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Javier Azúa Romeo *, Arantxa Andueza Armendáriz *, Irene Rodríguez Pérez *, Bárbara Angulo Biedma *

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Analysis of Genes Involved in Lung Cancer: Study of 101 Cases Through Massive Sequencing

Javier Azúa-Romeo [1,*], Arantxa Andueza Armendáriz [2,*], Irene Rodríguez [3,*], Bárbara Angulo [4,*]

[1] Chief Medical Oficer and Operational Chief of Pathology. Analiza. Madrid. Spain. Professor and Senior researcher. University of Zaragoza. Spain
[2] Pathology Department. Analiza. Madrid. Spain
[3] Head of Pathology Department in Madrid. Analiza. Madrid. Spain
[4] Molecular Diagnostic Department. Analiza. Madrid. Spain
* Correspondence: jazua@analizalab.com; arandueza@analizalab.com; irodriguez@analizalab.com; bangulo@analizalab.com

Abstract

Lung cancer is one of the most commonly diagnosed cancers worldwide and remains the leading cause of cancer-related death in both men and women. In 2022, approximately 2.5 million new cases of lung cancer and 1.8 million deaths due to the disease were estimated. Historically, lung cancer has been more frequent in men, although the difference between sexes has been decreasing, with tobacco use remaining the main etiological factor. Survival rates vary considerably depending on the stage at diagnosis and other factors, and overall prognosis remains poor, with a relatively low five-year survival rate compared to other types of cancer. In this work, the objective is to present current approaches to lung cancer diagnosis through the study of multiple genetic alterations and biomarkers, mainly detected by next-generation sequencing (NGS), which has significantly transformed cancer diagnostics by enabling high-throughput and cost-effective genomic analysis. In the context of lung cancer, NGS plays a crucial role in improving molecular characterization, guiding targeted therapies, and supporting personalized medicine strategies. Specifically, its relevance lies in the ability to provide a comprehensive genomic profile of the tumor, identify driver mutations, predict treatment response, detect co-occurring alterations, and assist in therapeutic stratification. A real-world case study was conducted including 101 patients diagnosed with lung cancer between 2023 and 2025 in a reference laboratory, whose tumors were analyzed using NGS. The most frequently altered genes identified were *KRAS*, *EGFR*, and *ALK*, together with other less common but clinically actionable alterations, as well as the evaluation of programmed death-ligand 1 (PD-L1) expression by immunohistochemistry. In summary, next-generation sequencing represents a fundamental tool in the diagnostic workflow of lung cancer, enabling comprehensive molecular profiling that supports personalized treatment selection and contributes to improved clinical management of patients.

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Introduction

Lung cancer is the most frequently diagnosed cancer and the leading cause of cancer-related death worldwide. The most recent data reported by the Global Cancer Observatory for 2022 indicate nearly 2.5 million new lung cancer cases globally, together with 1.8 million deaths attributed to the disease. This means that, of all diagnosed cases, 73.3% result in death due to lung cancer.

When compared with data from 2020, Europe remains the continent with the highest mortality rates (22.6 per 100,000 inhabitants in 2020 versus 21.4 per 100,000 inhabitants in 2022), followed by Asia and North America.

Globally, lung cancer mortality among men is decreasing, although it remains high, with 1.2 of the 1.8 million lung cancer–related deaths in 2022 occurring in men. In contrast, among women, there is a slight increase in incidence. [1,2]

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In Spain, lung cancer remains the leading cause of cancer-related death in both sexes, and the number of deaths due to this cause is estimated at 23,326 in 2024.

It was estimated that in 2025, the number of new lung cancer cases would be 34,506 cases (compared with 31,435 diagnosed in 2024), of which more than 30% would occur in women. In 2022, lung cancer became the third most commonly diagnosed cancer among Spanish women, and to date it remains so, thus reflecting the progressive increase in the incidence of this type of cancer in women. [3–5]

It should be noted that, in recent years, significant progress in both diagnosis and treatment, combined with long-term decreases in smoking prevalence, has been associated with globally declining mortality. Despite this, the prognosis for lung cancer remains relatively poor and 75% of patients are diagnosed at an advanced stage, with a relatively low five-year survival rate compared to other types of cancer. The problem lies mainly in the fact that the diagnostic tools currently used are not sufficiently sensitive and do not allow diagnosis at an early stage of the disease. This scenario highlights the importance of using new methodologies and approaches that allow rapid and accurate diagnosis, in order to be able to apply effective treatment as early as possible. In this case, the use of next-generation sequencing (NGS) is highlighted for the diagnosis of lung cancer through high-throughput genomic analysis. The detection of various mutations in each tumor, its molecular characterization, and the understanding of its behavior have represented a major advance in knowledge of the disease, in improving diagnosis and treatment, as well as in guiding personalized medicine approaches. [6,7]

Lung cancer mainly presents two histological variants: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Approximately 80–85% of cases correspond to NSCLC, a variant mainly composed of adenocarcinoma (the most common, ~50%), squamous cell carcinoma, large cell carcinoma, and neuroendocrine carcinoma. On the other hand, SCLC, also known as small cell lung cancer, accounts for a considerably smaller percentage of cases (15%). This type is characterized by more aggressive behavior and a high probability of early metastasis.

In addition to these two main variants, a small percentage (<5%) corresponds to other rare lung tumors. [6,8]

Most of the mutations present in lung cancer are associated with smoking and the use of tobacco products, specifically around 90%, although there are other factors that also contribute to pulmonary carcinogenesis, such as exposure to gases, asbestos, air pollution, or chronic infections. However, inherited genetic mutations that increase the risk of developing this type of cancer must also be considered. For example, mutations present in the germline, and therefore with an autosomal dominant inheritance pattern, are independent of smoking and environmental factors to which each individual has been exposed, and may promote the development of lung cancer. Li–Fraumeni syndrome and EGFR susceptibility syndrome harbor such mutations associated with lung cancer. It should also be noted that, although there is insufficient evidence to support a clear genetic involvement or to observe a Mendelian inheritance pattern in this type of neoplasm, first-degree relatives present an increased risk of developing the disease. [9,10]

The molecular basis of lung cancer lies in the gradual accumulation of genetic and epigenetic alterations within the cell nucleus. The need for tumor molecular characterization stems from the fact that the response to targeted therapies largely depends on the specific genotype of each tumor, which allows estimation of the likelihood of response to these treatments. Specifically, the detection of mutations in *EGFR*, *BRAF*, *KRAS*, and *MET*, together with the analysis of translocations involving *ALK*, *ROS1*, *RET*, and *NTRK*, has been incorporated into the diagnostic standards for non-small cell lung cancer (NSCLC), as inhibitors targeting these kinases are routinely used in clinical practice. Clinical outcomes are generally favorable, since most patients harboring appropriate genetic alterations in *EGFR*, *ALK*, *ROS1*, *BRAF*, *MET*, and related genes benefit from kinase inhibitors, enabling effective disease control. Conversely, the absence of actionable mutations in these genes substantially limits the likelihood of a therapeutic response. Detection of these molecular alterations

 doi:10.20944/preprints202601.0311.v1

is preferentially performed using NGS, which enables the simultaneous analysis of multiple genes from a single tumor sample, thereby facilitating comprehensive molecular characterization. [6,11,12]

In this context, lung adenocarcinoma exhibits a characteristic molecular profile in which activating mutations of the epidermal growth factor receptor (*EGFR*) gene stand out, being identified in approximately 10–16% of cases, with a significantly higher frequency in never-smokers. The most common sensitizing mutations affect exons 19 and 21 and confer high sensitivity to anti-*EGFR* tyrosine kinase inhibitors (TKIs).

Likewise, rearrangements of the *ALK* gene, present in approximately 2–5% of NSCLC cases, are more frequently observed in younger patients, women, and never-smokers, and give rise to oncogenic fusion proteins that activate multiple intracellular signaling pathways involved in cell proliferation and survival. The identification of these alterations predicts a favorable response to *ALK*-specific inhibitors, with significant improvements in survival even in advanced disease stages. Similarly, *ROS1* gene rearrangements, detected in approximately 1% of NSCLC cases, are mainly associated with adenocarcinomas in never-smokers and activate oncogenic cascades comparable to those observed in *ALK*-positive tumors.

Mutations in the proto-oncogene *BRAF* are observed in approximately 2% of lung carcinomas, predominantly in adenocarcinomas, with the *V600E* mutation being the most frequent and clinically relevant.

Conversely, *KRAS* mutations, more commonly found in smokers, are associated with a poorer prognosis and have historically represented a therapeutic challenge, although targeted therapies against specific variants have been developed in recent years. In addition, other less frequent driver alterations affecting *RET*, *MET*, and *NTRK* exist, the identification of which is essential due to the availability of highly effective targeted therapies. [6,7,11–14]

Generally, together with these molecular markers, an analysis of the expression of the PD-L1 (Programmed Death-Ligand 1) protein is performed. PD-L1 is an immunoregulatory ligand involved in tumor immune evasion mechanisms. PD-L1 determination is carried out using immunohistochemical techniques and allows estimation of the degree of inhibition of the T lymphocyte–mediated immune response by the tumor. This analysis, as with the aforementioned molecular markers, helps guide therapeutic decision-making based on the likelihood of tumor response to targeted treatments, in this case immune checkpoint inhibitors such as antibodies directed against PD-1 or PD-L1. In clinical practice, PD-1/PD-L1 inhibitors are routinely used in the treatment of NSCLC. These agents have demonstrated significant efficacy, particularly in patients with high PD-L1 expression or advanced-stage disease. [11,14]

The use of next-generation sequencing (NGS), in addition to providing a comprehensive genomic profile of the tumor and enabling prediction of its response to specific treatments, also allows dynamic monitoring of tumor progression. In this regard, it enables clinicians to detect the early emergence of mutations associated with therapeutic resistance, assess the molecular evolution of the disease over time, and adjust treatment strategies in a more precise and personalized manner, thereby contributing to improved clinical decision-making and optimization of therapeutic outcomes.

Material and Methods

A case study was conducted by selecting patients from our reference laboratory at Analiza. Specifically, the cohort consisted of patients diagnosed with any type of lung cancer at Analiza during the years 2023, 2024, and 2025. From all these patients, only those who underwent NGS for tumor molecular characterization and who had been previously diagnosed in our laboratory were selected.

To filter all cases and select those of interest, the Analiza Laboratory Information System (LIS) for Pathology, Atlas, was used. Cases were reviewed month by month for each year. An initial filter was applied based on the SNOMED topographic code, selecting only cases in which this field indicated “lung,” and subsequently selecting only those classified as malignant and coded in SNOMED as “malignant pathology,” “adenocarcinoma,” “carcinoma,” “small cell carcinoma,”

 doi:10.20944/preprints202601.0311.v1

"squamous cell carcinoma," "oat-cell carcinoma," among others. In this way, the total number of malignant lung neoplasms diagnosed in each month, and consequently in each year, was obtained.

Table 1. Sample size of the case study.

YEAR	LUNG CANCER CASES	NGS PERFORMED ON DIAGNOSED CASES	TOTAL NGS TESTS
2023	148	37	66
2024	198	49	180
2025	184	41	170
Total	**530**	**127**	**416**

After filtering the cases according to the aforementioned parameters, **127 case studies** were obtained. The difference compared with the "total NGS" column is due to the inclusion in that category of NGS analyses performed at Analiza without a prior diagnosis in our laboratory, which were therefore excluded from the study. In addition, among the 127 initially selected cases, those in which biopsy specimens did not contain sufficient tumor material for sequencing were excluded, as well as cases in which the analyzed neoplasms corresponded to metastases from primary tumors of other organs rather than primary lung tumors. The inclusion of such cases would not be appropriate for this study, as metastatic tumors of extrapulmonary origin present distinct molecular profiles determined by the tissue of origin, which could introduce bias into the molecular characterization specific to lung cancer and affect the validity of the results. Furthermore, "duplicate" cases (defined as NGS analyses performed on the same biopsy) were merged in order to consolidate results into a single case.

By applying these exclusion criteria, **26 cases were discarded**, resulting in a final case study sample size of **101 cases.**

For this investigation, the **Action OncoKitDx NGS panel** (Health in Code Group, Spain) and the **NextSeq 550 sequencing platform** (Illumina, USA) were used to perform the analysis and detect specific mutations in solid tumors across **59 genes** relevant to tumor development. This procedure requires DNA extraction from formalin-fixed, paraffin-embedded (FFPE) tissue. Subsequently, enzymatic fragmentation and enrichment of regions of interest are performed by hybridization with capture probes, followed by sequencing on the NextSeq 550 platform (Illumina) using cyclic reversible termination sequencing, enabling detection of mutations and rearrangements in the selected genes.

The alterations covered include point mutations (substitutions, deletions, or insertions), copy number variations (CNVs), and gene rearrangements, which have diagnostic and prognostic relevance and are therapeutically actionable, as they represent therapeutic targets and/or predictive biomarkers for approved targeted drugs or those under clinical development. In addition, the panel integrates microsatellite instability (MSI) analysis with potential predictive value in the context of immunotherapy, as well as pharmacogenetic studies through the analysis of variants associated with toxicity or efficacy of major chemotherapy treatments. The results of the study may support personalized therapeutic decision-making based on tumor genetic alterations.

The **Action OncoKitDx panel** includes:

- Sequencing of complete exonic regions of **55 genes**: *ALK, ARID1A, ATM, ATRX, BAP1, BRAF, BRCA1, BRCA2, CHEK2, CDH1, CTNNB1, EGFR, ERBB2, ESR1, FGFR1, FGFR2, FGFR3, FGFR4, GNA11, GNAQ, H3F3A, HIST1H3B, HIST1H3H, HRAS, IDH1, IDH2, KIT, KRAS, MAP2K1, MET,*

 doi:10.20944/preprints202601.0311.v1

MLH1, MSH2, MSH6, MTOR, MYC, NRAS, NTRK1, NTRK2, NTRK3, PALB2, PBRM1, PDGFRA, PIK3CA, PMS2 + 5'UTR, PTEN, POLD1, POLE, RET, ROS1, SDHA, SDHB, SDHD, TERT + 5'UTR, TP53, and *VHL*.

- Sequencing of hotspot regions of the genes *TSC1, TSC2,* and *AKT1*.
- Analysis of rearrangements in the genes *ALK, BRAF, EGFR, FGFR2, FGFR3, NRG1, NTRK1, NTRK2, NTRK3, RET,* and *ROS1*. The Action OncoKitDx panel uses probes covering intronic regions where breakpoints have most frequently been identified: intron 19 of *ALK*; introns 31–35 of *ROS1*; introns 9–11 of *RET*; intron 17 and the 3'UTR of *FGFR2*; intron 17 and the 3'UTR of *FGFR3*; introns 8–12 of *NTRK1*; introns 10 and 12 of *NTRK2*; introns 7–10 of *BRAF*; and introns 7, 23, 24, and 25 of *EGFR*.
- Microsatellite instability (MSI) analysis using a panel of **110 microsatellite regions**.
- Detection of CNVs (amplifications and deletions) in genes covered by the panel and analysis of large chromosomal alterations across the genome, including deletions or gains of entire chromosomes or chromosomal regions.
- Detection of variants related to patient pharmacogenetics to assess response or toxicity to chemotherapy treatments. Variants are analyzed in seven genes affecting response to treatments for tumors of different origins: *DPYD* (rs3918290, rs67376798, rs55886062, rs115232898, rs75017182), *XRCC1* (rs25487), *UGT1A1* (rs4148323), *CYP2D6* (rs3892097, rs5030655), *MTHFR* (rs1801133), *TPMT* (rs1142345, rs1800460, rs1800584, rs1800462), and *CYP2C9* (rs1799853, rs1057910).

The bioinformatic analysis of the results is performed using the Data Genomics platform, aligning the obtained sequences with the reference sequence and applying quality criteria for variant identification. Both the panel and the analysis software have in vitro diagnostic certification. In addition, the system allows detection of point mutations with an allele frequency of 5%, provided that the sample has at least 30% tumor cellularity and a sequencing depth greater than 200 reads. [15–22]

It should be noted that the use of massive sequencing for comprehensive molecular annotation of tumors is in accordance with the recommendations of the European Society for Medical Oncology (ESMO). [24]

Results

A total of 101 lung cancer cases diagnosed between 2023 and 2025 met the inclusion criteria and yielded next-generation sequencing (NGS) results of sufficient quality for analysis. All samples corresponded to primary lung neoplasms and met the minimum requirements for tumor cellularity and sequencing depth, allowing reliable detection of point mutations, copy number variations (CNVs), and gene rearrangements.

From the collected data, at least one clinically relevant genetic alteration was identified in 65 of the 101 cases (64.4%), whereas 36 cases (35.6%) did not show reportable alterations in the genes included in the Action OncoKitDx panel. Most tumors harbored a single genetic alteration (50 cases; 49.5%). However, 5 cases (5.0%) presented concomitant alterations affecting two or more genes. The observed combinations included KRAS–PIK3CA, KRAS–TSC1, FGFR1–PTEN, MET–TP53, and a complex profile involving BRAF, ARID1A, and PTEN. These multi-altered profiles reflect increased molecular complexity and potential biological heterogeneity of the tumors.

 doi:10.20944/preprints202601.0311.v1

Overall, alterations were heterogeneously distributed among the different driver genes, with a clear predominance of mutations in classical oncogenes associated with non-small cell lung cancer (NSCLC). The most frequently altered gene was KRAS, identified in 30 cases (29.7%), representing 46.2% of tumors with genetic alterations (30/65). EGFR alterations were detected in 10 cases (9.9%), accounting for 15.4% of tumors with alterations. ALK gene rearrangements were identified in 4 cases (4.0%). Less frequently, alterations were detected in MET, BRAF, and PTEN, with 3 cases each (3.0%). Other less common alterations included single mutations in NRAS, FGFR1, FGFR2, ARID1A, PIK3CA, ESR1, TSC1, and TP53, each present in approximately 1.0% of the total cohort.

Among tumors with KRAS mutations and interpretable protein annotation, the most prevalent alteration was KRAS G12C, detected in 14 cases, followed by G12D in 8 cases. Other less frequent variants included G12V, G13D, and G13C. These results reflect the high prevalence of mutations affecting codon 12 of KRAS and highlight the clinical relevance of the G12C variant, which is currently amenable to targeted therapy.

EGFR alterations predominantly corresponded to sensitizing mutations. Most cases presented exon 19 deletions, followed by L858R mutations in exon 21. A small number of cases showed exon 20 insertions, and one case exhibited EGFR copy number gain. This distribution is consistent with the molecular profile described in Western populations with lung adenocarcinoma.

ALK rearrangements were identified in 4 cases, all compatible with known oncogenic fusion events. BRAF alterations included activating variants, among them V600E, while MET alterations corresponded to mutations with potential clinical relevance. In addition, alterations in tumor suppressor genes such as PTEN, ARID1A, and TP53 were identified, either as isolated events or in combination with oncogenic drivers.

Regarding PD-L1, although its expression is determined by immunohistochemistry, the results were also analyzed. PD-L1 expression was positive in 41.1% of cases, while the remaining cases were negative or showed minimal expression. PD-L1 expression was observed in both tumors with actionable genetic alterations and those without them.

Discussion

The present case study provides an updated molecular characterization of lung cancer in a real-world cohort corresponding to the period 2023–2025, analyzed using next-generation sequencing (NGS). Overall, the results obtained are consistent with the international literature, although they also reveal certain particularities that warrant discussion within the current epidemiological and clinical context.

The most frequent genetic alteration in our cohort was *KRAS*, present in 29.7% of cases. This finding is consistent with multiple studies conducted in Western populations, in which *KRAS* has become established as the most prevalent driver oncogene in non-small cell lung cancer (NSCLC), with reported frequencies ranging from 25% to 35%. In particular, the high proportion of the *KRAS* G12C variant observed in our study is comparable to that described in large European and North American series, where this variant accounts for approximately 40–50% of *KRAS* mutations. This observation is of particular clinical relevance given the current availability of specific inhibitors targeting *KRAS* G12C, further reinforcing the need for detailed molecular characterization of these variants. [17,19,25–27]

EGFR mutations were detected in 9.9% of cases, a frequency consistent with that reported in European and Mediterranean populations, where rates range between 10% and 16%. The predominance of exon 19 deletions and L858R mutations in our cohort is in line with previous studies, which identify these variants as responsible for the majority of responses to anti-EGFR tyrosine kinase inhibitors. The lower frequency of EGFR mutations compared with Asian populations, where it can reach up to 40–50%, highlights the influence of ethnic and epidemiological factors on the molecular profile of lung cancer. [28,29]

ALK rearrangements, present in 4.0% of cases, fall within the range reported in the literature (2–7%), particularly in series predominantly including lung adenocarcinomas. Similarly, alterations in

 doi:10.20944/preprints202601.0311.v1

ROS1 and *BRAF*, detected in a limited number of cases, are consistent with the low but clinically relevant frequencies described in population-based studies, which underscore the importance of their identification given the high efficacy of the available targeted therapies. [30–32]

The identification of concomitant genetic alterations in a subset of tumors is consistent with recent NGS-based studies describing complex genomic profiles in a minority of patients with NSCLC. Although these tumors have traditionally been considered to be driven by a single dominant oncogene, the coexistence of multiple alterations may negatively influence treatment response and promote the development of resistance, particularly in the context of targeted therapies. [13,22]

Regarding PD-L1 expression, the proportion of positive cases observed in our cohort (47.1%) is comparable to that reported in international studies, in which PD-L1 positivity in NSCLC ranges between 40% and 60%, depending on the cutoff used. These results underscore the need to systematically integrate PD-L1 assessment together with genomic profiling in the initial diagnostic evaluation of lung cancer. [13,14,33]

It should be emphasized that, although not all NGS analyses performed were included as study cases based on the previously established exclusion criteria, the high number of sequencing analyses carried out (416) highlights that NGS is an increasingly adopted technique in routine clinical practice. This increase reflects the progressive integration of next-generation sequencing as a reference diagnostic tool in lung cancer.

In addition, as a limitation of the study, it should be noted that the total number of cases may have been underestimated. This refers to the fact that, following the diagnostic process, many lung cancer cases detected in the final months of 2025 are likely to require massive sequencing techniques. However, due to the high workload and the time required both for the administrative procedures associated with these tests and for their execution, they had not yet been performed at the time of study completion.

In conclusion, the results obtained in this study support the current recommendations of the European Society for Medical Oncology (ESMO) and many other scientific societies, which advocate the use of NGS as a first-line strategy in the molecular diagnosis of advanced lung cancer. The ability to simultaneously identify multiple actionable alterations from a single tumor sample represents a clear advantage over sequential approaches, particularly in samples with limited material.

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