RESEARCH ARTICLE

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Programmed Death Ligand -1 and Gene Mutation Characterization of Lung Malignancies in Patients at a Rural Hospital in Central India

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Abstract

Introduction: Lung malignancy is one of the most common neoplasms worldwide. Accurate histology sub-typing and identification of gene mutations in lung tumours are considered important to administer targeted therapy for improved clinical outcome. Our aim is to determine the frequency of *EGFR* mutation and Programmed death ligand-1 (*PD -L1*) status of lung malignancies in patients attending a rural hospital in Central India. **Materials and methods:** Formalin-fixed histology diagnosed lung malignancy (n=99) bronchoscopic/trucut lung biopsies were identified and the tissue blocks and slides were retrieved. Histology typing and staging of the lesions was assessed. *PD-L1* expression on biopsy was detected by immunohistochemistry using commercially available primary antibody. *PD-L1* expression was assessed and semi-quantified based on the intensity and proportion of tumour cells stained for the marker. *EGFR* gene mutation at exon19 and 21 was detected by polymerase chain reaction of tissue from paraffin blocks. Final analysis was performed on 87 biopsies for status of *EGFR* mutation and *PD-L1* expression. **Results:** The average age of lung malignancies patients was 63 years, with a preponderance of males. Advance disease in stage III and stage IV was more common in squamous cell carcinoma as compared to adenocarcinoma ($p < 0.01$). Mutations at exon 19-21 of the *EGFR* gene were detected in 7/87 (8%) cases of adenocarcinoma and all of these patients were non-smokers. A total of 52.9% of biopsies showed *PD-L1* expression, which was higher in adenocarcinoma patients ($p=0.04$), smokers ($p=0.00$), and stage II and III patients ($p= 0.00$). **Conclusion:** *EGFR* gene mutations at exon 19 or 21 are seen in lung adenocarcinoma cases. *PD-L1* expression was observed in *EGFR* mutated tissues. Our results should be further validated with large sample size and multicenter clinical data before extrapolation to design immunotherapy strategies.

Keywords: Lung malignancy- non-small cell lung carcinomas- epidermal growth factor receptor- PD-L1

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Introduction

Worldwide lung cancer is the leading cause of cancer-related deaths (1.76 million deaths/year) which accounts for approximately 18% of cancer deaths (Sung et al., 2020). In India, malignant lung diseases account for 5.9% of all cancer cases and 8.1% of all cancer-related deaths (Bray et al., 2018). The number of new lung cancer cases in India is predicted to rise to more than 100,000 by the year 2025 (Nath et al., 2022). In recent years, an accurate diagnosis with precise histology sub-typing, identification of gene mutations and molecular status of lung tumours facilities is possible to administer targeted therapy for better clinical outcome (Shim et al., 2017).

A germline mutation in the gene encoding epidermal growth factor receptor (*EGFR*) makes a person more susceptible to lung malignancies (Alexandrov et al., 2016). It occurs in approximately 20% to 30% of all lung adenocarcinoma, especially non-small cell lung cancer (NSCLC), which accounts for more than 80% of all lung malignancies (Schabath et al., 2019). *EGFR* mutation testing is considered useful predictive marker for better management with targeted tyrosine kinase inhibitors (Lee et al., 2015; Omar et al., 2022; Sukauichai et al., 2022) though few studies reported acquisition of *EGFR*-TKIs resistance and thus suggesting resistance mutation testing for improved treatment (Lee et al., 2021).

Programmed cell death protein-1 (PD-1) is an immune-inhibitory receptor that interacts with a ligand, programmed death ligand-1 (PD-L1), expressed on antigen-presenting cells and other immune cells (Han et al., 2020). The PD-1/ PD-L1 interaction is an important immune checkpoint that has been associated with adaptive immune resistance to various malignancies. In NSCLC,

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the expression of PD-L1 is increased, leading to immune evasion of tumour cells (Wei et al., 2018). PD-L1 is, therefore, a key predictive biomarker for anti PD-1/ PD-L1 treatment of NSCLC. However, studies from various settings offered conflicting findings for the predictive role of PD-L1 status and its relationship with tumour *EGFR* status (Inamura et al., 2016; Rangachari et al., 2017; Song et al., 2016).

Studies have describes the clinico-pathology characteristics of lung malignancies from various parts of India (Vasudevan et al.,2022; Nath et al., 2022; Bhopal et al., 2019; Shanmugapriya Shankar et al., 2014; Malik, 2013), but limited data is available on the clinical, pathology features and the types of mutation in lung malignancies from Central India.

We thus aim to determine the frequency of *EGFR* mutation and PD-L1 status of lung malignancies in patients attending a rural teaching hospital in Central India and describe their clinico-pathological characters.

Materials and Methods

Histologically diagnosed cases of lung malignancies on bronchoscopy/trucut biopsies between January 2012 and July 2018 were identified from the Department of Pathology, Ruxmaniben Deepchand Gardi Medical College, Ujjain, India. Formalin-fixed, paraffin-embedded tissue blocks were retreived and hematoxylin and eosin (H&E)-stained histological slides were prepared. Detailed clinical, pathologic, and imaging features, including tumour location and size, enlarged regional lymph nodes on CT /MRI, histologic subtype, and tumour grade, were recorded in a prepared data collection format for each patient from either the histology form or the patient's medical record. Any paraffin-embedded tissue blocks with very small remaining tissue were excluded from the study.

The histology slides were reviewed for histologic features, subtype, and tumour grade according to the World Health Organization classification system for lung tumours (Travis et al., 2015). Three to four consecutive 3µm-thick sections were prepared on especially loaded glass slides (DakoCytomation Denmark A/s, Glostrup, Denmark) for immunohistochemical staining and two to three 5 µm-thick sections were collected in 1.5 ml Eppendorf tubes for DNA extraction for the PCR procedure. For quality control, the cutting blade was cleaned between taking the samples.

The study was approved by the Institutional Review Board and the Institutional Ethics Committee. All experiments were performed in accordance with the relevant guidelines and regulations.

Polymerase Chain Reaction Assay

Mutations were examined in exons 19-21 of the *EGFR* gene from the paraffin-embedded tissue blocks sections. DNA extraction was performed using the D 3396 E.Z.N.A. Tissue DNA Kit (Omega Bio-tek, Inc. NorCross, GA, USA). Primers for the delE746-A750 mutation in exon 19 and a point mutation-specific primer for the L858R mutation in exon 21 of *EGFR* were used with the following sequence sets for delE746-A750: forward 5'-CACAATTGCCAGTTAACGTCTTC-3' (19DF) and reverse 5'-TGTTGGCTTTCGAGATGTTTG-3' (19DR3). The forward sequence for L858R was 5'-TCCCATGATGATCTGTCCCT-3' (21F2f) and the reverse sequence was CACCCAGCAGTTTGGTCC-3' (21ARMS3). Reaction mix contained 2 µl 10X PCR buffer, 0.5 µl dNTPs, 1 µl each of allele-specific primer (10 µM), 0.2 µl AmpliTaq® Gold DNA Polymerase (Applied Biosystems), 1 µl template DNA, and 14.3 µl dd H_2O in a total volume of 20 µl. PCR conditions were: 1 cycle at 94°C for 3 min followed by 35 cycles at 94°C for 1 min, at 59°C for 1 min, and at 72°C for 2 min and the final cycle at 72°C for 5 min for the delE746-A750 mutation and 1 cycle at 94°C for 3 min followed by 35 cycles at 94°C for 1 min, at 64°C for 1 min and at 72°C for 2 min and the final cycle at 72°C for 5 min for the L858R mutation. The PCR products were then visualized by electrophoresis on an agarose gel stained with ethidium bromide (2%) along with a 100bp DNA ladder to measure the size of the PCR product and viewed under ultraviolet light. The presence of an 113bp PCR product indicates that mutations are present in exon 19; similarly, a 166bp PCR product indicates mutations in exon 21. A known positive control and a negative control were included in the procedure for the quality of the procedure.

Immunohistochemistry

After de-paraffinization, paraffin-embedded tissue sections were hydrated with graded alcohols, washed in PBS, and treated with a blocker (endogenous peroxidase activity with 3% aqueous H_2O_2 solution) for 10 minutes. The sections were then incubated overnight in a humidified chamber at 40°C with a rabbit monoclonal antihuman antibody PD -L1 (Abcam, clone ZR3, UK) at a dilution of 1:200 of the primary antibody. After washing three times with phosphate-buffered saline, they were incubated with a general IgG-HRP polymer for one hour, followed by 3,3'-diaminobenzidine tetrahydrochloride for approximately 5 minutes. The sections were then counterstained with haematoxylin for one minute and then dehydrated in graded alcohols, clarified in xylene, and covered with a cover slip. The sections were washed three times with buffered saline between each step. Antibodies used for immunohistochemistry were -anti-human PD -L1 (clone E1L3N, Cell Signalling Technology, Danvers, MA, diluted 1 : 200).

Immunohistochemistry positivity and scoring

PD-L1 expression was assessed on each slide independently by two pathologists. Results were semi-quantified based on the intensity and proportion of tumour cells that exhibited membranous or cytoplasmic brownish-yellow staining. The proportion of stained cells was estimated in five randomly selected high-magnification fields of view (400×) of the sections, and the intensity of staining was recorded as: no staining =0, light yellow weak membrane staining =1, brownish yellow =2, and dark brown linear membrane staining =3. The intensity of staining was categorized as high (2-3) or low (0-1). Five percent of positive cells with a score of 2-3 were classified as positive.

Statistical analysis

Collected data were entered into Microsoft Excel 2010 (Microsoft Corporation, USA) and analyzed using the statistical software package Statistical Package for the Social Sciences (SPSS) version 21 (SPSS-Inc., Chicago, IL). Categorical variables were expressed as frequencies and percentages. The Pearson chi-square test was used to assess the association between different parameters, *EGFR* mutation status, or *PD -L1*. A p value of <0.05 was considered significant.

Results

A total of 99 bronchoscopic/trucified lung biopsies with a diagnosis of lung malignancy were identified, and tissue blocks and slides were obtained. Twelve patients were excluded because the biopsy material remaining on the tissue blocks was insufficient for further investigation. Eighty-seven cases were finally included in the further analysis and experiments (Figure 1).

Clinical characteristics

The clinical characteristics of the patients are summarized in Table 1. The mean age at the diagnosis was 63 years (with a range of 38 to 80 years). Most cases (52.7%) were in the 5th and 6th decades of life, with a male predominance (n=70/87, 80.4%) and a male-to-female ratio of 4.1:1. The majority (58.6%) of patients were farmers and 80% of patients were from rural areas. 50.5% of patients were current smokers, while 28.7% of patients were ex-smokers.

Patients presented with symptoms of cough (n=70/87, 80.4%), chest pain (n=62, 71.2%), dyspnoea (n=69, 79.3%), and haemoptysis (n=21, 24.2%). Other clinical systemic symptoms such as fever (23%), weight loss (17.2%), vomiting (16%), and hoarseness (9%) were also observed in combination with pulmonary symptoms. Most symptoms had been recorded for 15 days to 3 months. Computed tomography of the chest showed various lesions either alone or in combinations as mass on the right side of the lung (80%), pleural effusion (26.4%), consolidation (9.1%), pulmonary collapse (14.9%), or hilar protrusion (9.1%).

Histology, EGFR mutation, and PD-L1 expression

Table 1 shows the types of lesions detected by histology classification. The two main histological types were (i) NSCLC (n=83/87, 95.4%), including adenocarcinoma (n = 40/87, 48.1%), squamous cell carcinomas (n = 42/87, 50.6%), and large cell carcinomas (n=1/87, 1.2%), and (ii) SCLC (n=4/87, 4.6%). Histology type differed significantly (p=0.04) between men and women. Men were more frequently diagnosed with squamous cell carcinoma, while women were more frequently diagnosed with adenocarcinoma. No significant differences were found between the different histology types in terms of clinical symptoms. Biopsy revealed TNM stage I in 52.8% cases, Stage II in 32% in and stage III in 15% cases. Squamous cell carcinoma patients more commonly presented with stage III and IV as compared with adenocarcinoma patients ($p < 0.01$).

EGFR mutation and *PD-L1* expression were assessed in all biopsies. Mutations in exon 19 and exon 21 of the *EGFR* gene were detected in 7/87 (8%) cases, and all of these (17.5%, 7/40) biopsies had adenocarcinoma alterations. *EGFR* gene mutations were more common in non-smokers. High expression (≥ 5% of tumour cells) of PD-L1 was revealed in 17.2% (15/87) biopsies while 35.7% (31/87) biopsies showed low expression (< 5% of tumour cells) and 47.1% (41/87) biopsies had no *PD-L1* expression. No obvious statistical significance was found between *PD-L1* expression and gender, age group, and *EGFR* mutation status. However, adenocarcinoma patients (p=0.04), smokers (p=0.00), and stage II and III patients

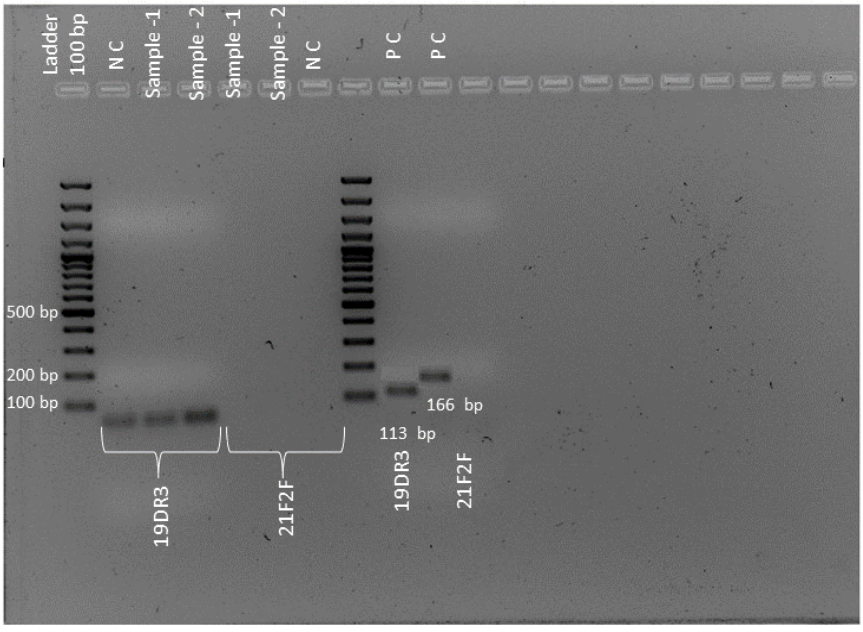

Figure 1. PCR Characterization of *EGFR* Mutation in Lung Carcinoma. NC, negative control; PC, positive control; bp, base pair; 19 DR3, exon 19 mutation; 21 F2F, exon 21 mutation

Table 1. Baseline Characteristics of Lung Carcinoma Patients Studied (N=87)

Characteristics	N (%)
Age	
<40 years	6 (6.8)
40-60 year	46 (52.8)
>60 year	35 (40)
Mean Age	63 year
Age Range	38-80year
Gender	
Male	70 (80.4)
Female	17 (19.5)
Ratio	4.1:1
Smoking Ratio	
Never smoker	18 (20.6)
Current smoker	44 (50.5)
Ex-smoker	25 (28.7)
Occupation	
Farmer	51 (58.6)
Laborer	15 (17.2)
Cotton mill worker	10 (11.4)
Housewife	07 (8.04)
Other	04 (4.59)
Associated Disease	
Diabetes Mellitus	19 (21.8)
Hypertension	15 (17.2)
COPD	12 (13.7)
PTB	15 (17.2)
Other	4 (4.5)
None	22 (25.2)
Symptom	
Cough	70 (80.4)
Chest Pain	62 (71.2)
Dyspnoea	69 (79.3)
Hemoptysis	21 (24.2)
Decreased Appetite	54 (62)
Fever	20 (22.9)
Weight loss	15 (17.2)
Vomiting	14 (16)
Hoarseness of voice	08 (9.1)
CT scan finding	
Consolidation	08 (9.1)
Collapse	13 (14.9)
Mass	77 (88.5)
Pleural effusion	23 (26.4)
Hilar Prominence	08 (9.1)
Histology Type*	
Adenocarcinoma	40 (48.1)
Squamous cell carcinoma	42 (50.6)
Large cell carcinoma	1 (1.2)
Small cell carcinoma	4 (4.6)
TNM staging*	
Stage I	46 (52.8)
Stage II	28 (32.1)
Stage III	13 (14.9)

*TNM, tumour, node, metatstsis

(p= 0.00) had significantly higher *PD-L1* expression (Table 2).

Discussion

Various studies around the world show *PD-L1* as a biomarker to predict checkpoint inhibitor immunotherapy, which has revolutionized the treatment strategy in oncology (Xu et al., 2019). Detection of *PD-L1* in lung tumours can predict good response to immunotherapy, which has much lower toxicity compared to chemotherapy (Xu et al., 2019). We detected *PD-L1* expression and *EGFR* mutation status in lung malignancies in a rural hospital in Central India. We found *EGFR* mutation in 8%

Table 2. Association between Clinical Features of Lung Carcinoma Patients and *PD-L1* Expression in Their Biopsies

Characteristics	PD-L1 expression (N=87)		p-value
	Positive n=46	Negative n=41	
Gender			
Male	38	32	0.59
Female	8	9	
Age group			
18-40	3	3	0.7
41-60	24	22	
61-80	15	20	
Smoking Habit			
Current Smokers	31	23	0
Ex-smokers	3	17	
Non-smokers	12	1	
Histology			
Adenocarcinoma	16	24	0.02
Squamous cell carcinoma	29	13	
Large cell carcinoma	0	1	
Small cell carcinoma	1	3	
TNM* Staging			
Stage I	10	36	0
Stage II	23	5	
Satge III	13	0	
EGFR expression status			
Positive	6	1	0.11
Negative	40	40	

*TNM, tumour, node, metatstsis

of NSCLC patients and *PD-L1* expression was detected in 52.8% (46/87) patient samples by immunohistochemistry using 22C3 PD -L1 assay.

In our study, > 80% of the patients were male (male:female ratio 4.1:1) with an age range of 36-80 years (mean age 63 years) as also observed in most of the studies from India (Vasudevan et al.,2022; Nath et al., 2022). Although tobacco smoking (50% current smokers) is considered as a major risk factor for lung malignancies, the prevalence (10-30%) of lung malignancies are now increasing among non-smokers as well (Ou, 2013) which could be because of exposure to environmental carcinogens as dust, insecticides, indoor smoke or cooking smoke from Chullas in Indian settings (Vasudevan et al., 2022). Cough (80%) is reported as the most common symptom but other systemic symptoms as fever (22.9%) and weight loss (17.2%) of unknown cause should also be methodically investigated to rule out any pulmonary malignancies (Malik et al., 2013).

We found 95.4% cases are of NSCLC types with almost as many adenocarcinoma (48.1%) as squamous cell carcinomas (50.6%), suggesting that the trend in India is changing from the predominant squamous cell carcinoma to more adenocarcinoma as in the Western world (Alberg et al., 2013; Shanmugapriyaetal, 2014). The precise histology subtype of lung tumours has a significant impact on patient management, as it has implications for selection of appropriate molecular tests for planning targeted therapy such as *EGFR* mutations are associated with adenocarcinoma (Neumann et al., 2022). *EGFR* tyrosine kinase inhibitors (TKIs), a therapy for patients with *EGFR*-mutated tumours, have shown reduced toxicity and improved prognosis compared with conventional chemotherapy, so therapy modulation based on genetic alterations has increased the importance of appropriate tumour classification.

Studies show that the most common mutation in NSCLC is in the *EGFR* gene at exon 19-21 (Tseng et al., 2017) Almost 90% of all *EGFR* mutations are deletions in exon 19 (E19 dels) or a leucine-to-arginine substitution in E21 (Li et al., 2008). The frequency of *EGFR* mutations varies between 6.25 and 80% of cases depending on ethnicity (Kawaguci et al., 2010; Kimura et al., 2006; Chougule et al., 2013; Omar et al., 2022). We found an *EGFR* mutation in 8% (7/87) of cases. All of these cases were female and non-smokers. It is known that *EGFR* gene mutations are more common in non-smokers (Bhopal, 2013). However, in our study, many patients were smokers (50.5%) or former smokers (28.7%), which could be the reason for the low incidence of mutations in our samples. Secondly, >80% of our cases were male showing squamous cell carcinoma lesions while *EGFR* mutations are mainly seen in adenocarcinoma. Choughule A et al., 2013 observed no significant difference in the incidence of *EGFR* mutations in different locations in India, although their study included few patients from central India (Chougule et al., 2013).While detection of gene mutations is useful for therapeutic and prognostic purposes, low sensitivity of *EGFR* mutation detection, as in our study, suggests a combination of genes or gene panels should be tested for the development of targeted therapies.

Expression of *PD-L1* in tumour cells is a biomarker for predicting immunotherapy (Xu et al., 2019). It is well documented that PD -L1 expressed on tumour cells would promote immune tolerance of tumours (Rangachari et al., 2017; Yang et al., 2014). We found that *PD-L1* expression level was significantly higher in squamous cell carcinomas (p=0.02) than in adenocarcinoma and higher grade tumours (p=0.00) (Jiang et al., 2017). This could be because of the reason that more number of squamous cell carcinoma patients carried stage III and IV ($p< 0.001$) lesions as compared to adenocarcinoma, and likewise, expression of *PD-L1* is significantly associated (p=0.00) with smokers who have a high likelihood of having again squamous cell carcinoma. Over-expression of *PD-L1* by tumour cells correlates with a worse prognosis. However, some studies have shown that although higher *PD-L1* expression level correlates with squamous cell carcinoma, but not with its disease stage. In adenocarcinoma, *PD-L1* expression showed higher expression in the poorly differentiated histology variant (Jiang et al., 2017; Pawelczyk et al., 2019).

In our study no obvious relation could be delineated for PDL-1 expression in *EGFR*-mutated tumours. Some studies showed that *EGFR*-mutated tumours showed high expression of *PD-L1* than in tumours without *EGFR* mutations (Song et al., 2016). This result is in contradiction with some previous studies (Malik et al., 2013). There are limited and controversial data on *PD-L1* expression in *EGFR*-mutated lung adenocarcinoma (Inamura et al., 2016; Rangachari et al., 2017; Yang et al., 2014; Song et al., 2016; Pawelczyk et al., 2019). Studies have shown that *PD-L1* expression with a threshold of 1% and 50% positivity was significantly higher in patients with *EGFR* wild type than in patients with *EGFR* mutants (Song, 2019), while others have found higher expression in tumours with *EGFR* mutants tumours (Song et al., 2016) and Yang et al found no association of *PD-L1* expression with *EGFR* mutation (Yang et al., 2014).

Our study have few limitations. We took retrospective cases and information from archival tissues and patient charts, so complete information might be missing for some cases. Being hospital based study most of the patients were in advanced stage of the disease. In addition, we do not have complete data on patients' treatment and outcomes. We tested few mutations and only *EGFR*, which leads to the possible inclusion of patients with other mutations (KRAS, TP53, ROS1, STK11, etc.) that may affect *PD-L1* expression. The number of cases and mutations detected was undersized and could lead to bias in the analysis. Despite these limitations, we, for the first time, evaluated the *PD-L1* expression and *EGFR* mutations status in various histology types of lung malignancies patients from central India.

In conclusion, the study revealed that in our region adenocarcinoma of the lung is as common as squamous cell carcinoma. *EGFR* gene mutations at exon 19 or 21 are seen in adenocarcinoma cases. *PD-L1* expression was seen in wild-type *EGFR* biopsies but there was no correlation between *PD-L1* expression and *EGFR* mutation. Our results should be further validated with large sample size

and multicenter clinical data before extrapolation.

Author Contribution Statement

MRP, PM, MK were involved in the conception and design of the study. PM initiated the formulation of the study. ML and PM were responsible for the histological work, analysis, and drafting of the first version of the manuscript. MRP critically formulated, analyzed the data, and revised the manuscript. PD, GB and SP were responsible for data collection. All authors read and approved the final version of the manuscript. .

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Ethical Clearance

The study was approved by the Institutional Review Board and the Institutional Ethics Committee. All experiments were performed in accordance with the relevant guidelines and regulations.

Availability of data and Materials

The datasets used and/or analysed during the study are available from the corresponding author on reasonable request.

Conflict of interest

The authors declare that they have no competing interests.

References

Alberg AJ, Brock MV, Ford JG, Samet JM, Spivack SD (2013). Epidemiology of lung cancer: Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. *Chest J*, **143**, e1S-e29S.

Alexandrov LB, Ju YS, Haase K, et al (2016). Mutational signatures associated with tobacco smoking in human cancer. *Science*, **354**, 618-22.

Bhopal A, Peake MD, Gilligan D, Cosford P (2019). Lung cancer in never-smokers: a hidden disease. *J R Soc Med*, **112**, 269-71.

Bray F, Ferlay J, Soerjomataram I, et al (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*, **68**, 394-424.

Choughule A, Noronha V, Joshi A, et al (2013). Epidermal growth factor receptor mutation subtypes and geographical distribution among Indian non-small cell lung cancer patients. *Indian J Cancer*, **50**, 107-11.

Chougule A, Prabhash K, Noronha V, et al (2013). Frequency of *EGFR* mutations in 907 lung adenocarcioma patients of Indian ethnicity. *PLoS One*, **8**, e76164.

Han Y, Liu D, Li L (2020). PD-1/*PD-L1* pathway: current researches in cancer. *Am J Cancer Res*, **10**, 727-42.

Inamura K, Yokouchi Y, Sakakibara R, et al (2016). Relationship of tumor PD-L1 expression with *EGFR* wild-type status and poor prognosis in lung adenocarcinoma. *Jpn J Clin Oncol*, **46**, 935-41.

Jiang L, Su X, Zhang T, et al (2017). PD-L1 expression and its relationship with oncogenic drivers in non-small cell lung cancer (NSCLC). *Oncotarget*, **8**, 26845-57.

Kawaguchi T, Matsumura A, Fukai S, et al (2010). Japanese ethnicity compared with Caucasian ethnicity and never-smoking status are independent favourable prognostic factors for overall survival in non-small cell lung cancer: a collaborative epidemiologic study of the National Hospital Organization Study Group for Lung Cancer (NHSGLC) in Japan and a Southern California Regional Cancer Registry databases. *J Thorac Oncol*, **5**, 1001-10.

Kimura H, Kasahara K, Kawaishi M, et al (2006). Detection of epidermal growth factor receptor mutations in serum as a predictor of the response to gefitinib in patients with non-small-cell lung cancer. *Clin Cancer Res*, **12**, 3915-21.

Lee CK, Wu YL, Ding PN, et al (2015). Impact of Specific Epidermal Growth Factor Receptor (*EGFR*) Mutations and Clinical Characteristics on Outcomes After Treatment With *EGFR* Tyrosine Kinase Inhibitors Versus Chemotherapy in *EGFR*-Mutant Lung Cancer: A Meta-Analysis. *J Clin Oncol*, **33**, 1958-65.

Li AR, Chitale D, Riely GJ, et al (2008). *EGFR* mutations in lung adenocarcinomas: clinical testing experience and relationship to *EGFR* gene copy number and immunohistochemical expression. *J Mol Diagn*, **10**, 242-8.

Malik PS, Sharma MC, Mohanti BK, et al (2013). Clinico-pathological profile of lung cancer at AIIMS: a changing paradigm in India. *Asian Pac J Cancer Prev*, **14**, 489-94.

Nath A, Sathishkumar K, Das P, Sudarshan KL, Mathur P (2022). A clinicoepidemiological profile of lung cancers in India - results from the National Cancer Registry Programme. *Indian J Med Res*, **155**, 264–72.

Neumann JM, Freitag H, Hartmann JS, et al (2022). Subtyping non-small cell lung cancer by histology-guided spatial metabolomics. *J Cancer Res Clin Oncol*, **148**, 351-60.

Omar A, Bahnassy A, Gaafer R, El Desouky E, Medhat A (2022). Assessing Mutations in Treatment Response-related Genes in Egyptian Patients with Non-small Cell Lung Cancer. *Asian Pac J Cancer Biol*, **7**, 125-32.

Ou SH (2013). Lung cancer in never-smokers. Does smoking history matter in the era of molecular diagnostics and targeted therapy?. *J Clin Pathol*, **66**, 839-46.

Pawelczyk K, Piotrowska A, Ciesielska U, et al (2019). Role of PD-L1 Expression in Non-Small Cell Lung Cancer and Their Prognostic Significance according to Clinicopathological Factors and Diagnostic Markers. *Int J Mol Sci*, **20**, 824.

Rangachari D, VanderLaan PA, Shea M, et al (2017). Correlation between Classic Driver Oncogene Mutations in *EGFR*, ALK, or ROS1 and 22C3-PD-L1 ≥50% Expression in Lung Adenocarcinoma. *J Thorac Oncol*, **12**, 878-83.

Rawat J, Sindhwani G, GaurD, DuaR, Saini S (2009). Clinico-pathological profile of lung cancer in Uttarakhand. *Lung India*, **26**, 74-6.

Schabath MB, Cote ML (2019). Cancer progress and priorities: lung cancer. *Cancer Epidemiol Biomark Prev*, **28**, 563–1579.

Shankar S, Thanasekaran V, Dhanasekar T, Duvoori P (2014). Clinicopathologic and IHC profile of NSCLC in a tertiary care medical centre in South India. *Lung India*, **31**, 23-8.

Sheikh S, Shah A, Arshed A, Makhdoomi R, Ahmad R (2010). Histological pattern of primary malignant lung tumours diagnosed in a tertiary care hospital: 10 year study. *Pac J Cancer Prev*, **11**, 1341-6.

Shim HS, Choi YL, Kim L, et al (2017). Korean Molecular Pathology Study Group. Molecular Testing of Lung Cancers. *J Pathol Transl Med*, **51**, 242-54.

Song P, Wu S, Zhang L, Zeng X, Wang J (2019). Correlation Between PD-L1 Expression and Clinicopathologic Features in 404 Patients with Lung Adenocarcinoma. *Interdiscip Sci*, **11**, 258-65.

Song Z, Yu X, Cheng G, Zhang Y (2016). Programmed death-ligand 1 expression associated with molecular characteristics in surgically resected lung adenocarcinoma. *J Transl Med*, **14**, 188.

Sukauichai S, Maneenil K, Supavavej A, et al (2022). *EGFR* Mutation-positive Lung Cancer in Real-world Treatment Outcomes: A Multicenter Study from Thailand. *Asian Pac J Cancer Care*, **7**, 643-50.

Sung H, Ferlay J, Siegel RL, et al (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*, **2021**.

Tseng CH, Chiang CJ, Tseng JS, et al (2017). *EGFR* mutation, smoking, and gender in advanced lung adenocarcinoma. *Oncotarget*, **8**, 98384-93.

Vasudevan S, Krishna V, Mehta A (2022). Lung Cancer in Non-Smokers: Clinicopathological and Survival Differences from Smokers. *Cureus*, **14**, e32417.

Wei SC, Duffy CR, Allison JP (2018). Fundamental Mechanisms of Immune Checkpoint Blockade Therapy. *Cancer Discov*, **8**, 1069-86.

Xu Y, Wan B, Chen X, et al (2019). The association of PD-L1 expression with the efficacy of anti-PD-1/PD-L1 immunotherapy and survival of non-small cell lung cancer patients: a meta-analysis of randomized controlled trials. *Transl Lung Cancer Res*, **8**, 413-28.

Yang CY, Lin MW, Chang YL, Wu CT, Yang PC (2014). Programmed cell death-ligand 1 expression in surgically resected stage I pulmonary adenocarcinoma and its correlation with driver mutations and clinical outcomes. *Eur J Cancer*, **50**, 1361-9.