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Article

Next-Generation Sequencing Reveals Distinct Molecular Patterns in Lung Cancer: KRAS-Driven Adenocarcinoma and Limited Comutations in EGFR-Mutated Tumors

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Abstract

Background: Lung cancer is a highly heterogeneous disease in which molecular characterization has become essential for guiding personalized therapies. The implementation of next-generation sequencing (NGS) allows the simultaneous detection of multiple genomic alterations, improving tumor profiling and therapeutic decision-making. This study aimed to characterize the molecular landscape of lung cancer using NGS and to evaluate its association with histological subtypes and programmed death-ligand 1 (PD-L1) expression. Methods: A retrospective observational study was conducted on 96 patients diagnosed with lung cancer between 2023 and 2025. Molecular profiling was performed using the Action OncoKitDx panel. Associations between genetic alterations, histological subtypes, and PD-L1 expression were analyzed using Fisher's exact test, with $p < 0.05$ considered statistically significant. Results: Adenocarcinoma was the most common histological subtype (67.7%), followed by squamous cell carcinoma (26%). The most common mutations were KRAS (34.4%), TP53 (29.2%), and EGFR (11.5%). KRAS mutations were significantly associated with adenocarcinoma ($p = 0.001$), while the absence of detectable mutations was associated with squamous cell carcinoma ($p = 0.002$). Co-mutations were identified in 22.9% of cases, with KRAS–TP53 being the most common combination. Tumors harboring EGFR mutations showed a significantly lower frequency of co-mutations ($p = 0.012$). No significant associations were found between PD-L1 expression and either histological subtypes or the analyzed genetic alterations. Conclusions: Lung cancer exhibits marked molecular heterogeneity, with a predominance of KRAS mutations in adenocarcinoma. The low frequency of co-mutations in EGFR-mutated tumors supports their role as dominant driver alterations. The lack of association between PD-L1 expression and genomic alterations highlights the complexity of its regulation and suggests the involvement of multiple biological factors. These findings reinforce the clinical value of NGS in comprehensive tumor profiling and in the development of precision medicine strategies.

Keywords: NGS; lung cancer; KRAS; EGFR; PD-L1

1. Introduction

Lung cancer is one of the leading public health problems worldwide. According to data from the Global Cancer Observatory (GLOBOCAN), in 2022 there were an estimated 2.48 million new cases and more than 1.8 million deaths, making it the most prevalent cancer in the world and the leading

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cause of cancer mortality globally. It is the most common cancer in men and the second most common in women, behind breast cancer. Smoking remains the primary risk factor, which has contributed to a gradual narrowing of the incidence gap between the two sexes today [1].

In this context, lung cancer should not be considered a single entity, but rather a heterogeneous group of neoplasms with different histological characteristics and clinical behavior. Traditionally, it has been classified into non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), with the former being the most common. Within NSCLC, the most common subtypes include adenocarcinoma and squamous cell carcinoma, in addition to less frequent ones such as adenosquamous carcinoma, large cell carcinoma, or anaplastic carcinoma. On the other hand, SCLC corresponds to high-grade neuroendocrine tumors with aggressive clinical behavior. In some cases, especially with limited samples, it is not possible to precisely determine the histological subtype, and the category "Not Otherwise Specified" (NOS) is used [2–4].

The characterization of these subtypes is based on histopathological examination supported by immunohistochemistry (IHC) techniques, which allow for the identification of specific marker expression and improve diagnostic accuracy, particularly in small or poorly differentiated samples. In this regard, markers such as TTF-1 and Napsin A are associated with adenocarcinomatous differentiation, while p40 and p63 are characteristic of squamous cell carcinoma; generally, these profiles show differential expression that facilitates their distinction. In cases where characteristics of both lineages coexist, a diagnosis of adenosquamous carcinoma may be considered, always in correlation with morphological findings. On the other hand, neuroendocrine tumors show expression of markers such as synaptophysin, chromogranin, and CD56 [3,5].

Until a few years ago, tumor characterization in lung cancer was based primarily on histological and immunohistochemical studies, with the latter being particularly relevant for the selection of immunotherapy treatments through the evaluation of biomarkers such as PD-L1. However, in recent years, knowledge of the molecular and alterations involved in lung cancer has taken on a fundamental role in its clinical management. Mutations in genes such as KRAS, EGFR, or TP53 act as oncogenic drivers and have both prognostic and therapeutic implications, enabling the identification of targets for targeted therapies. Overall, molecular characterization of the tumor is essential for the implementation of personalized medicine strategies [3,6,7].

In this context, Next Generation Sequencing (NGS) has become a fundamental tool for the simultaneous analysis of multiple genetic alterations, enabling a more comprehensive molecular characterization of the tumor. Its use has been progressively incorporated into clinical practice and recommended by international guidelines for the study of solid tumors. Therefore, the objective of this study is to analyze the molecular profile of patients with lung cancer using NGS, as well as to evaluate its association with different histological subtypes and PD-L1 expression [3,5–7].

2. Materials and Methods

A retrospective observational study was conducted by selecting patients from the Analiza reference laboratory. Specifically, the cohort consists of patients diagnosed with various histological subtypes of lung cancer at Analiza over the past three years (2023–2025). Of all patients, only those who underwent NGS for molecular tumor characterization and who had been previously diagnosed at that laboratory were selected.

To filter all cases and select those of interest, Analiza's Laboratory Information System (LIS) for Pathology, Atlas, was used. An initial filter was applied based on the SNOMED topographic code, selecting only cases where this field indicated "lung"; subsequently, only those classified as malignant and coded in SNOMED as "malignant pathology," "adenocarcinoma," "carcinoma," "small cell carcinoma," "squamous cell carcinoma," and "oat cell carcinoma," among others. In this way, the total number of malignant lung neoplasms diagnosed each year was obtained.

After filtering the cases according to the parameters mentioned above, 126 cases were obtained for the study. From this point, certain inclusion and exclusion criteria were applied; initially, cases were excluded in which the analyzed neoplasms corresponded to metastases from primary tumors

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in other organs rather than primary lung tumors (11 cases excluded, leaving a sample size of 114); since 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 specific molecular characterization of lung cancer and affect the validity of the results. Subsequently, cases in which the biopsy samples did not contain sufficient tumor material for sequencing were excluded (13 cases excluded, leaving a sample size of 101). Finally, cases in which PD-L1 was not analyzed were also excluded (5 cases excluded), due to insufficient biopsy size to perform both NGS and PD-L1 testing, in which NGS was prioritized. Applying these criteria resulted in a final sample size of 96 cases.

This study utilized the Action OncoKitDx high-throughput sequencing panel (Health in Code Group, Spain) in conjunction with the NextSeq 550 platform (Illumina, USA) to identify relevant genetic alterations in solid tumors by analyzing a set of genes involved in oncogenesis. The process began with DNA extraction from formalin-fixed, paraffin-embedded (FFPE) tissue samples. Subsequently, the genetic material underwent enzymatic fragmentation and a process of target region enrichment via capture with specific probes. Finally, sequencing was performed using reversible terminator synthesis technology on the aforementioned platform, enabling the precise identification of genomic alterations of interest.

The panel used enables the detection of various types of genetic alterations, including point variants (substitutions, insertions, and deletions), copy number variations (CNVs), and structural rearrangements. These alterations have both diagnostic and prognostic relevance, as well as therapeutic implications, as they constitute potential targets for targeted therapies or predictive biomarkers of response. Furthermore, the panel includes analysis of microsatellite instability (MSI), which is useful for selecting immunotherapies, as well as the study of pharmacogenetic variants related to the efficacy and toxicity of certain chemotherapeutic agents. Taken together, this approach enables a comprehensive molecular characterization of the tumor, facilitating therapeutic decision-making and optimizing the clinical management of cancer patients.

The Action OncoKitDx panel includes [2]:

· Whole-exome sequencing of 55 genes: ALK, ARID1A, ATM, ATRX, BAP1, BRAF, BRCA1, BRCA2, CHEK2, CDH1, CTNNB1, EGFR, ERBB2, ESRI, FGFR1, FGFR2, FGFR3, FGFR4, GNA11, GNAQ, H3F3A, HIST1H3B, HIST1H3H, HRAS, IDH1, IDH2, KIT, KRAS, MAP2K1, MET, 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 BVS.

· Sequencing of hotspot regions in the TSC1, TSC2, and AKT1 genes.

· Analysis of rearrangements in the ALK, BRAF, EGFR, FGFR2, FGFR3, NRG1, NTRK1, NTRK2, NTRK3, RET, and ROS1 genes. The Action OncoKitDx panel uses probes that cover the intronic regions where breakpoints have been most frequently identified: intron 19 of ALK; introns 31–35 of ROS1; introns 9–11 of RET; intron 17 and 3'UTR of FGFR2; FGFR3 intron 17 and 3'UTR; NTRK1 introns 8–12; NTRK2 introns 10 and 12; BRAF introns 7–10; and EGFR introns 7, 23, 24, and 25.

· 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 entire genome, including deletions or gains of entire chromosomes or chromosomal regions.

· Detection of variants related to the patient's pharmacogenetics to assess response or toxicity to chemotherapy treatments. Variants are analyzed in seven genes that affect 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).

Bioinformatic processing of the obtained sequences was performed using the Data Genomics platform, which allows for alignment with the reference genome and subsequent identification of variants through the application of quality filters. Both the panel used and the analysis software are

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certified for in vitro diagnostic use. The system enables the detection of point mutations with a minimum allelic frequency of 5%, provided that the samples have at least 30% tumor cell content and a sequencing depth greater than 200 reads. It should be noted that the use of next-generation sequencing for comprehensive molecular profiling of tumors is in accordance with the recommendations of the European Society for Medical Oncology (ESMO) [2,8–10].

Statistical analysis was performed using the Jamovi software (version 2.7.15.0). Categorical variables were analyzed using Fisher's exact test, due to the small sample size (96 cases) and the fact that the frequency of some categories was less than 5 cases for some variables. A p-value < 0.05 was considered statistically significant for assessing the association between the studied variables.

3. Results

The final sample size consisted of 96 cases, which were classified according to histological subtype (adenocarcinoma, squamous cell carcinoma, adenosquamous carcinoma, neuroendocrine carcinoma, anaplastic carcinoma, and "Not Otherwise Specified" (NOS)). The distribution of cases is shown in Table 1.

Table 1. Number of cases of the different histological subtypes of lung cancer.

HISTOLOGICAL SUBTYPE	Nº OF CASES
ADENOCARCINOMA	65
SQUAMOUS CELL C.	25
NEUROENDOCRINE C.	2
ADENOSQUAMOUS C.	2
ANAPLASTIC C.	1
NOS	1
TOTAL	**96**

The distribution of molecular alterations detected by NGS was evaluated in relation to histological subtype. The results are shown in Table 2.

Table 2. Distribution of mutations in absolute numbers according to histological subtype.

HISTOLOGICAL TYPE	Nº OF CASES	Absent	TP53	KRAS	EGFR	BRAF	FGFR1	ALK	MDM2
Adenocarcinoma	65	13	19	19	19	19	19	3	2
Adenosquamous c.	2	1							
Anaplastic c.	1		1	1	1	1	1		
Squamous cell c.	25	14	8	8	8	8	8		
Neuroendocrine c.	2	1							
NOS	1	1							
TOTAL	**96**	**30**	**28**	**28**	**28**	**28**	**28**	**3**	**2**

HISTOLOGICAL TYPE	Nº OF CASES	MET	PTEN	ARID1A	PIK3CA	TSC1	ATM	AKT
Adenocarcinoma	65	13	19	19	19	19	19	3
Adenosquamous c.	2	1						
Anaplastic c.	1		1	1	1	1	1	
Squamous cell c.	25	14	8	8	8	8	8	
Neuroendocrine c.	2	1						
NOS	1	1						
TOTAL	**96**	**30**	**28**	**28**	**28**	**28**	**28**	**3**

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This initial analysis revealed that the most common histological subtype is adenocarcinoma (67.71% of cases), followed by squamous cell carcinoma (26.04% of cases); in contrast, the remaining subtypes of NSCLC, as well as small cell lung cancer (SCLC) and neuroendocrine carcinoma, are much less common, with only isolated cases found.

Regarding the mutations analyzed by NGS, the most frequent were KRAS (34.38% of cases), TP53 (29.17% of cases), and EGFR (11.46% of cases). The remaining alterations were rare (BRAF, FGFR1, ALK, MDM2, MET, PTEN, ARID1A, PIK3CA, TSC1, ATM, AKT).

After analyzing an overview of the data obtained, a more comprehensive statistical analysis was performed based on the altered genes and histological subtypes, which are presented below.

The KRAS mutation showed a significant association with histological type (p=0.001), being more frequent in adenocarcinoma (Table 3). The absence of mutations also showed a significant association (p=0.002), with a higher proportion in squamous cell carcinomas (Table 4). The remaining mutations did not show significant associations with any of the histological subtypes analyzed (Appendix A, Tables A1–A13).

Table 3. Association between the KRAS mutation and histological subtype (Fisher's exact test).

HISTOLOGICAL TYPE	NO KRAS	KRAS	TOTAL
ADENOCARCINOMA	36	29	65
SQUAMOUS CELL C.	23	2	25
NEUROENDOCRINE C.	2	0	2
ADENOSQUAMOUS C.	1	1	2
ANAPLASTIC C.	0	1	1
NOS	1	0	1
TOTAL	**63**	**33**	**96**
FISHER'S EXACT TEST			**p=0,001**

Table 4. Association between the absence of mutations and histological subtype (Fisher's exact test).

HISTOLOGICAL TYPE	MUTATION	ABSCENCE	TOTAL
ADENOCARCINOMA	52	13	65
ADENOSQUAMOUS C.	1	1	2
ANAPLASTIC C.	1	0	1
SQUAMOUS CELL C.	11	14	25
NEUROENDOCRINE C.	1	1	2
NOS	0	1	1
TOTAL	**66**	**30**	**96**
FISHER'S EXACT TEST			**p=0,002**

Likewise, the association of mutations (22 cases in total) was analyzed according to the different histological subtypes listed below, of which 17 cases present co-mutations of 2 mutations (Table 5), 4 cases of co-mutations of 3 mutations (Table 6), and one case of up to 4 simultaneous mutations (Table 7).

Table 5. Co-mutations of 2 mutations.

HISTOLOGICAL TYPE	TP53 + MET	TP53 + KRAS	TP53 + ALK	TP53 + BRAF	TP53 + EGFR	KRAS + ATM	KRAS + PIK3CA	MDM2 + MET
Adenocarcinoma	1	8	1	1	1	1	1	1
Anaplastic c.		1						
Squamous cell c.					1			

Table 6. Co-mutations of 3 mutations.

HISTOLOGICAL TYPE	TP53 +	TP53 +	TP53 +	TP53 +

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	KRAS + TSC1	KRAS + PIK3CA	PTEN + FGFR1	PTEN + ARID1A
Adenocarcinoma		1		1
Anaplastic c.				
Squamous cell c.	1		1	

Table 7. Co-mutations of 4 mutations.

HISTOLOGICAL TYPE	TP53 + PTEN + ARID1A + BRAF
Adenocarcinoma	1

Regarding the number of molecular alterations per tumor, most cases had a single mutation (74 cases), while 22 cases (22.9%) had two or more mutations. No statistically significant differences were observed according to histology (p=0.255) (Table 8).

Table 8. Distribution of the number of mutations by histological subtype (Fisher's exact test).

HISTOLOGICAL TYPE	1 MUTATION	≥2 MUTATIONS	TOTAL
ADENOCARCINOMA	47	18	65
ADENOSQUAMOUS C.	2	0	2
ANAPLASTIC C.	0	1	1
SQUAMOUS CELL C.	22	3	25
NEUROENDOCRINE C.	2	0	2
NOS	1	0	1
TOTAL	**74**	**22**	**96**
FISHER'S EXACT TEST			**p=0,255**

Of all the cases studied, the most frequent co-mutation was KRAS + TP53 (11 cases), predominantly in adenocarcinoma, although this was not statistically significant (p=0.184) (Table 9). Furthermore, tumors with EGFR mutations rarely presented other associated molecular alterations; only 2 of the 11 EGFR-mutated tumors had co-mutations, showing a statistically significant association (p=0.012) (Table 10).

Table 9. Association between KRAS + TP53 co-mutation and histological subtype (Fisher's exact test).

HISTOLOGICAL TYPE	NO KRAS + TP53	KRAS + TP53	TOTAL
ADENOCARCINOMA	56	9	65
ADENOSQUAMOUS C.	2	0	2
ANAPLASTIC C.	0	1	1
SQUAMOUS CELL C.	24	1	25
NEUROENDOCRINE C.	2	0	2
NOS	1	0	1
TOTAL	**85**	**11**	**96**
FISHER'S EXACT TEST			**p=0,184**

Table 10. Distribution of co-mutationns in tumors with an EGFR mutation (Fisher's exact test).

EGFR MUTATION	EGFR CO-MUTATION		TOTAL
PRESENT	85	0	85
ABSENT	9	2	11
TOTAL	**94**	**2**	**96**
FISHER'S EXACT TEST			**p=0,012**

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Following a thorough analysis of the results obtained via NGS, we also analyzed PD-L1 expression according to histological subtypes (Tables 11 and 12). Of the total cases, 41 cases (42.7%) had negative PD-L1 expression (<1%), 42 cases (43.8%) had low PD-L1 expression (1–49%), and 13 cases (13.5%) had high PD-L1 expression (≥50%). Thus, it can be concluded that most tumors exhibit intermediate PD-L1 expression.

Table 11. Distribution of PD-L1 expression.

PD-L1 EXPRESSION	NUMBER OF CASES	% OF TOTAL
1-49%	42	43.8%
<1%	41	42.7%
≥50%	13	13.5%
TOTAL	**96**	**100%**

Table 12. Association between PD-L1 expression and histological subtype.

HISTOLOGICAL TYPE	PD-L1			TOTAL
	1-49%	<1%	≥50%	
ADENOCARCINOMA	23	31	11	65
ADENOSQUAMOUS C.	2	0	0	2
ANAPLASTIC C.	1	0	0	1
SQUAMOUS CELL C.	15	8	2	25
NEUROENDOCRINE C.	0	2	0	2
NOS	1	0	0	1
TOTAL	**42**	**41**	**13**	**96**
FISHER'S EXACT TEST				**p=0,185**

No significant associations were observed between PD-L1 expression and histological type (p=0.185), nor with TP53 (p=0.744), KRAS (p=0.508), or EGFR

(p=0.242) mutations. However, a trend toward lower PD-L1 expression was observed in tumors with EGFR mutations (Tables 13, 14, and 15).

Table 13. Association between the TP53 mutation and PD-L1 expression.

TP53	PD-L1			TOTAL
	1-49%	<1%	≥50%	
ABSENT	30	30	8	68
PRESENT	12	11	5	28
TOTAL	**42**	**41**	**13**	**96**
FISHER'S EXACT TEST				**p=0,744**

Table 14. Association between the KRAS mutation and PD-L1 expression.

KRAS	PD-L1			TOTAL
	1-49%	<1%	≥50%	
ABSENT	27	29	7	63
PRESENT	15	12	6	33
TOTAL	**42**	**41**	**13**	**96**
FISHER'S EXACT TEST				**p=0,508**

Table 15. Association between the EGFR mutation and PD-L1 expression.

TP53	PD-L1	TOTAL

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	1-49%	<1%	≥50%	
ABSENT	38	34	13	85
PRESENT	4	7	0	11
TOTAL	**42**	**41**	**13**	**96**
FISHER'S EXACT TEST				**p=0,242**

4. Discussion

The molecular characterization of lung cancer using next-generation sequencing techniques has transformed the diagnostic and therapeutic approach to this disease. In this context, the present study provides a comprehensive view of the molecular profile in a real-world clinical cohort, highlighting distinct patterns of genetic alterations based on histological subtype and their relationship with immunological biomarkers.

One of the most relevant findings is the association between KRAS mutations and adenocarcinoma (p=0.001), consistent with previous evidence identifying this gene as one of the main molecular drivers in this tumor subtype; with frequencies around 30%, and significantly more frequent in this histological subtype compared to others [10–13].

Another notable aspect is the higher proportion of tumors without detectable mutations in squamous cell carcinoma. This finding is consistent with recent studies describing that squamous cell lung carcinoma presents a distinct molecular profile, characterized by a lower frequency of classic driver mutations such as KRAS or EGFR, and greater genomic heterogeneity. Far from being interpreted as a true absence of genetic alterations, this result likely reflects the limitations of targeted sequencing panels in capturing the genomic complexity of this subtype. In this regard, squamous cell carcinoma could be characterized by a distinct molecular profile, with alterations less represented in standard panels or with alternative oncogenic mechanisms, underscoring the need for broader analytical strategies for its adequate characterization [13,14].

The remaining mutations did not show statistically significant differences across the histological subtypes analyzed; this may be due to the very small number of cases for the other subtypes because of their low prevalence (neuroendocrine c., anaplastic c.); a larger sample size would be needed to reassess this in the future. Various authors refer to the relationship between various genes such as ALK, BRAF, ROS1, or MET (associated with co-mutation alongside MDM2) in CPCNP tumors; however, most studies do not investigate this relationship more specifically with regard to the different histological subtypes. Some authors note that PIK3CA, FGFR1, or SOX2 are more frequently present in squamous cell carcinoma than in adenocarcinoma; or that mutations in JAK3, NRAS, or VHL1 are found exclusively in small cell carcinoma and not in large cell carcinoma [3,15–17].

Tumors with EGFR mutations showed a statistically significant lower frequency of co-mutations, supporting their role as dominant molecular drivers. This finding is consistent with the literature, which describes that EGFR mutations act as dominant molecular drivers and often exhibit mutual exclusivity with other genetic alterations. This phenomenon of "mutual exclusivity" has been widely described in the literature, reinforcing the validity of our results and providing additional evidence in a clinical practice context. Furthermore, it is described that EGFR mutations exhibit a pattern of mutual exclusivity with other molecular drivers, particularly with KRAS, as both alterations are generally mutually exclusive. The identification of this pattern has significant implications, as it suggests that EGFR-mutated tumors may exhibit a more defined oncogenic dependency, which can influence both response to targeted therapies and clinical course. However, rare cases of co-mutations have been described, such as the coexistence of EGFR with ALK or ROS1, with very low frequencies (less than 1%), indicating that, although uncommon, the presence of concomitant alterations is possible, requiring individualized dual-targeted therapies. This fact could explain the few cases of co-mutation observed in our cohort [18,19].

The presence of multiple mutations in a significant percentage of tumors in our cohort highlights the high molecular heterogeneity of lung cancer. Although no significant differences were observed

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between histological subtypes in terms of the number of mutations, the identification of recurrent patterns, such as the KRAS–TP53 co-mutation, suggests that genomic alterations do not occur in isolation but rather in coordinated patterns reflecting tumor biological complexity and clonal evolution. This phenomenon, widely described in high-throughput sequencing studies, may have relevant biological and clinical implications, particularly regarding response to therapies, including immunotherapy [20].

Our results highlight the tendency of KRAS to coexist with other alterations, particularly TP53, suggesting the presence of cooperative oncogenic programs, predominantly observed in adenocarcinoma, although without reaching statistical significance. This finding is consistent with the literature, which describes that the coexistence of mutations in KRAS and TP53 is relatively common in lung cancer; recent studies indicate that genomic alterations in lung cancer do not occur in isolation but tend to present in patterns of co-occurrence, particularly among genes such as EGFR, KRAS, and TP53. Furthermore, several studies have demonstrated that these co-mutations may have relevant clinical implications, particularly regarding response to immunotherapy, suggesting a potential role as prognostic and predictive biomarkers [20–22].

No significant associations were observed between PD-L1 expression and either the histological subtypes or the genetic alterations analyzed. This finding, far from undermining the results, highlights the complexity of the regulation of this biomarker. However, the literature describes that certain genetic alterations, particularly TP53 and KRAS, may be associated with higher PD-L1 expression and a higher tumor mutational burden, suggesting a more immunogenic tumor microenvironment. However, these findings are not consistent across different studies, suggesting that PD-L1 expression is influenced by multiple biological factors and not exclusively by specific genetic alterations [22–24].

In conclusion, this study provides a real-world integrative analysis of lung cancer molecular profiles using next-generation sequencing, highlighting distinct co-mutation patterns across histological subtypes. In particular, it identifies the enrichment of KRAS mutations in adenocarcinoma and the relative molecular exclusivity of EGFR-mutated tumors, supporting their role as dominant oncogenic drivers. Additionally, the lack of association between genomic alterations and PD-L1 expression underscores the complexity of immune biomarker regulation. These findings contribute to a better understanding of tumor heterogeneity and reinforce the clinical value of NGS in precision oncology.

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Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: We encourage all authors of articles published in MDPI journals to share their research data. In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. Where no new data were created, or where data is unavailable due to privacy or ethical restrictions, a statement is still required. Suggested Data Availability Statements are available in section "MDPI Research Data Policies" at https://www.mdpi.com/ethics.

Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

Associations between various mutations and histological subtypes of lung cancer.

The following tables show the contingency tables for the remaining genes analyzed, with no significant differences according to Fisher's exact test.

Table A1. Association between the TP53 mutation and histological subtype (Fisher's exact test).

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HISTOLOGICAL TYPE	NO TP53	TP53	TOTAL
ADENOCARCINOMA	46	19	65
ADENOSQUAMOUS	2	0	2
ANAPLASTIC C.	0	1	1
SQUAMOUS CELL C.	17	8	25
NEUROENDOCRINE C.	2	0	2
NOS	1	0	1
TOTAL	**68**	**28**	**96**
FISHER'S EXACT TEST			**p=0.678**

Table A2. Association between the EGFR mutation and histological subtype (Fisher's exact test).

HISTOLOGICAL TYPE	NO EGFR	EGFR	TOTAL
ADENOCARCINOMA	58	7	65
ADENOSQUAMOUS	2	0	2
ANAPLASTIC C.	1	0	1
SQUAMOUS CELL C.	22	3	25
NEUROENDOCRINE C.	1	1	2
NOS	1	0	1
TOTAL	**85**	**11**	**96**
FISHER'S EXACT TEST			**p=0.559**

Table A3. Association between the BRAF mutation and histological subtype (Fisher's exact test).

HISTOLOGICAL TYPE	NO BRAF	BRAF	TOTAL
ADENOCARCINOMA	62	3	65
ADENOSQUAMOUS	2	0	2
ANAPLASTIC C.	1	0	1
SQUAMOUS CELL C.	25	0	25
NEUROENDOCRINE C.	2	0	2
NOS	1	0	1
TOTAL	**93**	**3**	**96**
FISHER'S EXACT TEST			**p=0.636**

Table A4. Association between the FGFR1 mutation and histological subtype (Fisher's exact test).

HISTOLOGICAL TYPE	NO FGFR1	FGFR1	TOTAL
ADENOCARCINOMA	64	1	65
ADENOSQUAMOUS	2	0	2
ANAPLASTIC C.	1	0	1
SQUAMOUS CELL C.	24	1	25
NEUROENDOCRINE C.	2	0	2
NOS	1	0	1
TOTAL	**94**	**2**	**96**
FISHER'S EXACT TEST			**p=0.544**

Table A5. Association between the ALK mutation and histological subtype (Fisher's exact test).

HISTOLOGICAL TYPE	NO ALK	ALK	TOTAL
ADENOCARCINOMA	61	4	65
ADENOSQUAMOUS	2	0	2

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ANAPLASTIC C.	1	0	1
SQUAMOUS CELL C.	25	0	25
NEUROENDOCRINE C.	2	0	2
NOS	1	0	1
TOTAL	**92**	**4**	**96**
FISHER'S EXACT TEST			**p=0.671**

Table A6. Association between the MDM2 mutation and histological subtype (Fisher's exact test).

HISTOLOGICAL TYPE	NO MDM2	MDM2	TOTAL
ADENOCARCINOMA	63	2	65
ADENOSQUAMOUS	2	0	2
ANAPLASTIC C.	1	0	1
SQUAMOUS CELL C.	25	0	25
NEUROENDOCRINE C.	2	0	2
NOS	1	0	1
TOTAL	**94**	**2**	**96**
FISHER'S EXACT TEST			**p=1.000**

Table A7. Association between the MET mutation and histological subtype (Fisher's exact test).

HISTOLOGICAL TYPE	NO MET	MET	TOTAL
ADENOCARCINOMA	63	2	65
ADENOSQUAMOUS	2	0	2
ANAPLASTIC C.	1	0	1
SQUAMOUS CELL C.	25	0	25
NEUROENDOCRINE C.	2	0	2
NOS	1	0	1
TOTAL	**94**	**2**	**96**
FISHER'S EXACT TEST			**p=1.000**

Table A8. Association between the ARID1A mutation and histological subtype (Fisher's exact test).

HISTOLOGICAL TYPE	NO ARID1A	ARID1A	TOTAL
ADENOCARCINOMA	63	2	65
ADENOSQUAMOUS	2	0	2
ANAPLASTIC C.	1	0	1
SQUAMOUS CELL C.	25	0	25
NEUROENDOCRINE C.	2	0	2
NOS	1	0	1
TOTAL	**94**	**2**	**96**
FISHER'S EXACT TEST			**p=1.000**

Table A9. Association between the PTEN mutation and histological subtype (Fisher's exact test).

HISTOLOGICAL TYPE	NO PTEN	PTEN	TOTAL
ADENOCARCINOMA	63	2	65
ADENOSQUAMOUS	2	0	2
ANAPLASTIC C.	1	0	1
SQUAMOUS CELL C.	24	1	25
NEUROENDOCRINE C.	2	0	2

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NOS	1	0	1
TOTAL	**93**	**3**	**96**
FISHER'S EXACT TEST			**p=1.000**

Table A10. Association between the PIK3CA mutation and histological subtype (Fisher's exact test).

HISTOLOGICAL TYPE	NO PIK3CA	PIK3CA	TOTAL
ADENOCARCINOMA	63	2	65
ADENOSQUAMOUS	2	0	2
ANAPLASTIC C.	1	0	1
SQUAMOUS CELL C.	25	0	25
NEUROENDOCRINE C.	2	0	2
NOS	1	0	1
TOTAL	**94**	**2**	**96**
FISHER'S EXACT TEST			**p=1.000**

Table A11. Association between the TSC1 mutation and histological subtype (Fisher's exact test).

HISTOLOGICAL TYPE	NO TSC1	TSC1	TOTAL
ADENOCARCINOMA	65	0	65
ADENOSQUAMOUS	2	0	2
ANAPLASTIC C.	1	0	1
SQUAMOUS CELL C.	24	1	25
NEUROENDOCRINE C.	2	0	2
NOS	1	0	1
TOTAL	**95**	**1**	**96**
FISHER'S EXACT TEST			**p=0.323**

Table A12. Association between the ATM mutation and histological subtype (Fisher's exact test).

HISTOLOGICAL TYPE	NO ATM	ATM	TOTAL
ADENOCARCINOMA	64	1	65
ADENOSQUAMOUS	2	0	2
ANAPLASTIC C.	1	0	1
SQUAMOUS CELL C.	25	0	25
NEUROENDOCRINE C.	2	0	2
NOS	1	0	1
TOTAL	**95**	**1**	**96**
FISHER'S EXACT TEST			**p=1.000**

Table A13. Association between the AKT mutation and histological subtype (Fisher's exact test).

HISTOLOGICAL TYPE	NO AKT	AKT	TOTAL
ADENOCARCINOMA	64	1	65
ADENOSQUAMOUS	2	0	2
ANAPLASTIC C.	1	0	1
SQUAMOUS CELL C.	25	0	25
NEUROENDOCRINE C.	2	0	2
NOS	1	0	1
TOTAL	**95**	**1**	**96**

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FISHER'S EXACT TEST	**p=1.000**

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