RESEARCH PAPER

Evaluation Of Mutational Landscape In Non-Small Cell Lung Carcinoma And Its Co-Relation With PD-L1 Expressions

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Abstract: This retrospective study was a systematic evaluation of mutational landscape of non-small cell lung carcinoma (NSCLC) and its association with programmed death-ligand 1 (PD-L1) expression in an Indian patient cohort. One hundred and sixty histopathologically confirmed cases of NSCLC were examined with a specific next-generation sequencing (NGS) panel of 52 oncogenes and tumor suppressor genes and PD-L1 immunohistochemistry (22C3 clone). The most frequent type of alterations was single-nucleotide variations (SNVs) and small insertions/deletions observed in 72.5 % of cases, and fusions of genes in 21.3 %, and copy-number variations (CNVs) in 5.0. EGFR (33.1%) and KRAS (15.0%), PIK3CA (3.8%), and BRAF (3.8%) were the most common drivers. The fusions that are actionable (8.8%-5.6%-3.1%-2.5%) were ALK, RET, MET, and ROS1. 8.1% of tumors had co-occurring changes, most often SNV+CNV or SNV+fusion. It was possible to test PD-L1 status in 86 cases in which 44.2 were PD-L1 positive and 55.8 were PD-L1 negative. The mutations of KRAS, PIK3CA, and NRAS and ALK fusions enriched PD-L1 positive tumours, which is indicative of an immune-inflamed phenotype, whereas the mutations of EGFR were prevailing in PD-L1 negative tumours, which is characteristic of immune-excluded. Significant associations were statistically found between PD-L1 expression and individual mutational patterns ($p < 0.05$). In general, the research shows that the NSCLC presents a significant heterogeneity of genomic and immunologic features, and combined NGS-based profiling with PD-L1 measurement offers a quantitative model of refined patient stratification and accurate choice of therapy in the Indian population.

Keywords: Non-small cell lung carcinoma; next-generation sequencing; mutational landscape; PD-L1 expression; genomic heterogeneity; precision oncology.
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1. Introduction

Lung cancer is still a significant health concern in the world, is the leading cause of cancer deaths. Out of its types, non-small cell lung cancer (NSCLC) represents most of the cases and includes adenocarcinoma, squamous cell carcinoma and large-cell carcinoma. Although screening improvements have been achieved, surgical treatment, and systemic treatments, the overall prognosis of NSCLC is still unsatisfactory (Duma et al., 2019; Gridelli et al., 2015; Hendriks et al., 2024). This is due to the fact that the poor survival outcomes can be explained by the strong molecular and biological heterogeneity of the disease that determines its behaviour, response to therapy, and clinical outcome (Caswell & Swanton, 2017; Dagogo-Jack & Shaw, 2018; Diaz-Cano, 2012).

The pathogenesis of NSCLC is characterized by a large number of changes in the genes controlling cell growth, differentiation, and apoptosis. Oncogenic signalling is initiated and maintained by variations in the epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), KRAS, BRAF, ROS1, RET, and MET known to activate and inhibit the key intracellular pathways including the

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MAPK/ERK, PI3K/AKT, and JAK/STAT (Bou Antoun & Chioni, 2023; Montor et al., 2018; Yang et al., 2023). These mutations have changed the treatment paradigm because specific molecular lesions are targeted with targeted tyrosine kinase inhibitors (TKIs) (Ali, 2016; Yang et al., 2023). Nonetheless, the subsequent sensitivity to TKIs is frequently preceded by acquired resistance, which is often secondary to secondary mutations, bypass track activation, or other genetic events (Marrocco & Yarden, 2023; Westover et al., 2018). It proves that the evolution of the NSCLC is not motivated by one molecular event, but by coexisting genetic networks that define its course of evolution.

With the invention of next-generation sequencing (NGS) technology, it has become possible to screen at the same time a large panel of cancer-related genes, which has the benefit of providing a broad overview of the mutational spectrum within each tumor (Mandelker et al., 2017). NGS data analysis have identified that numerous cases of NSCLC have over single oncogenic alteration i.e. combination of EGFR point mutation and EGFR amplification or ALK and NRAS alterations in the same tumor (Ferreira et al., 2021; Scheffler et al., 2019). Such results indicate that there is clonal diversity present within tumors and that there exists the possibility of genetic events cooperating to regulate growth kinetics and response to treatment (Black & McGranahan, 2021; McGranahan & Swanton, 2017). Knowledge of these molecular interactions is needed to improve classification of diagnoses as well as to develop multi-targeted therapeutic tactics.

In line with the appreciation of genetic diversity, the contribution of the tumor immune microenvironment to NSCLC has also been noted to become more pronounced. PD-1 and PD-L1 are a fundamental regulating pair whereby the tumor cells have used to avoid the immunity(Bai et al., 2017; Caswell & Swanton, 2017; Yi et al., 2022). PD-L1 expression on cancer cells inhibits the activity of cytotoxic T-cells, which enable the immune system to evade the immune system (Juneja et al., 2017). Therefore, PD-L1 has become a quality indicator in use as a biomarker when identifying patients who can be treated with immune checkpoint inhibitors (Doroshow et al., 2021; Meng et al., 2015; Sacher & Gandhi, 2016). However, the correlation between expression of PD-L1 and genomic changes is not homogenous. The tumors with EGFR or ALK mutations are normally less PD-L1 expressive and less responsive to immunotherapy, whereas the ones with KRAS, BRAF mutations or PIK3CA mutations are more likely to have high PD-L1 expression and inflammatory tumor microenvironment (Liu et al., 2022; Rangachari et al., 2017). These correlations imply that the molecular contributors of NSCLC are highly interconnected with its immunological phenotype in a complex interplay, which determines the reaction to therapies ADDIN ZOTERO_ITEM CSL_CITATION Continuous revision of the histologic and stage-wise classification of lung cancer by the World Health Organization (WHO) provides the foundation for therapeutic advances by promoting molecular targeted and immunotherapies and ensuring accurate diagnosis. Cancer epidemiologic data provide helpful information for cancer prevention, diagnosis, and management, supporting health-care interventions. Global cancer mortality projections from 2016 to 2060 show that cancer will overtake ischemic heart diseases (IHD) as the leading cause of death (18.9 million) immediately after 2030, surpassing non-small cell lung cancer (NSCLC), which accounts for 85 percent of lung cancers. The clinical stage at the diagnosis is the main prognostic factor in NSCLC therapies. Advanced early diagnostic methods are essential as the initial stages of cancer show reduced mortality compared to the advanced stages. Sophisticated approaches to proper histological classification and NSCLC management have improved clinical efficiency. Although immune checkpoint inhibitors (ICIs) and targeted molecular therapies have refined the therapeutic management of late-stage NSCLC, the specificity and sensitivity of cancer biomarkers should be improved by focusing on prospective studies, followed by their use as therapeutic tools. The liquid biopsy candidates such as circulating tumor cells (CTCs), circulating cell-free tumor DNA (cfDNA), tumor educated platelets (TEP), and extracellular vesicles (EVs) possess cancer-derived biomolecules and aid in tracing: driver mutations leading to cancer, acquired resistance caused by various generations of therapeutic agents, refractory disease, prognosis, and (Padinharayil et al., 2023).

The changes in environmental exposure, tobacco consumption, diet, and genetic ancestry may affect the occurrence and pattern of oncogenic mutations and the PD-L1 expression pattern (Shklovskaya & Rizos, 2020). In Western and East-Asian cohorts, the studies have indicated that there is a significant variation in EGFR mutation rates as well as in the rate of ALK and ROS1 rearrangements, whereas similar statistics are scarce in the Indian population (Aggarwal et al., 2024; De Guzman, 2025; Mehta et al., 2020). Lack of sufficient genomic-immunologic correlation studies limits possibilities of developing population-specific therapeutic algorithms as well as discovery of new combinatorial biomarkers applicable to local populations of patients.

The current study aims at explaining association between PD-L1 expression, mutational heterogeneity and clinicopathological variables in NSCLC. The study includes 160 histopathologically validated NSCLC, which is studied by means of an Ion Torrent-based targeted sequencing panel consisting of 52 oncogenes and tumor suppressor genes. The analysis compares single nucleotide variants (SNVs), copy number variations (CNVs), and gene fusions and then compares mutational profiles of PD-L1-positive and PD-L1-negative groups. The comorbid genetic changes are also discussed and their potential impact on the aggressiveness of disease and distribution of tumor subtypes are also noted.

Such interrelated molecular and immunological characteristics deserve a close evaluation in order to understand more clearly the heterogeneity of NSCLC. The description of the pattern of PD-L1 expression in relation to particular mutational patterns provides new information on the mechanism of immune resistance and can be used to optimise biomarker-based therapeutic strategies. This type of integrative profiling can help in the creation of tailored treatment regimens in the future that involve combination of molecular-targeted inhibitors and immune-modulating therapies, to enhance patient outcomes.

2. Materials and Methods

2.1 Study Design and Patient Selection

It was a retrospective analysis of 160 patients who had non-small cell lung cancer (NSCLC) conducted via a molecular profiling technique. All the cases were histopathologically identified as either adenocarcinoma or squamous cell carcinoma using the hematoxylin and eosin (H&E)-stained sections. Molecular testing of tumor tissues was received on formalin-fixed paraffin-embedded (FFPE) tissues. Informed consent of the patients was obtained. Inclusion criteria were sufficient tumor content (≥20% viable tumor cells) and yield nuclear acids to be subject to sequencing. Small cell

carcinoma patients, poor tissue samples, and deteriorated nucleic acid were not included in the study. Clinical files and pathology reports were used to get demographic and clinical data, such as age, gender, primary tumor site, histological subtype, and metastatic status.

2.2 Tissue Processing and Nucleic Acid Extraction

FFPE tissue blocks were cut into sections of between 5-10 μm and representative areas of the tumor were determined by a certified pathologist. Xylene and graded ethanol washes were done to perform deparaffinization, which was followed by proteinase K tissue digestion at 56^0 C. The manufacturer of the Thermo Fisher Invitrogen PureLink Genomic DNA Mini Kit and RecoverAll Total Nucleic Acid Isolation Kit were used to extract DNA and RNA respectively, and extract DNA and RNA according to the instructions of the manufacturer.

NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA) was used to measure the purity and concentration of nucleic acids with A260/A280 ratios of 1.8 to 2.0. To check the integrity of the extracted material, the Qubit 3.0 Fluorometer and Agilent TapeStation 4200 were used to measure the quality of extracted material before library preparation.

2.3 Library Preparation and Next-Generation Sequencing

DNA and RNA samples of high quality were prepared by library, using the Ion AmpliSeq Library Kit Plus (Thermo Fisher Scientific). A multigene panel of 52 oncogenes and tumor suppressor genes with a high probability of being relevant to NSCLC was used to detect single nucleotide variants (SNVs), multiple deletions/insertions (indels), copy number variations (CNVs), and fusions of genes.

After the amplification, Qubit dsDNA HS Assay Kit was used to quantify the libraries and they were normalized to equimolar concentrations. The Ion Chef System was used to prepare the templates and load the chips and sequencing was done on the Ion S5 XL platform by using Ion 540 chips. The Torrent Suite Software (version 5.12) was used to process the sequencing data, and the data were aligned to the GRCh37 human reference genome.

Analysis was done with the Ion Reporter Software (version 5.10) with read depth, base quality, and allele frequency predefined filters that were used to call variants and annotate them. The variants were categorized as pathogenic, likely pathogenic or of uncertain importance according to databases such as ClinVar, COSMIC and OncoKB. CNVs were obtained as normalized ratios of read depths, whereas gene fusions were obtained as RNA-based fusion assays of known rearrangement mates (ALK, RET, ROS1, MET, NTRK13, and FGFR3).

2.4 PD-L1 Immunohistochemical Analysis

The expression of PD-L1 was measured on 4 mm FFPE tissue sections with a monoclonal anti-PD-L1 antibody (clone 22C3, Dako/Agilent Technologies, USA). The Dako Autostainer Link 48 staining was done according to standard protocols. The %age of viable tumor cells with membranous staining was divided by the entire %age of the viable tumor cells to obtain the Tumor Proportion Score (TPS).

Cases with PD-L1 expression of 100/100 cell cases were classified as PD-L1 positive and those with 100/100 cells with <100 cell staining as PD-L1 negative. The slides were evaluated by two independent pathologists who graded them without knowing in order to ensure reproducibility.

2.5 Data Integration and Analytical Framework

In each patient, there was a combination of molecular results with PD-L1 status of expression and clinical results. Genetic changes were classified under SNVs, Indels, CNVs, and fused genes. The rate of each change was counted on the whole cohort and then stratified based on the presence of PD-L1 and histopathological subtype.

The analysis was performed through co-occurrence to determine overlapping genetic events in a tumor. Mutations were also compared based on age distribution, tumor location, and metastatic to determine correlations of the molecular profile with the disease aggressiveness.

2.6 Statistical Analysis

The statistical test was conducted with the SPSS software (version 26.0, IBM Corp., USA) and GraphPad Prism (version 9.0). The categorical and continuous variables were summarized using descriptive statistics. The Chi-square test or Fisher exact test was used to assess the links between the expression of PD-L1 and the personal gene variations.

PD-L1-positive and PD-L1-negative groups were compared to determine any differences in the frequency of mutation, co-occurrence and tumor features. Continuous variables like age were compared by the use of the independent samples t-test or the Mann-Whitney U test according to the normality of the data. The threshold of statistical significance was p-value that was below 0.05.

Data were checked on completeness and consistency before statistical computation and graphical representation generated to show distributions of mutations, PD-L1 correlations and co-occurrence patterns by subgroups.

2.7 Quality Control and Validation

Sequencing quality measures were assessed at several points, such as the library quantification, sequencing depth, and variant calling depth. Variants that had a minimum read depth of 500x, score on base quality 30x and allele frequency 5% and above were analyzed. The final dataset did not include known artifacts, synonymous variants and polymorphisms that were already available in population databases like gnomAD.

Each sequencing batch had internal controls and reference standards to guarantee technical reliability. Interobserver concordance testing was used to confirm the reproducibility of PD-L1 scoring. All the analytical procedures were based on CAP-accredited laboratory standards to ensure diagnostic validity.

3. Results and Discussion

3.1 Patient Demographics and Clinicopathological Characteristics

One hundred and sixty (160) patients with non-small cell lung cancer (NSCLC) were studied in order to ascertain the demographic and clinicopathological features of the group. Patient ages varied between 30 and 88 years, with the mean age of 61.7 years and the median age of 62 years, which indicates that the age representation of the population that was under study was mostly middle-aged and old. The pattern of distribution indicates that the correlation between increasing age and aggregate impact of carcinogen exposure and genetic unsteadiness resulting in the development of NSCLC has been established well.

According to Table 3.1, the age group of 61-70years posted the highest number of cases (30.63%), then the 51-60years (28.12%), and then the 41-50years (26.13%). The patients in 71-80-year age bracket were 20 % of all patients with younger people 30-40 years and 41-50 years were only 13.75 %

showing that younger populations had lower disease burden. The distribution also showed that incidence decreased gradually after the eighth decade, which fits in with difficulties of survival and less clinical reporting with age.

Table 3.1: Demographic and Clinicopathological Characteristics of NSCLC Patients

Parameter	Observation
Total Patients	160
Mean Age (years)	61.7
Median Age (years)	62
Age Range (years)	30-88
Predominant Age Group	61-70 years (30.63%)

Table 3.1(a) provides the detailed age distribution of the patients with NSCLC. The data show that the majority of cases were in the age range of 51 and 70 years, which makes almost 60% of the entire cohort, which means that NSCLC in this area is concentrated in the population of the later middle age. This trend is in line with other epidemiological studies conducted in the past, in which malignant transformation is caused by cumulative exposure over decades to carcinogens like tobacco smoke, environmental pollutants, and workplace agents. The graphical representation of the same dataset (Figure 3.1) depicts a distinct unimodal, where the value of the age category in the range of 61-70 is maximum, the age category of 51-60 is just less than that, followed by an age category of 41-50 and the age category of 21-40. The histogram depicts the anticipated epidemiological trend of NSCLC, in which age is an important prognostic and etiologic predictor. The age distribution is associated with the accumulation of DNA damage, epigenetic changes, and decreased cell repair functions, promoting the risk of malignant transformation of the pulmonary epithelial cells. he research carried out also points out that an alarming trend is developing in regard to the use of tobacco and its effects on neoplastic changes that have seen 13.75 % of the young generation become victims.

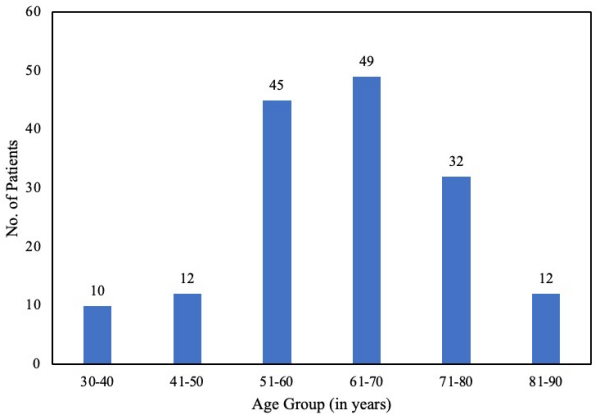

Figure 3.1: Age Distribution of NSCLC Patients

The male-female ratio of the present cohort was around 1.4:1, which means that the male predisposition to NSCLC remains evident according to previous reports on the higher susceptibility of men to long-term smoking and occupational exposure to carcinogenic substances such as asbestos, silica, heavy metals and society taboos. Nevertheless, the %age of female adenocarcinoma cases identified in this case raises an argument indicating an increasing trend of non-smoking related NSCLC in women due to environmental and hormonal influence. Histopathological analysis showed that adenocarcinoma was the most common subtype (73 %), and squamous cell carcinoma (23 %) and unclassified or poorly differentiated forms were the other subtypes (4 %). Adenocarcinomas were typically moderately differentiated, and most commonly arose in peripheral areas of the lungs, squamous carcinomas were distributed in well, moderate and poorly differentiated forms. The metastatic dissemination was found in a considerable %age of the cases, mostly in pleura and liver (add mediastinum), which is consistent with the usual metastatic pattern of NSCLC, which inclines to hematogenous spread to visceral organs. The trends identified highlight the clinical aggressiveness of late-stage NSCLC and the need to use molecular diagnostics in targeted therapy early.

3.2 PD-L1 Expression Patterns and Mutational (SNVs, Indels, CNVs, Fusions and Co-occurrences) Distribution

The molecular and immunohistochemical characterization of the 160 non-small-cell lung cancer (NSCLC) samples showed the strong genomic heterogeneity with the unstable PD-L1 expression. Here, the distribution of PD-L1 status and its mutational classes are systematically provided and a comprehensive analysis of SNVs/Indels, copy-number variations (CNVs), gene fusions, and their interactions between them is analyzed.

3.2.1 PD-L1 Expression Status

Out of the 160 NSCLC cases evaluated, 86 tumor patients had PD-L1 immunohistochemistry (IHC) results (53.7 %), whereas 74 tumor cases (46.3 %) were Not tested according to the requirement by the clinician. In the assessable cases, there was 38 (44.2) PD-L1 positive cases and 48 (55.8) PD-L1 negative that represent 23.8 and 30.0 % of the overall cohort, respectively. This distribution is consistent with the global ranges of PD-L1 expression (2540 % heterogeneous) and the immunophenotype of NSCLC. PD-L1 positivity is activation of the PD-1/PD-L1 axis which can often be due to KRAS, PIK3CA, or MET, whereas PD-L1 negativity is commonly linked to EGFR-mutant, immune-excluded tumours. The fact that a large %age of untested cases occurred underscores the technical variability of IHC testing and points to the necessity of standardized PD-L1 testing and combination with genomic profiling to improve the decision-making process in therapeutic options.

Table 3.2 PD-L1 Expression Summary (n = 160)

PD-L1 Expression Category	No. of Cases	%age (%)	Interpretation
Positive	38	23.8	Immunologically active; potential ICI responders

Negative	48	30	Immune-excluded phenotype; often EGFR-driven

3.2.2 Distribution of Genetic Alteration Classes

The examination of the 160 NSCLC samples using genomic profiling showed that a wide range of molecular changes including single-nucleotide variants (SNVs), small insertions/deletions (Indels), gene fusions and copy-number variations (CNVs) were observed. These ones were SNVs/Indels (116 cases, 72.5%), gene fusions (34 cases, 21.3%), CNVs (8 cases, 5.0%), and no detectable alterations (2 cases, 1.2%). This change hierarchy reflects the mutagenesis-based pathogenesis of NSCLC, in which base replacements and short indels in oncogenic hotspots, especially those of EGFR, KRAS, and PIK3CA, are the major molecular force.

The comparatively low incidence of structural rearrangements (fusions) and CNVs implies that although these events may play a vital role in individual subgroups (e.g., ALK, RET, ROS1, or MET fusions), most tumors of the NSCLC in this cohort are driven by point mutations disrupting important signalling cascades (e.g. MAPK, PI3K-AKT-mTOR, and JAK-STAT). The minor group (1.2 %) that cannot be identified by variants in the sequencing panel is probably either tumors whose alterations are beyond the scope of the sequencing panel or those whose alterations are mediated by epigenetic and non-coding regulatory mechanisms. This distribution, as a whole, supports the idea that SNV/Indel-induced oncogenesis is the molecular basis of NSCLC, and fusions and CNVs are less common but complementing contributors to tumorigenic signaling diversity.

Table 3.3 Alteration Class Summary

Alteration Class	No. of Cases	%age (%)	Key Interpretation
SNV / Indel	116	72.5	Dominant alteration type; nucleotide-level mutagenesis driving oncogenesis
Gene Fusion	34	21.3	Structural rearrangements activating kinase pathways (e.g., ALK, RET, ROS1)
Copy-Number Variation (CNV)	8	5	Gene amplifications influencing signal intensity and therapeutic resistance
Not Tested	74	46.3	Test not required
Total	160	100	—
No Alteration Detected	2	1.2	Possible low tumor purity or off-panel/epigenetic drivers
Total	160	100	—

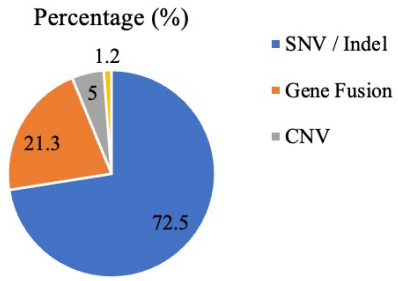

Fig. 3.2 Distribution of alteration classes in the NSCLC cohort

3.2.3 SNV / Indel Spectrum and Functional Significance

Genomic profiling revealed a mutation-dominant NSCLC landscape, primarily driven by EGFR and KRAS alterations. EGFR mutations were most frequent (53 cases; 33.1%), followed by KRAS (15.0%) and PIK3CA (3.8%), while BRAF (3.8%), MET (1.9%), NRAS (1.9%), ERBB2 (1.3%), FGFR2 (1.9%), and CTNNB1 (1.9%) occurred less often. These results confirm that SNVs/Indels (72.5%) represent the principal oncogenic class in NSCLC.

EGFR mutations, located mainly in exons 18–21, activate the RAS–RAF–MEK–ERK and PI3K–AKT–mTOR pathways, promoting proliferation and survival—typical of Asian, non-smoker adenocarcinomas. KRAS mutations, predominantly at codons G12 and G13, act downstream of RTKs and are mutually exclusive with EGFR, defining an inflammatory, PD-L1–high subset amenable to immunotherapy. PIK3CA variants enhance cell survival and confer resistance to TKIs, while BRAF, NRAS, and ERBB2 mutations activate parallel MAPK and receptor kinase signaling routes. Rare FGFR2 and CTNNB1 mutations drive EMT and invasion through FGF and Wnt pathways.

Overall, the cohort exhibits an EGFR–KRAS–PI3K–MAPK–mTOR signaling axis dominance, reflecting the characteristic Asian molecular phenotype of NSCLC, with clear therapeutic implications for TKI- and ICI-based precision oncology.

Genomics profiling showed the presence of mutation dominant landscape of NSCLC mainly caused by EGFR and KRAS changes. They were most common with EGFR (53; 33.1%), KRAS (15.0%), and PIK3CA (3.8%), then BRAF (3.8%), MET (1.9%), NRAS (1.9%), ERBB2 (1.3%), FGFR2 (1.9%), and CTNNB1 (1.9%). These findings corroborate the fact that SNVs/Indels (72.5%) are the major oncogenic group in NSCLC.

Activation of the RAS–RAF–MEK–ERK and PI3K–AKT–mTOR pathways by EGFR mutations, primarily in exons 1821, are characteristic of proliferation and survival, which are typical of Asian, non-smoker adenocarcinomas. KRAS mutations, which are mostly at codon G12 and G13, are RTK downstream and mutually exclusive with EGFR, and these characteristics define an inflammatory, PD-L1-high subset that is subject to immunotherapy. PIK3CA mutations increase cell survival and provide resistance to TKIs, whereas BRAF, NRAS, and ERBB2 mutations stimulate cross-linkage between MAPK and receptor kinase signaling pathways. FGF and Wnt induce EMT and invasion via rare FGFR2 and CTNNB1 mutations.

In general, the cohort presents an RAS–RAF–MEK–ERK and PI3K–AKT–mTOR axis of signatures, which depicts the typical Asian molecular phenotype of NSCLC, and has a direct

clinical implication of TKI- and ICI-mediated precision oncology.

Table 3.4 Top SNV/Indel Frequencies

Gene	No. of Cases	Percentage (%)	Functional Role
EGFR	53	33.1	Activates MAPK & PI3K–AKT–mTOR; key TKI target
KRAS	24	15	RAS pathway activation; mutually exclusive with EGFR
PIK3CA	6	3.8	Promotes survival & therapy resistance
BRAF	6	3.8	MEK–ERK activation; targetable (V600E)
MET	3	1.9	RTK activation; drives EMT & TKI resistance
NRAS	3	1.9	Parallel RAS–MAPK activation
ERBB2	2	1.3	HER2 signaling; targetable subset
FGFR2	3	1.9	FGF pathway; promotes angiogenesis
CTNNB1	3	1.9	Wnt/β-catenin activation; invasion & EMT
Others	9	5.6	Secondary or passenger variants

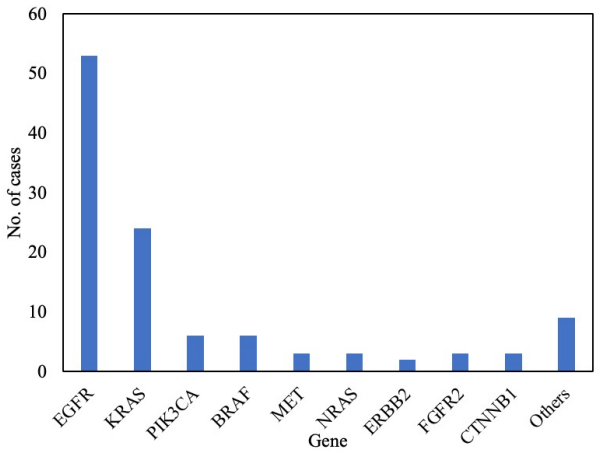

Fig. 3.3 Top SNV/Indel gene frequencies in the cohort

3.2.4 Gene Fusion Profile

Systematic genomic screening revealed 34 fusion-positive cases of NSCLC (21.3%), which marks the importance of chromosomal rearrangements as oncogenic factors. The most common ones were ALK fusion (14 cases; 8.8%), then came RET (9 cases; 5.6%), MET (5 cases; 3.1%), ROS1 (4 cases; 2.5%), and rare EGFR (1.3%), FGFR3 (1.3%), and NTRK1 (0.6%) fusions. These mutations result in constitutively active kinase chimeras that persistently signal via MAPK, PI3K AKT and JAK SAT pathways, inducing unregulated proliferation and survival.

The most actionable subset was ALK rearrangements (mostly EML4–ALK) which are in line with the patterns of Asian incidence in NSCLC, and are highly sensitive to ALKs inhibitors including crizotinib, alectinib, and lorlatinib. The fusions of RET and ROS1 showed similar activities of kinase activation, which were responsive to selpercatinib, pralsetinib, and entrectinib, respectively. The most frequent resistance mechanisms were MET fusions (3.1%), exon 14 skipping variants and NTRK1 fusions were rare but pan-tumor events treatable with larotrectinib or entrectinib.

Table 3.5 Gene Fusion Frequencies

Fusion Type	No. of Cases	%age (%)	Functional / Therapeutic Significance
ALK	14	8.8	Constitutive kinase signaling; sensitive to ALK-TKIs
RET	9	5.6	KIF5B–RET, CCDC6–RET; responsive to selpercatinib/pralsetinib
MET	5	3.1	MET activation or exon 14 skipping; EGFR-TKI resistance driver
ROS1	4	2.5	CD74–ROS1, EZR–ROS1; targetable with crizotinib/entrectinib
EGFR (Fusion)	2	1.3	Enhances receptor dimerization and signaling
FGFR3 (Fusion)	2	1.3	FGF pathway activation; emerging FGFR inhibitor target
NTRK1 (Fusion)	1	0.6	Pan-cancer actionable; larotrectinib sensitive
Others (Rare)	3	1.9	Includes atypical or low-frequency fusions such as ETV6–NTRK3, BRAF–KIAA1549, and FGFR2–BICC1, which are involved in aberrant kinase activation or transcriptional dysregulation; rare but potentially targetable with next-generation kinase inhibitors depending on the fusion partner and pathway activation profile

3.2.5 Copy-Number Variations (CNVs)

The importance of CNV analysis revealed that only a small yet biologically meaningful fraction of genomic amplifications leads to the regulation of the oncogenic signaling intensity and therapeutic resistance. Out of the 160 cases of NSCLC, the 8 of them (5.0%) had CNV events and these were mostly EGFR,

ERBB2 (HER2) and FGFR2 loci. Namely, EGFR amplification was the most common one (6 cases; 3.8%), then ERBB2 amplification (2 cases; 1.3%), and FGFR2 copy gain (1 case; 0.6%). These amplifications are amplifying enhancers of oncogenic pathways, commonly coupled with complement to the activity of SNVs to increase the signal output and aggressive tumor phenotypes.

The most common CNV is EGFR amplification and in most cases it behaves in co-existence with L858R mutations, EGFR exon 19 deletions or both. This twofold change augmentation of receptor activity by adding gene dosage and overexpressing proteins stimulates continued activation of MAPK and PI3K–AKT–mTOR cascades. Clinically, the EGFR amplification correlates with resistance to the first and the second generations of TKIs and possibly as a secondary effect occurs after therapy, thus longitudinal tracking of CNV is necessary during treatment.

ERBB2 amplification (2 cases) enhances receptor dimerization and hyperactivation of underlying proliferative pathways which is frequently associated with poor prognosis but sensitivity to the HER2-targeted therapy trastuzumab deruxtecan or afatinib. Although rare, FGFR2 copy gain is able to initiate the FGF signaling axis, and stimulate angiogenesis and epithelial mesenchymal transition (EMT), and can be inhibited by new types of FGFRSN inhibitors.

Table 3.6 CNV Summary

Gene (CNV)	No. of Cases	%	Functional / Therapeutic Significance
EGFR Amplification	6	3.8	Increases receptor dosage and TKI resistance; common co-driver with EGFR SNVs
ERBB2 (HER2) Amplification	2	1.3	Enhances HER2 signaling; targetable with HER2 inhibitors
FGFR2 Copy Gain	1	0.6	Activates FGF pathway; promotes EMT and angiogenesis
Others / non-significant	—	—	Minor CNVs with uncertain clinical impact
Total	8	5	Reflects low-frequency but high-impact genomic amplifications

3.2.6 Co-occurrence Analysis

Co-occurrence analysis showed that, whereas the majority of NSCLC tumors were events caused by single dominant alteration, a minority of events were associated with multi-drivers events with increased molecular complexity. Of 160 cases (147, or 91.9% had a single alteration type and 13, or 8.1% had multiple alterations): SNV/Indel (5.0%), Fusion (5.0%), and CNV (2.5%) or Fusion (0.6%).

Single-dose tumors are in line with the concept of oncogene tumor progression (e.g., EGFR, ALK, KRAS), whereas group mixtures are reflective of clonal changes at the expense of an outdated signaling (an element of therapeutic resistance).

Examples of these are EGFR SNV + CNV, an amplifier of receptor signaling, and EGFR + MET or ALK + NRAS dual changes, which permit an offsetting effect on pathways.

All these co-alterations cause convergent signaling through MAPK, PI3K-AKT and JAK-STAT signaling that persists due to focused inhibition. Such complexity in the clinical has led to the need to treat with combination or sequential targeted therapy (e.g., EGFR + MET or ALK + MEK blockade). Therefore, the implementation of multi-driver SNV, fusion, and CNV detection combined to create a complete genomic profiling approach is critical to precision therapy in multi-driver NSCLC.

Table 3.7 Co-occurrence Summary

Alteration Combination	No. of Cases	%age (%)	Functional Interpretation
Single alteration (SNV / Fusion / CNV)	147	91.9	Classical oncogene addiction; single dominant driver (EGFR, ALK, KRAS)
SNV + Fusion	8	5	Parallel activation of signaling pathways; compensatory resistance mechanism
SNV + CNV	**4**	**2.5**	Quantitative amplification of existing driver mutation (e.g., EGFR SNV + CNV)
Fusion + CNV	1	0.6	Rare; dual kinase activation leading to high aggressiveness
Total	160	100	Majority single-driver; minority multi-driver with adaptive complexity

3.2.7 PD-L1-Stratified Mutational Patterns

The stratified analysis of PD-L1 showed that there were different molecular-immune associations in the NSCLC cohort. Out of 86 cases, which could be assessed, 38 cases were PD-L1 positive and 48 cases were PD-L1 negative with typical genomic signatures.

PD-L1-positive tumors showed increased mutational diversity with frequent KRAS (4), PIK3CA (3), NRAS (2), MET (2) and ALK fusions (3). These mutations trigger the NF-KB- and STAT3-pathways and trigger a cytokine release (IL-6, IFN-g) and promote the up-regulation of PD-L1 to provide an inflamed microenvironment to immune checkpoint inhibitors (ICIs).

By contrast, PD-L1-negative tumours were EGFR-mutant (20 cases) which have an immune-excluder phenotype with inhibited interferon signatures and an inferior ICI responsiveness despite high oncogenic activity. The mutations of other groups like ALK and BRAF were found in both groups meaning that there was context-dependent immune modulation.

All in all, the KRAS- and PIK3CA-driven tumors are associated with the PD-L1 positivity and immune activation, whereas EGFR-driven tumors are PD-L1-low and immune-cold. Mutation profiling in combination with PD-L1 can be used to achieve a deeper stratification of the therapeutic approach which includes the use of targeted therapy in cases

of EGFR-dominant, PD-L1-negative, and ICI-based therapy in cases of KRAS/PIK3CA-driven, PD-L1-positive tumors.

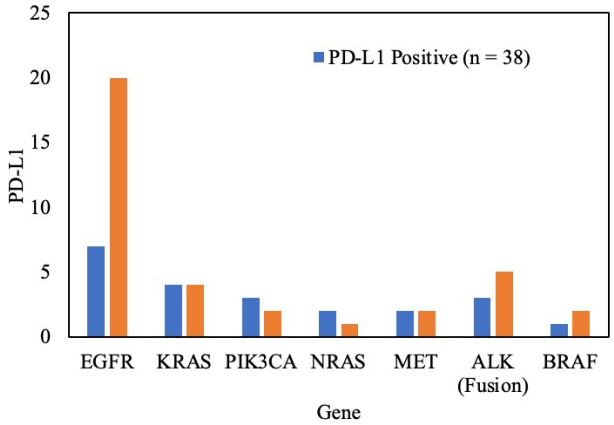

Figure 3.3 PD-L1-Stratified Distribution of Key Genes

3.2.8 Integrated Discussion and Clinical Implications

Combined molecular and immunohistochemical analysis of 160 cases of NSCLC unveils two- axis heterogeneity that is controlled by genomic changes and immune checkpoint interactions. The mutation was mutation dominant, SNVs/Indels (72.5) were the dominant type of alteration with fusions (21.3) and CNVs (5.0) coming in second and third position respectively depicting that nucleotide-based mutagenesis was the leading oncogenic event in NSCLC. This molecular pattern (high rate of EGFR mutations -33.1 % and relatively low rate of KRAS -15 %) indicates a typical pattern of Asiatic population of NSCLCs, whereas a major subset, which is sensitive to tyrosine kinase inhibitors (TKIs), consists of fusion-driven tumors (e.g., ALK, RET, ROS1).

Molecular redundancy shown in 8.1 % of tumors with EGFR SNV + CNV or EGFR + MET fusions will induce parallel signaling cascades (MAPK, PI3K5AKT, JAK5STAT) and keep growth and drug resistance. This promotes the principle of convergence of functional pathways, which highlights the importance of combining or sequencing therapy (e.g. EGFR + MET blockade) in multi- driver tumours.

The PD-L1 stratified analysis contributes to the immunogenomic interaction of NSCLC further. KRAS, PIK3CA, and NRAS-enriched PDL1-positive tumors demonstrate inflammatory response due to cytokines and are immunologically hot, which is associated with positive outcomes with immune checkpoint inhibitor (ICI) use. Conversely, PD-L1-negative and EGFR-driven tumors are cases of immune-exclusion phenotypes, with active oncogenic signaling presumably silencing interferon signaling and antigen presentation, limiting the effect of ICI. Such observations reiterate that the PD-L1 expression would not suffice to foretell the immunotherapy results without considering the genomic background.

These findings, clinically, confirm the significance of multi-dimensional molecular profiling that is capable of capturing SNVs, fusions, CNVs, and PD-L1 expression at the same time. This type of integrative diagnostics enables accurate tumor subtyping, directs individualized treatment approaches, and rational combination therapy, such as EGFR-TKIs and anti-PD-1/PD-L1 antibodies or MET inhibitors in order to overcome resistance and enhance survival.

Lastly, the conjunction of oncogenic signals and immune modulation determines the pathogenesis and response to therapy with NSCLC. The combination of mutational drivers and variable PD-L1 expression is characteristic of separate biological subgroups of EGFR-drived immune-cold and KRAS/PIK3CA-drived immune-active populations, which would have to be managed in a specific manner. Thus, extensive genomic-immunologic integration is the basis of next-generation precision oncology in NSCLC.

3.3 PD-L1 Positive and Negative–Stratified Mutational Patterns Across NSCLC Subtypes

The layers of PD-L1 expression with genetic modification underlying the expression across the subtypes of NSCLC are valuable in revealing the interaction between tumor biology and the immune phenotype. Out of the 86 cases that were assessable, 38 cases were PD-L1 positive (44.2%) and 48 cases were PD-L1 negative (55.8%), which is a heterogeneous pattern of immune landscape that is dictated by molecular subtype.

The tumors of the PD-L1-positive group had a wide spectrum of mutations as KRAS (4 cases), PIK3CA (3 cases), NRAS (2 cases), MET (2 cases), and ALK fusions (3 cases). These changes have been shown to activate signaling via the NF- 5 B, STAT3, and PI3K -AKT-mTOR pathways, which are capable of increasing cytokine activity and stimulating the expression of PD-L1. The mutations that caused such tumors typically exhibited the characteristics of the so-called immune-inflamed phenotype, which is increased immune cell infiltration and activation of the interferon pathway. The above molecular-immune dependence implies that it is possible that such tumors can respond better to active immune checkpoint inhibitors (ICIs) because of active immune interactions in the tumor microenvironment.

Comparatively, the PD-L1 negative group was substantially characterized by the presence of EGFR-mutant tumor (20 cases). These are generally oncogenic and low-immune-activated tumors that can be referred to as immune-excluded or cold. Mechanistically, EGFR activation inhibits interferon signals and antigen presentation leading to lessening of PD-L1 expression and loss of responsiveness in ICIs. The other mutations which included ALK fusions, BRAF and MET were identified in both types of PD-L1 and could be attributed to their unpredictable impact on immune regulation based on co-occurring genetic or environmental factors.

Adenocarcinomas were the most common PD-L1-positive, mutational, and KRAS/PIK3CA mutant cases (in each case across the histological subtypes). By contrast, the squamous cell carcinomas were less frequently PD-L1-positive and less frequently contained driver mutations, but were frequently characterized by a greater mutational load caused by neogenomic genomic damage, which might enable the immune surveillance of neoantigen expression events as opposed to conventional oncogenic signaling.

Therefore, the immune-active and mutation-diverse tumors were observed in PD-L1-positive NSCLCs, and EGFR-based and immune-muted tumors were predominant in PD-L1-negative tumors. The results of the study underscore the idea that PD-L1 expression needs to be regarded as a part of the overall genetic setting to make clinical decisions. Tumors that are EGFR mutated and exhibit a low PD-L1 level are better able to respond to EGFR therapies whereas those with KRAS, PIK3CA or NRAS mutation and the high PD-L1 expressors can benefit more with immunotherapy, or social methods.

Table 3.8: PD-L1 Positive vs. Negative: Mutational Distribution Across NSCLC Subtypes

Gene / Alteration	PD-L1 Positive (n = 38)	PD-L1 Negative (n = 48)	Dominant Subtype Association	Clinical Interpretation
EGFR	**7**	**20**	Adenocarcinoma	Immune-excluded phenotype; good TKI response, low ICI benefit
KRAS	**4**	**4**	Adenocarcinoma	Immune-inflamed; good response to ICIs and KRAS inhibitors
PIK3CA	**3**	**2**	Adenocarcinoma / Squamous	Activates AKT/mTOR pathway; contributes to PD-L1 expression
NRAS	**2**	**1**	Adenocarcinoma	Enhances inflammatory signaling and PD-L1 induction
MET	**2**	**2**	Adenocarcinoma	Dual role in oncogenic activation and immune regulation
ALK (Fusion)	**3**	**5**	Adenocarcinoma	Mixed PD-L1 expression; sensitive to ALK-TKIs
BRAF	**1**	**2**	Adenocarcinoma	Limited immune activity; moderate targetability
ERBB2 (Ampl./ Mut.)	**1**	**1**	Adenocarcinoma	HER2 signaling; poor immune activation
FGFR2	**1**	**0**	Squamous / Mixed	Promotes angiogenesis; potential target for FGFR inhibitors
CTNNB1	**1**	**0**	Adenocarcinoma	Wnt/β-catenin activation; linked to immune exclusion

3.4 Correlative Insights Between Molecular Alterations and Tumor Behavior

The relationship between molecular changes and tumor phenotype in non-small cell lung cancer (NSCLC) emphasizes the fact that certain genetic changes determine the biological aggressiveness, immune interaction, and the therapeutic response of the tumor. Differentiated mutation-based signals affecting the pace of cellular multiplication, angiogenesis, metastasis, and susceptibility to target-based and immune-based treatment. The clinical phenotype and mutational and structural data of the cohort reveal a distinct association between the genomic architecture and clinical phenotype in the NSCLC subtypes.

The most common genetic changes were EGFR mutations which were most common in adenocarcinoma and in most cases, non-smokers and female patients. These tumors exhibited active PI3K–AKT-mTOR and the MAPK signaling, which resulted in increased cell division and extended survival. EGFR-mutant tumors were generally lowly expressed PD-L1, less immune-cell infiltrated, and less exposed to interferon, which created an immune-excluded phenotype. The growth pattern of tumors in such cases indicated a great reliance on receptor tyrosine kinase activation as opposed to immune modulation. EGFR-driven tumors were sensitive to the tyrosine kinase inhibitor, or TKI, including erlotinib, gefitinib, and osimertinib. Secondary molecular alterations including T790M mutation or amplification of MET tended to arise throughout therapy, which is an adaptive resistance mechanism that indicates plasticity.

KRAS mutations characterized a unique molecular and immunologic subtype of high cellular activity and inflammatory signalling. The NF-KB, JAK-STAT, and MAPK pathways were activated leading to enhanced expression of pro-inflammatory cytokines such as interleukin-6 and interferon-gamma and the release of PD-L1. These tumors were associated with an immune active and proliferative phenotype, implying sensitivity to immune checkpoint inhibitors (ICIs). KRAS-driven NSCLCs were characterized by an increase in metabolic rates in tumors, tumor-infiltrating lymphocytes density, and increased chances of epithelial-mesenchymal transition (EMT). The combination of PIK3CA or TP53 mutations enhanced this inflammatory phenotype,

and generated highly adaptive and metabolically active tumor behaviour with unpredictable response to treatment.

PIK3CA mutation and ERBB2 amplification were other tumor progression mechanisms. Mutations in PIK3CA enhanced AKT/mTOR signaling that facilitated angiogenesis, cell growth and survival in stress. ERBB2 (HER2) overexpression increased the intensity of receptor signaling and provided aggressive growth with less apoptosis. Tumors that contained these mutations were mostly linked to quick progression of the disease and low rate of immune infiltration. Molecular targeting of the HER 2 with trastuzumab or afatinib showed better treatment response in these subsets together with cytotoxic therapy or anti-angiogenic therapy.

A structurally driven oncogenic subtype was described as manifested by ALK, RET and ROS1 fusions, which are common in patients with adenocarcinoma and no history of smoking. The resulting rearrangements generated constitutively active kinase fusion proteins that were incessantly activating the downstream RAS–RAF–MEK and PI3K–AKT pathways. Tumours containing such fusions were highly proliferative in nature and relied on fusion event to grow. The expression of PD-L1 has been different across these cases and in most cases, it can be associated with the activation of the STAT3 pathway. Clinical response to TKIs including crizotinib, alectinib and lorlatinib was strong, but resistance as a result of secondary mutation in the kinase domain or bypassing via activation of MET or EGFR was common and disease recurrence ensued.

Aggressive behavior and redundant signaling were related with alterations in BRAF, NRAS, and MET. The activation of MEKERK cascade (median effect) by BRAF mutations induced mitotic acceleration and metastatic potential. NRAS mutations stimulated downstream MAPK actively and in some cases were associated with immune activation depending on co-alterations. MET exon 14 deletions and amplification of the gene increased invasive ability by stimulating epithelial mesenchymal transition signaling, and acted as primary resistances and secondary mechanisms of resistance in EGFR-mutant tumors.

Amplifications of the oncogenes EGFR, ERBB2, and MET were related to a powerful oncogenic signaling response and poorer therapy response. CNVs enhanced density of receptors, ligand-independent activation and enhanced downstream signaling. The presence of CNVs and SNVs in the same gene increased oncogenic signaling to produce tumors with high growth rates, high probability of recurrence, and low drug sensitivity. These data suggest that the effect and dosage of genes are significant determinants of tumor aggressiveness and therapeutic resistance.

Less common but also associated mutations in CTNNB1 and FGFR2 were associated with particular biological behaviors. The activation of CTNNB1 caused a sustained Wnt/ 0 -catenin signaling, which led to T-cell exclusion and suppressed immune surveillance. Changes in FGFR2 stimulated angiogenic signaling, stromal remodeling and migration, implying an increase in invasive capacity. These infrequent mutations helped to increase intratumor heterogeneity and immunotolerance.

The pathophysiology of the NSCLC tumors is highly determined by the character of their underlying drives and the extent of immune suppression linked to them. The tumors with alterations of EGFR- and ERBB2 were highly proliferative yet immune silent and were optimal in treating them using targeted inhibitors. Mutations KRAS-, PIK3CA-, and NRAS-mutated tumors had both proliferative and immunogenic phenotypes to support immunotherapy-based regimens. The fusion-driven tumors which were reliant on certain kinase activation were sensitive to targeted inhibitors however they were subject to secondary resistance. Combination therapies were demonstrated to be essential since CNV-positive tumors depicted increased signaling and rapid tumor progression. Genetic changes and tumor phenotype allow defining the molecular context of the NSCLC development and offer the concept of personalized therapeutic interventions, combining the molecular along with immune-based strategies.

Table 3.9: Relationship Between Key Molecular Alterations and Tumor Behavior in NSCLC

Molecular Alteration	Dominant Pathway	PD-L1 / Immune Profile	Behavioral Features	Therapeutic Response
EGFR	MAPK, PI3K–AKT	Low PD-L1 (immune-cold)	Rapid proliferation; early metastasis	Sensitive to TKIs; develops resistance via MET amplification
KRAS	MAPK, NF-κB, STAT3	High PD-L1 (immune-inflamed)	Aggressive, inflammatory; variable differentiation	Responds to ICIs; benefits from KRAS and PD-1 combination therapy
ALK / RET / ROS1 Fusions	Tyrosine kinase signaling	Intermediate PD-L1	Highly proliferative; single-driver dependence	Strong response to TKIs; resistance via secondary mutations
PIK3CA	PI3K–AKT–mTOR	Moderate PD-L1	Promotes angiogenesis and resistance	Potential benefit from PI3K/mTOR inhibitors
ERBB2 Amplification	HER2 signaling	Low PD-L1	Rapid progression; invasive phenotype	Responsive to HER2-targeted drugs
MET Amplification / Mutation	PI3K–AKT, JAK–STAT	Variable	Promotes EMT and TKI resistance	Targetable by MET inhibitors

BRAF / NRAS	MAPK	Moderate PD-L1	Increased metastatic potential	Sensitive to BRAF/MEK inhibition
CTNNB1 / FGFR2	Wnt / FGF	Low PD-L1	Immune exclusion; stromal invasion	Poor ICI response; potential FGFR inhibitors effective

3.5 Integrated Discussion and Clinical Implications

The combination of molecular and immunological results of the NSCLC cohort characterizes a distinct relationship between the genomic structure of the tumor, the immune microenvironment and the related therapeutic behavior. The analysis confirms the presence of a concerted effect of oncogenic signaling, variations in gene dosage, and immune suppression control tumor development and treatment response, and no single-mutation dominance. The interaction of these molecular systems gives significant understanding of the heterogeneity of NSCLC and it is the basis of designing the mechanism-oriented therapeutic approaches that are specific.

EGFR mutant-driven tumors expressed a unique, molecularly active, but immunologically silent phenotype, i.e. robust proliferative rumination and weak immune contact. Continuous stimulation of MAPK and PI3K–AKT/mTOR signaling stimulated the accelerated tumor growth and early metastasis. Nevertheless, inhibition of antigen-presenting machine and interferon-dependent signaling inhibited immune cell infiltration leading to the low levels of PD-L1 and the reduced effect of immune checkpoint blockage. These tumours were responsive to EGFR-targeted tyrosine kinase inhibitors (TKIs) but were especially more likely to develop the secondary resistance, especially, making replacements via T790M substitutions, MET amplifications or ERBB2 co-activations. The clinical implication is that sequential or combination therapy is required with the introduction of third-generation TKIs together with MET or HER2 inhibitors to delay resistance and increase therapeutic effect.

On the tumors of the KRAS-driven, a different biological orientation was observed. The constitutive stimulation of the RAS pathway caused the release of cytokines and the inflammatory signaling of NF-KB, producing an immune-inflamed phenotype with moderate and high PD-L1 expression respectively. These tumors were observed to be more responsive to immune checkpoint inhibitors (ICIs) and were a molecular subgroup on which immune-targeted and molecular-targeted therapies could be used synergistically. KRAS inhibitors (e.g., sotorasib, adagrasib) and PD-1 blockage used as combination therapy can be clinically relevant in this subgroup as they have the effect of blocking the pathway and also activating immunotherapy. The interaction between KRAS and PIK3CA signaling also emphasized the fact that several oncogenic nodes may interact to tune tumor metabolism and immune tone which requires a dual-pathway suppression approach.

Fusion-positive subgroup (with defects in ALK, RET, and ROS1) was a structurally away oncogenic family characterized by chimeric protective action and significant reliance on solitary driver incidences. TKIs like crizotinib, alectinib and lorlatinib showed high response to these tumors indicating high pathway addiction. Long-term response was however curtailed by the development of secondary kinase domain mutations and alternate signaling activation. These results indicate that the expression of PD-L1 in these cases is also variable, and that fusions can indirectly regulate the immune microenvironment under the control of transcriptional activity of STAT3. Combined molecular immunologic approach, sequential TKI/ICI administration, is one approach that might improve the durability of response and disease control in this molecular class.

The PIK3CA, ERBB2 and MET-mutated tumors had a predilection to adaptive resistance and quantitative signaling amplification. The presence of CNVs in these genes enhanced the strength of receptor activation and downstream signaling and stimulated the rapid progression of tumors and reduced drug susceptibility. These genomic amplifications more especially when combined with activation of SNVs led to saturation of signals enabling bypass of therapeutic inhibition. Frequent re-examination of the molecules with the next-generation sequencing (NGS) or liquid biopsy would assist in detecting these emerging CNV-based resistance mechanisms as they occur, enabling appropriate therapeutic adjustment. The clinical value of multi-target monitoring can be supported by therapeutic applicability by targeting these amplifications using PI3K/mTOR, HER2, or MET inhibitors.

Tumors with BRAF, NRAS or CTNNB1 mutations had inconsistent and severe phenotypes. Activation of BRAF maintained mitogen-activated protein kinase signaling and stimulated acceleration of the cell-cycle, and changes in NRAS enhanced identical pathways with partial immune interactions. Activation of Wnt/ 8 -catenin signaling by Ctnnb1 mutations generated immune exclusion and decreased T -cell infiltration, which accounted for their poor immunotherapy response despite active proliferation. These results unanimously affirm that not every oncogenic prototyped network has the same potential to activate the immune system and the capability of the tumor to generate immunity determined by the type of signaling network instead of by the number of mutations alone.

Combination of the PD-L1 expression patterns with the molecular data created two significant NSCLC immunogenomic phenotypes. The predominant group of EGFR- and ERBB2 was an immune-silent, oncogene-driven phenotype, having low PD-L1 and little response to ICIs but a high response to precision-targeted TKIs. Contrary to that, the KRAS-, PIK3CA-, and NRAS-dominant group had an immune-active, inflammation-related phenotype, which is characterized by elevated levels of PD-L1, cytokine signaling, and positive ICI responsive profiles. The intermediate immune behavior of tumors that were driven by fusion represented partial activation of immune-recognition markers through fusion with other cells in tumors. Numerous driver events being detected in coexistence with each other in selected tumors (such as EGFR SNV + CNV or EGFR + MET fusions) described functional convergence and redundancy of pathways, evoking the necessity of combination therapeutic interventions, which may take care of parallel and sequential structures of activation.

The general clinical implication of these observations is the fact that the molecular behavior of tumors is a multidimensional process. Detection of mutational patterns, signaling cross-talk, and immune features are critical factors of interplay in the determination of the therapeutic result in NSCLC, not only through the expression of a single driver

mutation. Strict molecular-immunologic profiling, which involves the use of genomic sequencing and PD-L1 analysis tools can be used to efficiently stratify patients based on their response to therapy. This unified framework aids in the design of rational therapy, TKIs against the tumors of EGFR/ALK, ICIs against the tumors of KRAS/PIK3CA, and dual-inhibition programs against the tumors of multi-driving or CNV-enriched. There is also a need to continue genomic surveillance of the first resistance and adaptation of treatment regimens.

The analysis confirms that genomic signaling activity and immune microenvironmental adaptation result in the dynamics of the progression and response to treatment of NSCLC. Generation EGFR-mutant and fusion-driven tumors on constitutive molecular activation led to survival whereas KRAS- and PIK3CA- mutant tumors include oncogenic signaling with inflammatory responses. Copy-number amplification is another enhancement of proliferative signaling and hastens resistance. Such insights combined will create the foundation of the shift of clinical practice beyond mutation-specific therapy to multi-axis precision oncology in which the molecular drivers and the immune status of the patient are considered to provide a long-term and effective control of the pulmonary cancer progression.

4. Conclusions

Based on the integrated analysis of genomic alterations and PD-L1 expression patterns, the following conclusions are drawn:

1. As the initial study showed, genomic heterogeneity in the NSCLC cohort was pronounced, and SNVs/Indels were the most frequent type of altered (72.5%), followed by gene fusions (21.3%) and CNVs (5.0%), which once again verified nucleotide-level mutagenesis as the prevailing oncogenic mechanism.
2. Mutations of EGFR made up the highest frequency of drivers (33.1%) and were most commonly linked to PD-L1-negative, immune-excluding tumors, which suggested that the preferential advantage of tyrosine kinase-inhibitor based targeted therapy over immunotherapy.
3. PD-L1 positive tumors exhibited KRAS, PIK3CA, and NRAS changes more often, and this suggests that they are immune-inflamed and more likely to respond to immune checkpoint inhibitors.
4. ALK-RET, ROS1-MET gene fusions were also actionable (21.3%) and oncogenic drivers likely to be highly therapeutic.
5. Along with co-occurring genomic changes with 8.1 % of cases, molecular redundancy and pathway convergence were found, which emphasize the importance of combination or sequential targeted treatment methods.
6. Combinatory study of genomic changes and PD-L1 expression will be an effective paradigm of precision oncology to provide better patient stratification and drug therapy maximization in NSCLC.

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