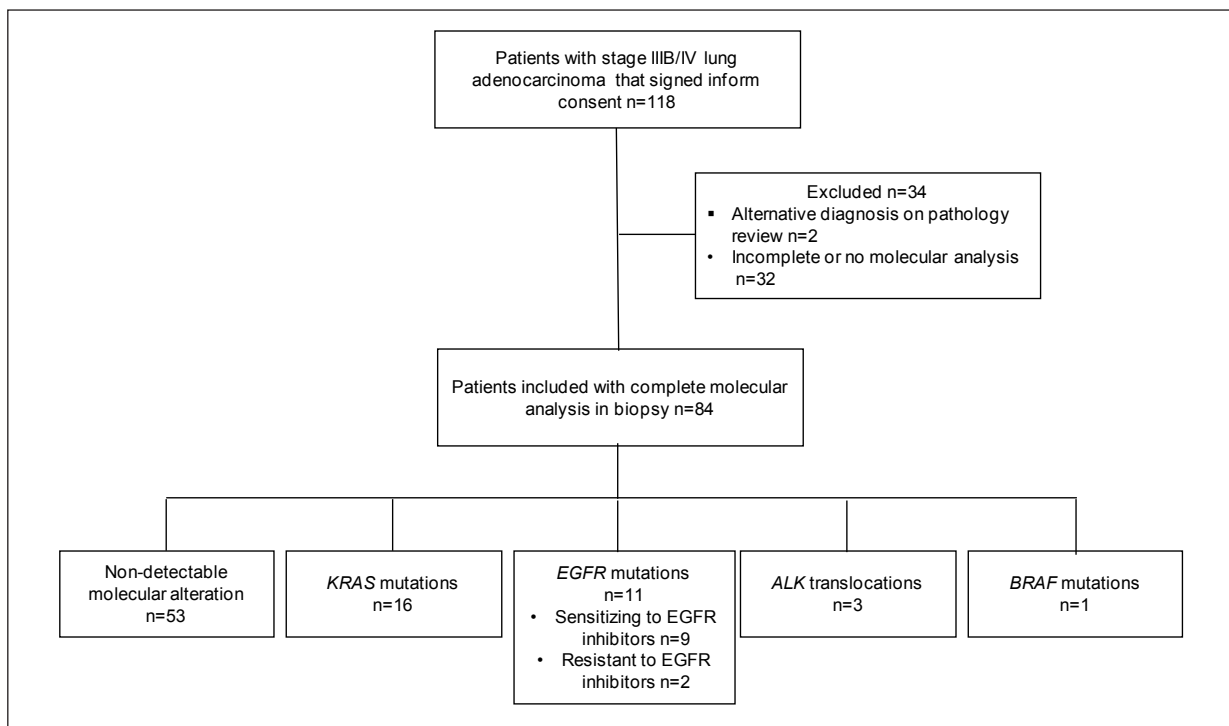


Fig. 1.– Patients and samples workflow

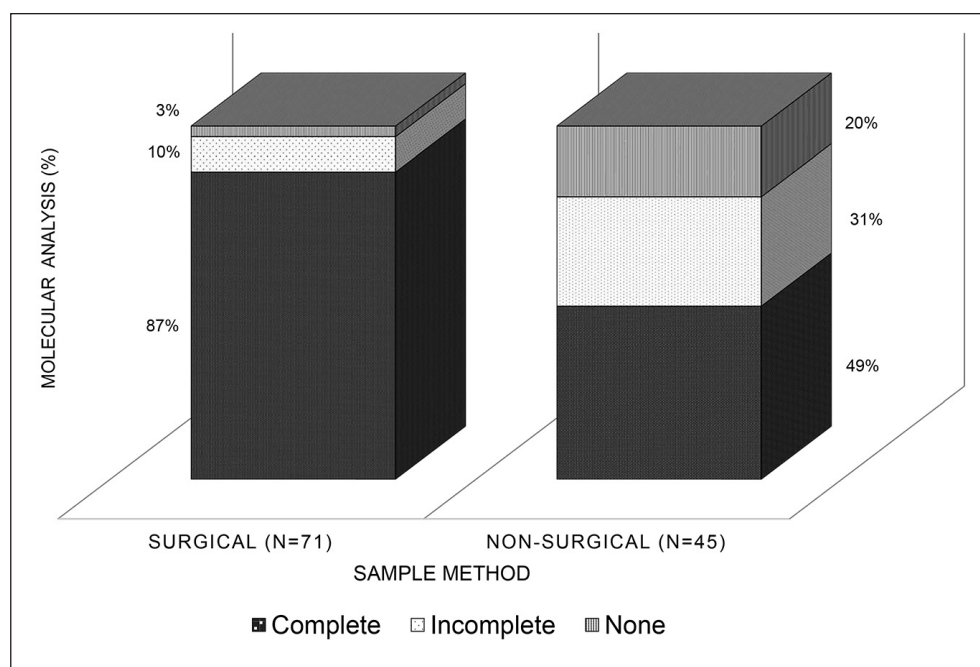


Fig. 2.– Yield of biopsy sampling method for molecular analysis

Globally, 71 patients (84%) received at least one line of systemic therapy for advanced disease: 43 (51%) received one, 18 (21%) two lines, 8 (10%) three lines and 2 (2%) received 4 lines of therapy. Carboplatin or cisplatin plus pemetrexed was the preferred first line regimen (77%). Other first line treatments were platinum-based combinations of paclitaxel or pemetrexed with bevacizumab (8%), and carboplatin given concomitantly with taxanes (6%).

In patients with non-detectable alterations ($n = 53$) and *KRAS* mutant tumors ($n = 16$), 46 (87%) and 15 (94%) received chemotherapy, respectively. Seven patients did not receive chemotherapy treatment because of clinical deterioration and poor performance status. In the group of patients with *EGFR* mutations ($n = 11$), three did not meet clinical criteria for treatment with TKI due to the presence of *EGFR* TKI resistant exon 20 insertions ($n = 2$) and surgical resection of a single brain metastasis in a patient without evidence of extracranial disease ($n = 1$). Among 8 patients with clinical indication of treatment with *EGFR* TKI, 3 received treatment with erlotinib, one with gefitinib and one with afatinib. Among patients with *ALK* translocation, one received *ALK* TKI crizotinib and the remaining received chemotherapy. Reasons for not receiving *EGFR/ALK* inhibitors were: rapid clinical deterioration ($n = 3$), lack of access to TKI by insurance delay ($n = 1$) and false negative testing with *ALK* 5A4 antibody ($n = 1$).

The median duration of follow-up for individuals included in the analysis was 42.4 months [IQR: 27.0-64.2].

At study completion, 71 patients had died (85%). Median overall survival for the whole population ($n = 84$) was 10.3 months [IQR: 5.6-20.2]. In the group of patients with tumors that did not have detectable molecular alterations, median overall survival was 10.8 months [IQR: 6.0-20.3]. Patients with *KRAS* mutated tumors had a median overall survival of 9.6 [IQR: 3.7-16.1] months (HR 1.08, 95% CI 0.55-2.12; $p = 0.82$).

In patients receiving at least one line of systemic therapy ($n = 67$), the median overall survival for the group of patients without detectable molecular alterations ($n = 46$) was 13.65 months [IQR: 6.4-20.0] (Fig. 4). In the *KRAS* mutant subgroup, the median overall survival of patients receiving systemic treatment ($n = 15$) was 10.3 [IQR: 3.7-16.8] months (HR: 1.08, 95% CI 0.54-2.18; $p = 0.80$). In patients with *EGFR* mutant and *ALK* rearranged tumors treated with TKI ($n = 6$), the median overall survival was 32.5 [IQR: 19.6 – 38.4] months (HR: 0.27, 95% IC 0.08 – 0.9, $p = 0.033$).

Discussion

The understanding of the local prevalence of molecular alterations in patients with lung adenocarcinoma is relevant to optimize their cancer care. In this study, around 14% of patients had an actionable *EGFR* mutation or *ALK* fusion, rendering further opportunities of treatment with

TABLE 2.– Clinical characteristics of patients with complete molecular analysis

Characteristics	N° of patients	%
Age at diagnosis (median)	64 [IQR: 57.7 - 71]	
Sex		
Men	46	55
Women	38	45
Ethnicity		
Caucasian	78	93
Native American	5	6
Asian	1	1
Disease presentation		
Upfront advanced disease	54	64
Relapsed disease at inclusion	30	36
Smoking habit		
Current	28	33
Former	40	48
Never	16	19
PS ECOG		
0	35	42
1	35	42
2	14	16
Weight loss > 10%	15	18
Stage at inclusion		
IIIB	11	13
IV	73	87
Number of metastasis sites		
1	56	67
2	19	23
3 or more	9	11
Site of metastasis		
Bone	32	38
Lymph nodes	28	33
Lung	18	21
CNS	17	20
Pleura	13	15
Liver	11	13
Adrenal gland	6	7
Kidney	1	1
Peritoneum	1	1

PS ECOG: Performance status according to the Eastern Cooperative Oncology Group scoring system;
CNS: Central nervous system

tyrosine kinase inhibitors. The prevalence of EGFR and ALK mutations in this study was similar to that reported previously in other series from Argentina^{27,31}. Interestingly, the prevalence of EGFR mutations seems lower compared to other countries in Latin-America²⁷. To our knowledge, this is the first study to report results of KRAS mutations in Argentinean population, The prevalence of KRAS mutations in our population were similar to the reported in

Mexico, Colombia, Peru and Brazil and lower compared to TCGA and French cohorts^{27,29}. The prevalence of EGFR mutations and ALK rearrangements in our population was similar to that reported in studies from other western countries in Europe and North America^{9,32}.

The study of targetable molecular drivers allows to optimize patients care by providing personalized cancer therapies. In our study, patients whose tumors harbored

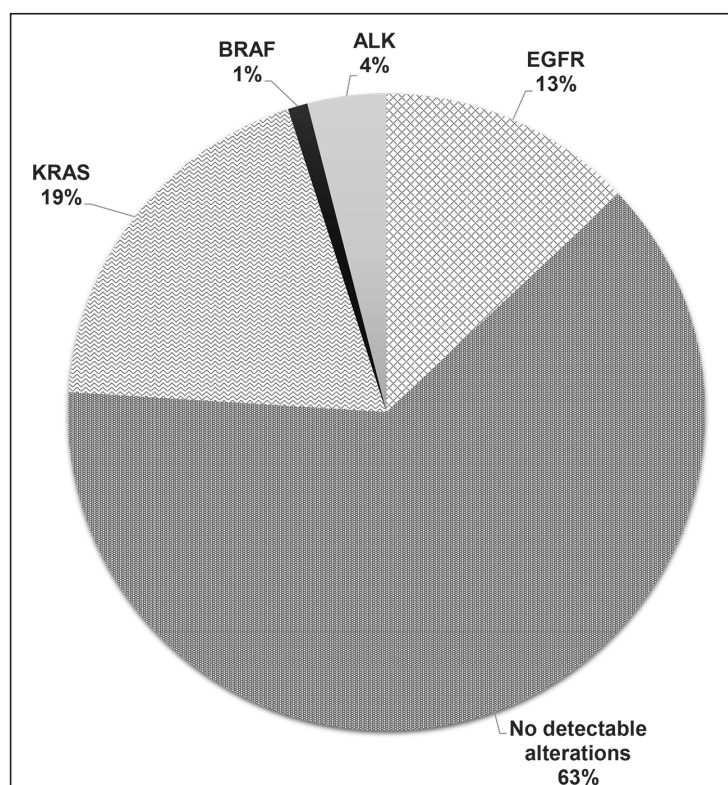


Fig. 3.– Distribution of molecular subtypes in tumor samples with complete analysis (n=84)

targetable molecular drivers experienced prolonged survival when treated with kinase inhibitors, in accordance with clinical studies with first line EGFR and ALK inhibitors³³. The overall survival of patients with EGFR mutant lung cancer treated upfront with gefitinib, in the phase III study with the longest follow up, was 34.8³⁴. Also, impressive long-term survival can be achieved with ALK inhibitors, with a 4-year survival rate of 56.6% for patients treated upfront with crizotinib in the PROFILE 1014 trial³⁵. We also observed that patients without detectable drivers treated with chemotherapy had had similar survival outcomes to those reported for patients treated with platinum-pemetrexed and maintenance therapy (~ 13 months) in phase III clinical trials³⁶.

Adequate tissue sampling and processing are crucial to perform multiple techniques required for accurate histological diagnosis and molecular profiling of limited tumor tissue. In this study, we performed multiple DNA based PCR amplifications, FISH and IHC techniques on limited biopsy material. Surgical samples conferred a higher rate of success for complete molecular profiling compared to non-surgical biopsies. However, in clinical practice, percutaneous and endoscopic samples are the

most common diagnostic procedures. In this initial study, around 50% of non-surgical biopsies were insufficient for a complete *KRAS*, *EGFR*, *BRAF* and *ALK* tumor profiling. In addition to these biomarkers, current clinical standard practice requires testing for programmed death-ligand 1 (PD-L1) expression by IHC, and *ROS1* fusions by FISH demanding the use of additional tissue. With the use of real time PCR and next generation sequencing, the required total amount of DNA needed per sample is diminishing, increasing the efficiency of molecular diagnostics. However, PCR and Sanger sequencing remain the available methodology in many laboratories. These observations are relevant to establish the quality and size of tumor samples and to improve multidisciplinary diagnostic strategies in each institution³⁷.

Our study has limitations, it is a single institution study from an academic hospital in the city of Buenos Aires, therefore we cannot extrapolate the prevalence of molecular alterations to the entire country. In addition, the small sample and the relative low proportion of patients with EGFR/ALK alterations treated with kinase inhibitors may overestimate the magnitude of the benefit of this strategy in our cohort.

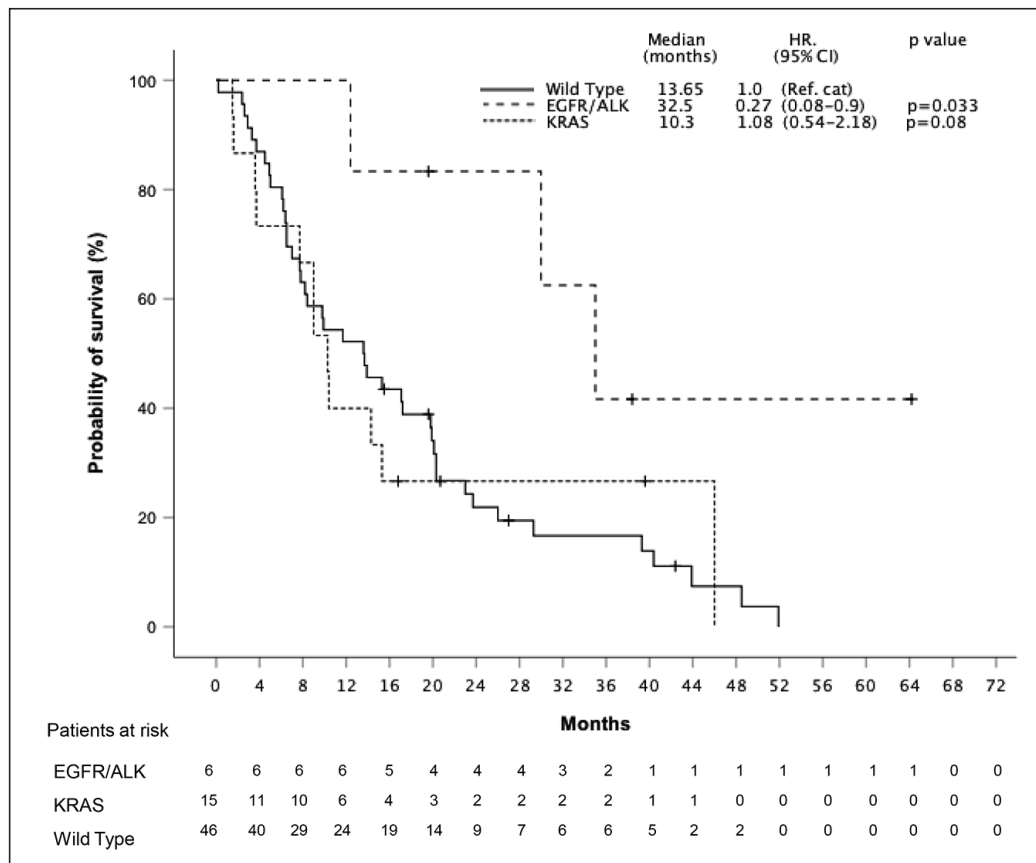


Fig. 4.— Survival outcomes of the 67 patients with complete molecular analysis that received at least one line of therapy according to molecular subtype. EGFR/ALK TKI in patients with sensitizing EGFR mutations or ALK translocations and chemotherapy in patients with KRAS mutations or wild type tumors

To our knowledge, this study is the first study in our population to report the prevalence of *EGFR*, *KRAS* and *BRAF* mutations together with *ALK* translocations, and to study the patient's characteristics and outcomes according to molecular subtype and therapeutic strategy in our country.

In conclusion, the prevalence of *EGFR*, *KRAS* and *BRAF* mutations and *ALK* translocations observed in this single center study is comparable to that reported in western countries. Patients whose tumors harbor *EGFR* sensitizing mutations and *ALK* translocation have a longer survival when treated with targeted therapies. The study of multiple genes is feasible and demands optimization of tumor sampling.

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Conflicts of Interest: None to declare

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LA TAPA

Passiflora caerulea, L. 1753

Sello postal de Argentina, 2009

Sello postal, Serie Fauna y Flora. Especies nativas, 2009. Diseño Magdalena Pérez del Cerro. Fotografía Nilce Silvana Enrietti. Imprenta Letra Viva, Buenos Aires. Volante Filatélico Nro. 1139. Correo Argentino. Jalil GA., Göttig JL. Catálogo Especializado de Sellos Postales de la República Argentina. 1856-2010. N° 3746. Sello y fotografía Dr. Claudio Zuckerberg. Digitalización BB.

Flor de *Passiflora caerulea*, L. 1753 (pasionaria azul, flor de la pasión, mbucuruyá, granadilla). La pasionaria es una planta enredadera, nativa de Sudamérica. Crece espontáneamente o cultivada. La flor, por sus características, está asociada a imaginativas leyendas y a la simbología cristiana de la pasión de Jesús: los estilos representan los tres clavos usados para clavarlo, las cinco anteras las cinco heridas; la corola la corona de espinas, los zarcillos el látigo con que fue flagelado, etc. Los frutos se han incorporado a la gastronomía por sus "maravillosos" beneficios, según los que patrocinan su consumo. A las infusiones de flores, hojas y corteza de las ramas se le atribuyen propiedades medicinales: ansiolíticas, sedantes, antiespasmódicas, somníferas, y muchas más. Hay preparaciones farmacéuticas de venta libre en la forma de extractos, líquidos, comprimidos y jarabes. En estos sedantes "naturales" se combinan los extractos de pasionaria con los de valeriana, tilo, espino (*Crataegus*), sauce, y, en alguno, vitamina B1. El lector interesado encuentra en *PubMed*, el 7/10/2018: *passion flower*, 659 referencias, y en *passion fruit*, 747. Y hay un misterio en la historia del arte: ¿Quién y cuándo agregó la pasionaria en un cuadro de Joos van Cleve (c. 1485-1540) datado 1530-35. En Europa, en los años 1530-35, no se conocía ni la flor real o en un herbario, ni una descripción escrita o una imagen.

(Michel E. Abrams en:

1. www.flwildflowers.com/vanclave/. 2. www.flwildflowers.com/clues/).