

Lung Cancer

A Real-World Molecular Epidemiological Study of Non-Small-Cell Lung Cancer (NSCLC) Patients from Western India

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Abstract



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Keywords

- ▶ NSCLC
- ▶ KRAS
- ▶ EGFR
- ▶ EML4-ALK
- ▶ ROS 1
- ▶ PD-L1

Background The molecular characterization of advanced non-small-cell lung cancer (NSCLC) has unveiled genomic alterations such as *EGFR* gene mutations, *KRAS* gene mutations, *ROS1* gene rearrangements, *EML4-ALK* rearrangements, and altered *MET* signaling. The objective of this molecular epidemiological study was to report the clinical, pathological, and molecular profile of NSCLC patients from western India.

Materials and Methods This real-world study of NSCLC patients was performed at a chemotherapy day-care center in western India. The clinical, pathological, and molecular data were collected from the patient's medical records after obtaining the Ethics Committee permission for the study. The study was conducted according to the ethical principles stated in the latest version of Helsinki Declaration, and the applicable guidelines for good clinical practice.

Results A total of 182 (58.7%) men and 128 (41.3%) women with a median age of 63 years (range: 22–93 years) were included in the study. Of the total 310 patients, 195 (62.9%) were nonsmokers whereas 81 (26.1%) had a past history of smoking. *EGFR*, *EML4-ALK* Fusion Gene, *KRAS*, *ROS1* gene rearrangement, and *PD-L1* were positive in 42 (22.3%), 12 (9%), 2 (28.6%), 3 (12.5%), and 3 (25%) patients, respectively. One patient had concurrent *EGFR* mutation along with *ROS1* gene rearrangement.

Conclusion Oncogenic driver mutations are present in Indian NSCLC patients. Molecular testing should be performed for all patients of advanced NSCLC to identify those that can benefit from newer generation of targeted or immunotherapies.

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Introduction

The purpose of this molecular epidemiological study is to report the clinicopathological and molecular profile of non-small-cell lung cancer (NSCLC) patients in community oncology setup from western India.

Materials and Methods

This real-world study of NSCLC patients was performed at the Mumbai Oncocare Center, a chemotherapy day-care center in western India. The clinical, pathological, and molecular data were collected from the patient's medical records after obtaining the Ethics Committee permission (reference number NSCLC/MOC/2K19RS05) for the study. The study was conducted according to the ethical principles stated in the latest version of Helsinki Declaration, and the applicable guidelines for good clinical practice.

Formalin-fixed, paraffin-embedded tissue blocks were used for the mutation analysis. *EGFR* and *KRAS* mutations were tested using polymerase chain reaction (PCR)/Sanger sequencing method. Deoxyribonucleic acid (DNA) was extracted using a commercially available blood DNA extraction kit, following the manufacturer's instructions. A nested PCR protocol was followed using gene-specific primers to amplify the desired region of the *EGFR* gene that is required to be sequenced. Four PCR amplicons, each representing Exons 18, 19, 20, and 21, were generated. A similar protocol was followed to amplify the target region of the *KRAS* gene that is represented by a 214bp PCR amplicon. The PCR products were purified using an exonuclease and an alkaline phosphatase enzyme and sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, ThermoFisher Scientific). The sequenced products were purified by ethanol precipitation, air dried, and suspended in water. Sequence analysis was performed on an Applied Biosystems genetic analyzer as per the standard protocol provided by the manufacturer. *EGFR* mutations (Exon 18[G719A/C/S]; Exon 19 Deletions; Exon 20 [T790M]; Exon 21 [L858R (CTG to CGG) and L861Q (CTG to CAG)]) and *KRAS* mutations (12 Gly12Asp [GGT > GAT]; 12 Gly12Ala [GGT > GCT]; 12 Gly12Val [GGT > GTT]; 12 Gly12Ser [GGT > AGT]; 12 Gly12Arg [GGT > CGT]; 12 Gly12Cys [GGT > TGT]; 13 Gly13Asp [GGC > GAC]) were then detected using a standard wild-type transcript ID, respectively, as reference to detect the presence or absence of the mutations. *ALK* gene rearrangement was detected using the Vysis *ALK* Break Apart Rearrangement Probe Kit (Abbott Molecular, Abbott Park, Illinois, United States). Cases were considered positive for *ALK* rearrangement when >15% of a minimum of 100 tumor cells showed split signals or single red signals. *ROS1* fluorescence in situ hybridization assay was performed using a dual-color break-apart probe (ZytoLight Spec *ROS1*; ZytoVision, Germany). For *PD-L1* immunohistochemistry (IHC) assay, sections of 4 μ m thickness were stained with anti-*PD-L1* (SP263) rabbit monoclonal primary antibody and a matched rabbit immunoglobulin G-negative control with an OptiView DAB IHC Detection Kit on the Ventana BenchMark ULTRA automated staining platform. For the SP142 assay, sections

were stained with anti-*PD-L1* (SP142) rabbit monoclonal primary antibody and a matched rabbit immunoglobulin G-negative control with an OptiView DAB IHC Detection Kit followed by an OptiView Amplification Kit, on the Ventana BenchMark ULTRA automated staining platform.

Categorical variables were analyzed using Pearson chi-squared or Fisher's exact test, whichever was appropriate, and presented in the form of number and percentage. Logistic regression was performed to compare the study groups. The width of the resultant confidence intervals (CIs) for parameters to be estimated was constructed with a significance level of 0.05, that is, a 95% CI. Furthermore, multiple logistic regression was performed to check the significance of independent variables. Statistical analysis was performed using SAS version 9.4 (SAS Institute Inc. Mumbai Oncocare Centre, 2nd Floor, Majithia Apartments, God's Gift Premises, Co-op. Society Ltd, Swami Vivekananda Rd, above Irla, Vile Parle, Mumbai, Maharashtra 400056).

Results

A total of 310 NSCLC patients were included in the study. There were 182 (58.7%) men and 128 (41.3%) women with a median age of 63 years (range: 22–93 years). Of the 310 patients, 81 (26.1%) were smokers whereas 195 (62.9%) were nonsmokers. Of the study population, 249 (80.3%) patients had adenocarcinoma whereas 48 (15.5%), 5 (1.6%), and 8 (2.6%) patients, respectively, had squamous cell carcinoma, adenosquamous carcinoma, and poorly differentiated carcinoma. Stage at presentation was III and IV in 25 (8.1%) and 285 (91.9%) patients, respectively. Performance status was 0, 1, 2, and 3 in 5 (1.6%), 82 (26.5%), 61 (19.7%), and 15 (4.8%) patients, respectively.

EGFR, *EML4-ALK* Fusion Gene, *KRAS*, *ROS1* gene rearrangement, and *PD-L1* were positive in 42 (22.3%), 12 (9%), 2 (28.6%), 3 (12.5%), and 3 (25%) patients, respectively. One patient had concurrent *EGFR* mutation along with *ROS1* gene rearrangement.

The distributions of *EGFR* mutations, *EML4-ALK* Fusion Gene, *ROS1* gene rearrangement, *KRAS*, and *PD-L1* were studied with regard to individual variable of gender, age, tobacco chewing, smoking history, alcohol intake, histology stage, and performance status. *EGFR* mutations showed significant association with adenocarcinoma histology ($p < 0.05$) whereas *EML4-ALK* Fusion Gene showed significant association with age ($p = 0.0025$) and alcohol intake ($p = 0.0057$). *ROS1* gene rearrangement showed significant association with tobacco chewing ($p = 0.0047$).

Discussion

The incidence of *KRAS* gene mutation in NSCLC has been reported in the range of 24% whereas the incidence of *EGFR* mutations has been reported in the range of 13%, 47%, and 51.8% across Caucasian, Asian, and Indian population, respectively.^{1–5} With an incidence of *KRAS* gene mutation of 28.6%, the results of our study are consistent with the previously published reports. The 22.3% incidence of *EGFR* mutations in our study is way below the previously published reports from India. The *ALK* gene

rearrangement incidence of 9% in our study confirms the findings of previously published reports. A higher rate of *EGFR* mutation and *ALK* gene rearrangement rate have been reported in females as compared with males along with a positive correlation with never smokers as compared with smokers in the previously published studies.²⁻⁵ The prevalence of *ROS1* gene in NSCLC has been reported in the range of 0.9–2.6%.^{6,7} Among Indian population, three independent studies by Suryavanshi et al,⁸ Joshi et al,⁹ and Mehta et al¹⁰ have reported *ROS1*-rearranged NSCLC in 2.9%, 4.1%, and 2.82% patients, respectively. Contrary to the previous reports, the incidence of 12.5% for the *ROS1* gene rearrangement in our study is the highest reported incidence to date. The results of Express study in locally advanced or metastatic NSCLC showed a real-world prevalence of *PD-L1* expression $\geq 50\%$ and $\geq 1\%$ in 50% and 52% patients, respectively.¹¹ The 25% incidence of *PD-L1* expression in our study is way below the findings of Express study.

Our study demonstrated a positive correlation of *EGFR* mutation in adenocarcinoma histology group (20.21%, $p=0.0102$). Similarly, a positive correlation of *EML4-ALK* fusion gene was observed for the age group of 40–60 years (7.46%, $p=0.0025$) as well as teetotaler group (7.46%, $p=0.0057$). A positive correlation of *ROS1* gene rearrangement was observed in tobacco chewing group although the same was equally distributed among tobacco users and nontobacco users. One case in the present study had concurrent *EGFR* mutation along with *ROS1* gene rearrangement. This observation is consistent with two of the previously published reports.^{6,10} Although a greater number of females in our study had *EGFR* mutation and *ROS1* gene arrangements, the results were not statistically significant. Similarly, our study did not demonstrate a significant association of never smokers vis-à-vis *EGFR* mutation, *EML4-ALK* Fusion Gene, and *ROS1* gene arrangements although the same has been reported in the literature.^{2-5,10}

Conclusion

Our study reports the clinical, pathological, and molecular profile of NSCLC patients at a chemotherapy day-care center in western India. The molecular characterization of advanced NSCLC has unveiled different genomic alterations that are paving the way for improved treatment outcomes as well as personalized treatment to the individual patient. Our study reassures the importance of molecular testing in the NSCLC with an aim to identify patients that can benefit from newer generation of targeted or immunotherapies.

Ethical Approval

The study was conducted according to the ethical principles stated in the latest version of Helsinki Declaration, and the applicable guidelines for good clinical practice.

Conflict of Interest

None declared.

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