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Mutation Analysis of Advanced Non-Small Cell Lung Cancer: A Retrospective Observational Study from South India

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Abstract

Introduction Lung cancer is a leading cause of cancer-related mortality worldwide, with non-small cell lung cancer (NSCLC) being the predominant type. Comprehensive genomic profiling plays a crucial role in identifying actionable mutations to guide personalized treatment strategies, especially in regions like India where late-stage diagnoses are common.

Objective This retrospective observational study aimed to determine the prevalence of actionable mutations in patients with advanced NSCLC from a tertiary cancer care center in South India.

Materials and Methods A retrospective observational study was conducted at the Department of Medical Oncology, Government Medical College, Kozhikode, South India. The study included 50 histologically confirmed patients with advanced NSCLC who underwent molecular testing between November 2022 and July 2024.

Results Among the 50 patients, adenocarcinoma was the predominant histological subtype (70%), with *TP53* and *EGFR* mutations found in 42 and 34%, respectively. Co-mutations, including *TP53* + *EGFR* exon 19 deletions, were observed in 4% of cases. Low PD-L1 expression (<1%) was identified in 78% of patients, suggesting limited eligibility for single agent immunotherapy.

Conclusion Comprehensive genomic profiling is largely inaccessible to most patients in India due to high costs, but targeted next-generation sequencing (NGS) panels offer a cost-effective way to optimize treatment. This study highlights the heterogeneity of mutations in NSCLC in South Indian patients and showcases the importance of targeted NGS panels in optimizing therapeutic strategies.

Keywords
- non-small cell lung cancer
- mutations
- personalized treatment
- immunotherapy
- EGFR mutations

Introduction

According to GLOBOCAN 2022, lung cancer was the most frequently diagnosed cancer in 2022, responsible for almost 2.5 million new cases, with an estimated 1.8 million deaths.[1] Non-small cell lung cancer (NSCLC) is the most frequently diagnosed type of lung cancer, with adenocarcinoma identified as the predominant histological subtype.[2] In India, an alarming 75% of patients with lung cancer are diagnosed at advanced stages. Around 66% patients are diagnosed at stage

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IV.[3,4] Approximately only 3.5 to 7.2% patients are diagnosed at an early stage, which considerably limits treatment options and survival chances. In India, patients with lung cancer have a median survival period of 8.8 months, improving to 12.6 months with specific treatments. The 5-year survival rates range from 92% at stage I to as low as 3 to 8% at stage IV.[3,5]

International guidelines published by National Comprehensive Cancer Network and European Society for Medical Oncology recommend molecular testing for clinically relevant biomarkers in advanced NSCLC.[6,7] International Consensus Guidelines also emphasize the critical role of molecular testing in the management of NSCLC, particularly in identifying actionable genetic alterations such as EGFR (epidermal growth factor receptor) mutations, ALK and ROS-1 rearrangements, and PD-L1 overexpression.[8] EGFR mutations are typically analyzed using real-time polymerase chain reaction (qPCR) or Sanger sequencing, focusing on hotspot regions such as exons 19 and 21. For ALK rearrangements, immunohistochemistry (IHC) serves as a rapid and cost-effective primary diagnostic tool, while fluorescence in situ hybridization (FISH) remains the gold standard due to its high specificity. Similarly, ROS1 rearrangements are detected via FISH, ensuring precise identification of chromosomal alterations. The implementation of these methodologies enables the identification of targetable mutations and optimizes therapeutic strategies for patients with NSCLC.[9]

In India, considerable efforts have been made to characterize the clinical and molecular profile of patients with NSCLC to enhance treatment outcomes. A study conducted at All India Institute of Medical Sciences, New Delhi analyzed 1,862 lung cancer cases (2008–2018), identifying adenocarcinoma (34%) as the most common subtype, followed by squamous cell carcinoma (28.6%).[3] Over the 10-year period, there was a marked increase in the incidence of adenocarcinoma from 9.5% in 2008 to 35.9% in 2018, indicating a shift in the histopathological landscape of lung cancer in India. The systematic review and meta-analysis by Raman et al found that approximately 40% of patients with adenocarcinoma and 30% of patients with NSCLC in India harbor actionable mutations in EGFR or ALK. The pooled prevalence rates for EGFR mutations were 28.7% in adenocarcinoma and 24.2% in NSCLC, while ALK rearrangements were found in 8.3% of adenocarcinoma cases.[10] The prevalence of EGFR mutations in India mirrors global trends, showing higher rates in Asian populations (30–40%) compared with Caucasians (15–20%).[11]

Recent advancements in immunotherapy and precision medicine, including development of first- and second-line targeted therapies for metastatic NSCLC, have significantly enhanced patient survival outcomes compared with traditional chemotherapy. This highlights the critical need for timely identification of actionable mutations to facilitate biomarker-guided treatments and improve overall survival (OS) rates.[12,13]

In this study, we conducted a comprehensive analysis of the mutational landscape in advanced NSCLC patients, focusing on identifying key actionable genetic alterations. With these findings, we aimed to provide insights into the molecular mechanisms of NSCLC and emphasize the importance of integrating molecular testing into routine clinical practice for improved patient outcomes.

Materials and Methods

Study Design and Setting

In this retrospective observational study, conducted at a single tertiary cancer care center in South India, the prevalence of actionable mutations in patients with advanced NSCLC was analyzed. The study was performed in the Department of Medical Oncology at the Government Medical College, Kozhikode, South India, between November 2022 and July 2024.

Inclusion and Exclusion Criteria

The study population included patients with histologically confirmed advanced NSCLC (stage III unresectable/IV) who had undergone molecular testing for driver mutations using next-generation sequencing (NGS). Patients who had not undergone molecular testing were excluded from the study. Within the study period, we had 50 patients satisfying the inclusion criteria and included them.

Variables

Relevant data were extracted from medical records, including demographic information (age, sex, and smoking history), clinical characteristics (stage, laterality, tumor location, lobe, metastatic sites, histopathology, and IHC), and results of mutation analysis. Mutations analyzed included *ALK, BRAF, EGFR, ERBB2, FGFR3, KEAP1, KRAS, MAP2K1, MET, NRAS, NTRK1, NTRK2, NTRK3, PIK3CA, TP53, RET, ROS1,* and *STK11* by NGS. NGS assay was done from two laboratories by custom hybrid capture technique to a minimum depth of 250X on Illumina NovaSeq X plus (Illumina Inc., San Diego, California, United States) and AmpliSeq technology using the Ion S5 system (Thermo Fisher Scientific Inc. Waltham, Massachusetts, United States) to a minimum depth of 500X. Sequences are processed using a customized and validated analysis pipeline designed to accurately detect all classes of genomic alterations (single nucleotide variants, insertions, deletions, copy number variations, and fusions). IHC for PD-L1 was done using VENTANA SP263 Assay (Roche Holdings AG, Basel, Switzerland).

Outcomes

Primary outcome is to find out the prevalence of actionable genomic alteration and PD-L1 status in advanced NSCLC in our population. Secondary outcome of the study is to find out the prevalence of actionable genomic alteration in different NSCLC histologic types.

Statistical Analysis

All statistical analyses were performed using the SPSS version 20 (IBM, Armonk, New York, United States). Descriptive statistics were used to summarize demographic and clinical data. Frequency analysis was conducted to determine the distribution of driver mutations.

Ethical Approval

The study protocol was approved by the Institutional Ethics Committee of the Government Medical College, Kozhikode (Ref. No. GMCKKD/RP 2024/IEC/307). All procedures performed in the study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Results

Out of 72 advanced biopsy-proven NSCLC cases registered during the study period, testing was recommended for all patients. A total of 50 patients, who had available test results, were included in the study. Six patients could not be tested due to insufficient tumor content in the biopsy block and 16 due to various other reasons like patient death, financial constraints, lost to follow-up, etc. Majority of the patients were in the age group of 61 to 70 years with a male predominance of 66%. Most patients were nonsmokers, and the majority of the cohort presented with stage IV disease. Adenocarcinoma was the most common histological subtype. Further details on baseline demographic and clinical characteristics are summarized in ►**Table 1**.

Table 1 Baseline demographic details

Variables	Frequency, *N* (%)
Age	
<40	3 (6%)
41–50	5 (10%)
51–60	11 (22%)
61–70	23 (46%)
71–80	7 (14%)
>80	1 (2%)
Gender	
Male	33 (66%)
Female	17 (34%)
Smoking	
Yes	22 (44%)
No	28 (56%)
Stage	
III	9 (18%)
IV	41 (82%)
Laterality	
Right lung	32 (64%)
Left lung	15 (30%)
Unknown	3 (6%)
Lobe	
Upper	21 (42%)
Middle	1 (2%)
Lower	24 (48%)
Unknown	4 (8%)
Location	
Peripheral	24 (48%)
Central	16 (32%)
Not specified	10 (20%)
Histopathology (HPR)	
Adenocarcinoma	35 (70%)
Squamous cell carcinoma	12 (24%)
NSCLC-NOS	2 (4%)
Poorly differentiated	1 (2%)
IHC	
TTF1 or napsin A positive	25 (50%)
P40 or P63 positive	5 (10%)

Abbreviations: IHC, immunohistochemistry; NSCLC-NOS, non-small cell lung cancer-not otherwise specified; TTF1, thyroid transcription factor 1.

Mutational Analysis

Among the mutations identified in patients with advanced NSCLC, the most frequently altered genes comprised TP53 (42%), EGFR (34%), ALK (10%), PIK3CA (10%), MET (4%), KRAS (4%), RET (4%), ROS1 (2%), and BRAF (2%) (►**Fig. 1**).

Mutation Distribution by Histological Subtype

Mutation Analysis in Adenocarcinoma

In the study of 35 patients with adenocarcinoma, genomic alterations were observed as both single-gene mutations and combinations of mutations. The most common alterations included EGFR exon 19 deletions and TP53 mutations, in 16 and 8% of patients, respectively. Additionally, complex mutational profiles were observed, with combinations such as TP53 + EGFR exon 19 del + PIK3CA and TP53 + ERBB2 exon 20, each present in 4% of cases (►**Table 2**).

Mutation Analysis in Squamous Cell Carcinoma

In squamous cell carcinoma patients, TP53 mutations were the most frequently observed, occurring in 12% of cases. A small number of patients exhibited mutations in PIK3CA, ERBB2, and KRAS, each identified in 2% of cases. Additionally, a single patient (2%) presented with a combination of TP53 + EGFR (L858R) mutations (►**Table 3**).

Mutation in Other Subtypes

In the study, one patient (2%) with poorly differentiated carcinoma had an EGFR exon 19 deletion. Two patients (4%) were diagnosed with Not Otherwise Specified subtype of NSCLC, and no mutation was detected in their samples.

PD-L1 Expression Profile

PD-L1 expression testing was conducted in all 50 patients and 39 patients (78%) had low PD-L1 expression (<1%; ►**Fig. 2**).

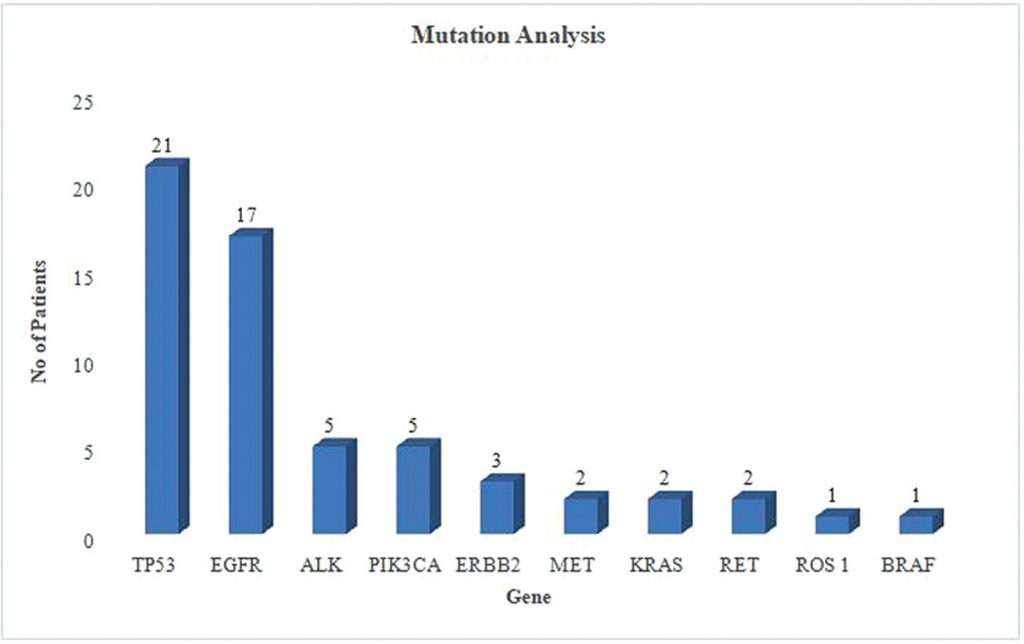

Fig. 1 Distribution of gene mutations.

Table 2 Mutations in adenocarcinoma patients ($N = 35$)

Genomic alteration	Frequency, *n* (%)
No mutation	4 (8%)
EGFR exon 19 del	8 (16%)
TP53	4 (8%)
ALK	3 (6%)
RET	2 (4%)
MET	1 (2%)
BRAF	1 (2%)
TP53 + EGFR exon 19 del + PIK3CA	2 (4%)
TP53 + EGFR L858R	1 (2%)
TP53 + ERBB2 exon 20	2 (4%)
TP53 + MET	1 (2%)
TP53 + ALK + PIK3CA	1 (2%)
ROS1 + PIK3CA	1 (2%)
TP53 + ALK	1 (2%)
KRAS + PIK3CA	1 (2%)
TP53 + EGFR exon 19 del	1 (2%)
TP53 + EGFR S768I, G719A	1 (2%)

Table 3 Mutation in squamous cell carcinoma patients ($N = 12$)

Genomic alteration	Frequency, *n* (%)
No mutation	2 (4%)
TP53	6 (12%)
PIK3CA	1 (2%)
ERBB2	1 (2%)
KRAS	1 (2%)
TP53 + EGFR L858R	1 (2%)

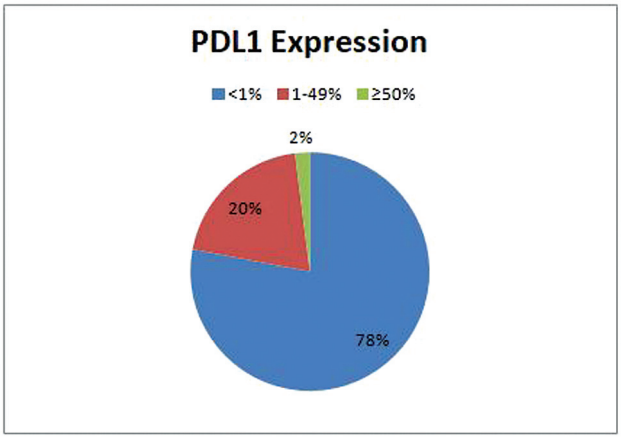

Fig. 2 PDL1 expression levels.

Discussion

Genomic profiling has emerged as a cornerstone in the management of NSCLC, enabling personalized treatment strategies tailored to specific molecular alterations. The average age of our patients was 60 years, which is similar to that reported in previous Indian studies.[14,15] Similarly, the male predominance in our study is similar to other Indian reports.[16,17] This study revealed significant heterogeneity in the mutational profiles of advanced NSCLC among South Indian patients, with TP53 and EGFR mutations emerging as the most common.

TP53 mutations are widely recognized as tumor suppressor gene alterations associated with poor prognosis in

various cancers, including NSCLC. These mutations are particularly prevalent in smokers, reflecting the mutagenic effects of tobacco carcinogens.[18] TP53 mutations are known to influence treatment response, with shorter progression-free survival (PFS) and OS, necessitating novel therapeutic strategies. EGFR mutations, on the other hand, are pivotal in the pathogenesis of adenocarcinoma, particularly in nonsmoking Asian populations. These mutations, including exon 19 deletions and L858R substitutions, drive tumorigenesis and serve as primary targets for tyrosine kinase inhibitors (TKIs).[19]

The predominance of TP53 (42%) and EGFR (34%) mutations observed in our study is consistent with findings from other studies. For instance, Lai et al reported a similar predominance of adenocarcinoma (91.8%) and high frequencies of EGFR (63%) and TP53 (50.7%) mutations in a Taiwanese cohort.[20] Similarly, Kaler et al detected EGFR mutations in 38.67% of patients with NSCLC, while Rajadurai et al identified EGFR mutations in 46.5% of cases, with adenocarcinoma comprising 84% of their cohort.[21,22] These studies emphasize the pivotal role of EGFR mutations in adenocarcinoma tumorigenesis, particularly in nonsmoking populations, suggesting a potential genetic predisposition unique to Asian populations.

In an Indian context, Sharma et al analyzed 53 patients with lung adenocarcinoma and identified TP53 and EGFR mutations as the most frequent, with prevalence rates of 52.8 and 50.9%, respectively.[23] Their findings also highlighted exon 19 deletions and L858R as the most common EGFR mutations, collectively accounting for 20.7% of cases. Demographic differences, such as a higher proportion of female patients (47.3%) compared with our cohort (34%), reflect potential regional and sample-specific variations. Roy et al further highlighted differences in molecular profiles between Indian and U.S.-based South Asian cohorts, with EGFR mutation prevalence closely aligning with our findings.[24]

The findings of this study highlight the critical role of comprehensive genomic profiling in NSCLC management. Identifying mutations in the EGFR gene allows for the selection of first-line TKIs, such as gefitinib, erlotinib, and osimertinib, which have demonstrated substantial improvement in PFS compared with chemotherapy.[25,26] Additionally, addressing resistance mechanisms, including EGFR amplification and MET activation, through tailored therapeutic strategies can further optimize outcomes. The high prevalence of TP53 mutations underscores the need for innovative treatment approaches. In patients with EGFR and TP53 co-mutations, combining EGFR-TKIs with chemotherapy has shown improved response rates and survival outcomes.[27,28] Furthermore, the low PD-L1 expression observed in most of our cohort limits eligibility for immune checkpoint inhibitors, emphasizing the importance of genomic-driven treatment selection.

This pioneering study from South India underscores the critical role of genomic profiling in shaping personalized treatments for NSCLC, especially given the substantial prevalence of TP53 and EGFR mutations in the region.

While the findings offer valuable insights, the study's limitations, including small sample size (50 patients) and single-center design, may affect generalizability and the ability to assess prognostic outcomes. We could not do NGS for all patients due to financial constraints and low tumor content. Future multicenter studies with larger cohorts and extended follow-up are essential to validate these findings and further refine mutation-based therapeutic strategies.

Conclusion

In 2025, testing for EGFR, ALK, ROS, and PD-L1 is insufficient for making treatment decisions in metastatic NSCLC due to the presence of multiple genomic alterations. Ideally, all patients should undergo comprehensive genomic profiling; however, in India, financial constraints limit access for many patients. In this context, targeted gene panels using the latest NGS technology with hybrid capture and PD-L1 by IHC can help identify relevant genomic alterations at a reduced cost, enabling effective personalized treatment decisions.

Patients' Consent

Written informed consents of patients were not taken as it is a retrospective observational study, and data were collected from hospital records.

Funding

None.

Conflict of Interest

None declared.

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