WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.63
Volume 15, Issue 14, 1419-1447. Research Article ISSN 2277–7105

PDL1 IMMUNOHISTOCHEMICAL EXPRESSION IN NON-SMALL CELL LUNG CANCER AND ITS CLINICOPATHOLOGICAL CORRELATION

***[1]Hadeel Habeeb Gatea, [2]Dr. Sura A. M. AL-Namel, [3]Dr. Saad Abdulbaqi**

*[1]M. B. Ch. B.

[2]Consultant Pathologist, AL-Kuwaiti Teaching Hospital, Basrah.

[3]Consultant Histopathologist.

Article Received on 15 June 2026,
Article Revised on 05 July 2026,
Article Published on 16 July 2026,

https://doi.org/10.5281/zenodo.21409302

***Corresponding Author**

Hadeel Habeeb Gatea

M. B. Ch. B.

How to cite this Article: *[1]Hadeel Habeeb Gatea, [2]Dr. Sura A.M. AL-Namel, [3]Dr. Saad Abdulbaqi. (2026). Pdl1 Immunohistochemical Expression In Non-Small Cell Lung Cancer And Its Clinicopathological Correlation. World Journal of Pharmaceutical Research, 15(14), 1419–1447.

ABSTRACT

Background: Programmed Death ligand Protein-1 (PDL-1) is a type I transmembrane protein that plays a central role in immune regulation. By interacting with its ligands, PDL-1 downregulates adaptive immune responses through suppression of cytokine release and inhibition of T-cell activation. This mechanism facilitates immune escape, which is critical in the initiation and progression of lung cancer. **Aim of study:** Evaluation of programmed cell death ligand 1 immunohistochemical expression in non-small cell lung cancer and its correlation with different clinical and histopathological parameters. **Methods:** This retrospective analysis included 47 previously diagnosed NSCLC cases. Tissue samples obtained during the period from June 2025 to the end of December 2025 using immunohistochemistry to assess PDL-1 expression. Expression levels were categorized into defined scores and statistically correlated with clinicopathological parameters. **Results: PDL-1 expression was identified in 61.7% of cases. No significant correlation was obs**erved between PDL-1 expression and patient age, sex, or overall cancer type. However, a significant association was found between level of PDL-1 expression and histological subtype. High PDL-1 expression was detected in 80% of squamous cell carcinoma cases, whereas low expression predominated in adenocarcinoma (83.3%). **Conclusion:** Level of PDL-1 expression in NSCLC is significantly linked to tumor subtype, with squamous cell carcinoma showing higher levels compared to adenocarcinoma.

No meaningful relationship was observed with patient age or sex, suggesting that PDL-1 expression is primarily influenced by tumor-intrinsic characteristics rather than demographic factors.

1.1 Overview

For the past four decades, platinum-based systemic chemotherapy has constituted the cornerstone of treatment for advanced-stage lung cancer. However, its clinical efficacy remains limited, with a median overall survival of approximately 12 months.[1] Approximately 40% of patients with non-small cell lung cancer (NSCLC) harbor oncogenic driver mutations, which represent actionable therapeutic targets. Targeted therapies directed against specific molecular alterations such as EGFR, ALK, and ROS1 have demonstrated significantly superior clinical outcomes compared to conventional chemotherapy.[2]

In recent years, the blockade of the programmed cell death protein 1 (PD-1) and its ligand PDL-1 has emerged as a promising immunotherapeutic strategy in NSCLC management. PD-1 is an inhibitory receptor expressed on activated T lymphocytes, while PDL-1 is frequently upregulated on tumor cells. The interaction between these two molecules plays a critical role in tumor immune evasion by suppressing T cell-mediated anti-tumor responses, thereby facilitating tumor progression and metastasis. The therapeutic inhibition of this pathway using monoclonal antibodies forms the basis of immune checkpoint blockade therapy in NSCLC.[3]

According to the National Comprehensive Cancer Network (NCCN) guidelines, pembrolizumab, a humanized monoclonal antibody targeting PDL-1, is recommended as a first-line monotherapy in patients with metastatic NSCLC lacking actionable driver mutations and exhibiting PD-L1 expression.[4] The reported prevalence of PD-L1 expression among NSCLC patients varies considerably, ranging from 13% to 70%.[5] A recent meta-analysis has highlighted the heterogeneity of PD-L1 expression based on diverse clinical characteristics, histological subtypes, and tumor differentiation grades.

In the present study, we conducted immunohistochemical (IHC) analysis to evaluate PD-L1 expression in a retrospective study of 47 NSCLC patients and investigated its correlation with various clinicopathological parameters.[6]

1.2 AIM OF STUDY

Evaluation of programmed cell death ligand 1 immunohistochemical expression in non-small cell lung cancer and its correlation with different clinical and histopathological parameters.

1.3 Literature review

1.3.1. Lung cancer: Epidemiology, Etiology, and Classification

Lung cancer represents the most frequently diagnosed malignant tumor worldwide and continues to be the primary cause of cancer-related deaths. According to the latest GLOBOCAN data, it accounts for roughly 12.4% of all new cancer cases and 18.7% of cancer-related mortality, with an estimated 2.4 million new diagnoses and 1.8 million deaths globally.[7] In men, lung cancer is the leading cause of both incidence and mortality. In women, it is the second most commonly diagnosed cancer, after breast cancer, but remains the top cause of cancer-related death.[8]

In Iraq, The total number of new cases of cancer in year 2022 was 37.382, lung cancer was the second most common cancer in both sexes with incidence rate 7.5 % 1.2 and cause 12.1% of cancer related death.[9]

1.3.2 Etiology and Risk Factors

1. **Smoking:** Tobacco use is the main cause of lung cancer, responsible for approximately 80% of cases. Cigarette smoke contains numerous carcinogens that trigger multiple molecular pathways leading to tumor development. There is usually a latency of 10–30 years between smoking initiation and lung cancer onset. Notably, risk remains elevated for decades after cessation.[10] Only a minority of smokers develop lung cancer, highlighting potential genetic susceptibility. Passive smoking, including exposure at home or work, also significantly increases risk.[11] Recently, third-hand smoke—residues of tobacco smoke on surfaces—has emerged as a concern, as it can persist for months and re-enter the air, posing health hazards, especially for children.[12]
2. **Gender:** While smoking accounts for most cases in men, a significant number of women with lung cancer have never smoked, suggesting gender-specific susceptibility factors. Hormonal influences, such as estrogen, may play a role, though evidence remains under investigation.[13]
3. **Environmental and Occupational Exposures:** Radon, asbestos, arsenic, chromium, nickel, and polycyclic aromatic hydrocarbons are recognized carcinogens associated with lung cancer.[14] Co-exposure to tobacco and substances like asbestos further increases

risk. Indoor and outdoor air pollution, including industrial emissions, vehicle exhaust, and cooking fumes, have also been implicated.[15] Previous exposure to ionizing radiation, for example during treatment for Hodgkin lymphoma or breast cancer, elevates the risk.[16]

4. **Dietary Factors:** Diet-related deficiencies, particularly low antioxidant levels (vitamins A, C, E), may reduce the body's ability to counteract oxidative stress, contributing to carcinogenesis.[17]
5. **Genetic and Hereditary Factors**: A family history of lung cancer increases risk, and inherited syndromes, such as Li-Fraumeni syndrome, can predispose individuals to multiple cancers, including lung cancer.[18]
6. **Race and Ethnicity:** Lung cancer incidence and mortality differ across populations. Higher rates are observed among Black and Polynesian populations, whereas Japanese populations generally show lower incidence. African Americans tend to experience higher mortality than White Americans, likely due to a combination of genetic, environmental, and socioeconomic factors.[19]
7. **Other Risk Factors**: Additional contributors include chronic pulmonary diseases (e.g., COPD, pulmonary fibrosis, tuberculosis), HIV infection, excessive alcohol consumption, and potentially oncogenic viruses such as human papillomavirus (HPV).[20,21]

1.3.3 Diagnosis

The diagnosis of lung cancer typically combines clinical evaluation with imaging and histopathological confirmation. Biopsy of the primary pulmonary mass is standard, often supplemented by lymph node, pleural, or distant metastatic tissue sampling.[22]

- **Clinical presentation:** Patients frequently present at advanced stages with poor prognosis. Common symptoms include persistent cough, shortness of breath, and hemoptysis, with digital clubbing serving as a more specific sign. Manifestations may arise from the primary tumor, metastases, or paraneoplastic syndromes. Clinicians should remain vigilant in individuals with risk factors such as smoking or occupational exposures.[23]

- **Radiologic Evaluation**

1. Chest X-ray.
2. CT Scan (Computed tomography).
3. PET Scan(Positron Emission Tomography) .[24]

- **Histopathology and Immunohistochemistry**: Essential for definitive diagnosis and molecular profiling. Techniques include bronchoscopy (with advanced methods for peripheral nodules), thoracoscopy, mediastinoscopy, transthoracic needle biopsy, and open lung biopsy when less invasive methods are inconclusive.[25]

1.3.4 Histopathological Classification of lung cancer

Lung cancer is primarily classified based on histopathological characteristics into two major categories: Non-Small Cell Lung Cancer (NSCLC) and Small Cell Lung Cancer (SCLC). This classification is crucial for determining prognosis and guiding treatment strategies.[26]

Table 1.1: the 2021 WHO Classification of Lung Cancer.[27]

Main type	Subtype	Nots
Non-small cell lung Ca(NSCLC) 85%	Adenocarcinoma Squamous cell carcinoma Large cell carcinoma	Most common is Adenocarcinoma. non-Smoker) Squamous linked to smoking
Small cell lung Ca (SCLC) 15%	Pure small cell Ca Combined small cell Ca	Very aggressive , rapid metastasis
Rare type	Neuroendocrine tumor Pulmonary sarcomatoid Lung sarcoma and lymphoma	

1- **adenocarcinoma:** Lung adenocarcinoma is the most common histological subtype of non-small cell lung carcinoma (NSCLC). It arises from glandular epithelial cells and is typically located in the peripheral regions of the lung. Histologically, lung adenocarcinoma demonstrates considerable heterogeneity, with several recognized growth patterns that have important prognostic and therapeutic implications.[28]

The main histological growth patterns include lepidic, acinar, papillary, micropapillary, and solid patterns.[29]

Tumor cells are typically cuboidal to columnar with enlarged, hyperchromatic nuclei and prominent nucleoli. Cytoplasmic and extracellular mucin production is a defining feature of adenocarcinoma and can be demonstrated using special stains such as periodic acid–Schiff (PAS) or Alcian blue. The tumor stroma may show desmoplastic reaction, necrosis, and evidence of invasion into adjacent structure, From a molecular perspective, lung adenocarcinoma frequently harbors driver mutations such as EGFR (epidermal growth factors receptor) mutations, ALK (anaplastic lymphoma kinase) rearrangements, and KRAS mutations.[30]

2- **squamous cell carcinoma:** Lung squamous cell carcinoma (SCC) is the second most common subtype of non-small cell lung carcinoma (NSCLC) and is strongly associated with cigarette smoking. It typically arises in the central bronchi and is characterized by distinct histopathological features reflecting squamous differentiation.[31]

Histologically, SCC is defined by the presence of keratinization and/or intercellular bridges. Tumor cells are polygonal with abundant eosinophilic cytoplasm, irregular hyperchromatic nuclei, and prominent nucleoli. Well-differentiated tumors show keratin pearl formation, whereas poorly differentiated SCC may lack obvious keratinization and require immunohistochemical confirmation.[32]

The growth pattern of SCC is generally solid, often accompanied by extensive tumor necrosis and stromal desmoplasia.

Molecular alterations commonly seen in adenocarcinoma, such as EGFR mutations and ALK rearrangements, are rare in SCC. Instead, SCC frequently exhibits complex genomic alterations related to tobacco exposure, resulting in a high tumor mutational burden (TMB).[33]

1.3.5 Histologic Grading of Lung Cancer

According to the 2021 WHO classification and IASLC recommendations, invasive lung adenocarcinoma is graded into three tiers based on the predominant histologic pattern and the presence of a high-grade component: Grade 1 (well-differentiated) with a predominant lepidic pattern, Grade 2 (moderately differentiated) with predominant acinar or papillary patterns, and Grade 3 (poorly differentiated) with predominant solid or micropapillary patterns or the presence of >20% high-grade components. Mucinous differentiation does not independently affect tumor grade but should be documented.[34]

Lung squamous cell carcinoma is commonly graded into three tiers based on the degree of keratinization, intercellular bridges, and nuclear atypia. Small cell lung carcinoma (SCLC) is not graded, as it is considered a high-grade neuroendocrine carcinoma by definition. Large cell carcinoma is a diagnosis of exclusion and is regarded as a high-grade tumor. Pulmonary neuroendocrine tumors are graded based on mitotic count and necrosis into typical carcinoid, atypical carcinoid, and large cell neuroendocrine carcinoma (LCNEC).[35]

Typical carcinoid: <2 mitoses/2 mm², no necrosis and Ki-67 <5%.

Atypical carcinoid: 2–10 mitoses/2 mm² and/or focal necrosis and Ki-67 5%-20%.
Large cell neuroendocrine carcinoma (LCNEC): >10 mitosis/2 mm² and high Ki-67 >30%.[36]

1.3.6 Staging of Non-small Cell Lung Cancer

The staging of non-small cell lung cancer (NSCLC) is fundamental for guiding treatment decisions and estimating prognosis. The latest iteration, the 9th edition of the TNM classification (2024), introduces more detailed tumor descriptors, refined lymph node stratification, and enhanced metastatic categories to improve clinical accuracy. Staging complements histologic grading by providing anatomical context for the disease.[37]

Table 1.2: TNM 9TH EDITION (NSCLC TNM Staging).[38]

T – Primary Tumor

Stage	Description
Tis	Carcinoma in situ
T1	Tumor ≤ 3 cm, surrounded by lung or visceral pleura
T1a	Tumor ≤ 1 cm
T1b	Tumor > 1 cm but ≤ 2 cm
T1c	Tumor > 2 cm but ≤ 3 cm
T2	Tumor > 3 cm but ≤ 5 cm or invasion of main bronchus, visceral pleura, or atelectasis
T2a	Tumor > 3 cm but ≤ 4 cm
T2b	Tumor > 4 cm but ≤ 5 cm
T3	Tumor > 5 cm but ≤ 7 cm or invasion of chest wall, phrenic nerve, or same-lobe nodules
T4	Tumor > 7 cm or invasion of mediastinum, heart, great vessels, or nodules in another ipsilateral lobe

N – Regional Lymph Nodes

Stage	Description
N0	No regional lymph node metastasis
N1	Ipsilateral peribronchial, hilar, or intrapulmonary lymph nodes
N2	Ipsilateral mediastinal or subcarinal lymph nodes
N3	Contralateral mediastinal/hilar or supraclavicular lymph nodes

M – Distant Metastasis

Stage	Description
M0	No distant metastasis
M1a	Contralateral lung nodules or malignant pleural/pericardial effusion
M1b	Single extrathoracic metastasis
M1c	Multiple extrathoracic metastases

Building on the TNM classification, the American Joint Committee on Cancer (AJCC) has defined a clinical cancer staging system. This system has slowly changed over time, with the

advances in diagnosis and treatment improvement. In the past decade, it was updated in 2010 and 2017.

Staging definitions in the newest version ('The Eighth Edition AJCC Cancer Staging Manual'14) are summarized in Table 1.6.

Table 1.3: Clinical staging of NSCLC.[39]

AJCC stage	TNM standard	Stage description
Occult (hidden)	Tx, N0, M0	The primary tumour cannot be assessed, or cancer cells are seen in lung fluids (e.g. sputum sample), but the cancer is not found with other tests, so its location cannot be determined.
0	Tis, N0, M0	The tumour is found only in the top layers of cells lining the air passages.
IA	T1mi, N0, M0 T1, N0, M0	The cancer is a minimally invasive carcinoma, or the tumour is no longer than 3cm.
IB	T2a, N0, M0	The tumour has one/more following features: - Larger than 3cm but less than 4cm across - Grown into the main bronchus, but with more than 2cm from the carina + less than 4cm across - Grown into the visceral pleura + less than 4cm across
IIA	T2b, N0, M0	The tumour has one/more following features: - Larger than 4cm but less than 5cm across - Grown into the main bronchus, but with more than 2cm from the carina + less than 5cm across - Grown into the visceral pleura + less than 5cm across
IIB	T1, N1, M0 T2, N1, M0 T3, N0, M0	The tumour has one/more following features: - Any above description in IA/IB/IIA + cancer has spread to the ipsilateral hilar lymph nodes (where the bronchus enters the lung) - Larger than 5cm but less than 7cm across - Grown into the chest wall, parietal pleura, phrenic nerve, or parietal pericardium - Have two or more separate nodules in the same lobe
IIIA	T1, N2, M0 T2, N2, M0 T3, N1, M0 T4, N0/N1, M0	The tumour has one/more following features: - Any above description in IA/IB/IIA + cancer has spread to the ipsilateral lymph nodes around the carina or mediastinum - Any above description in T3 IIB (T3, N0, M0) + cancer has spread to the ipsilateral hilar lymph nodes - Larger than 7cm across - Grown into the mediastinum, heart, trachea, oesophagus, diaphragm, backbone, carina, or the large vessels near the heart (e.g. aorta) - Have two or more separate nodules in the different lobes

IIIB	T1, N3, M0 T2, N3, M0 T3, N2, M0 T4, N2, M0	The tumour has one/more following features: - Any above description in IA/IB/IIA + cancer has spread to lymph nodes in any undescribed parts of the body - Any above description in T3 IIB (T3, N0, M0) or T4 IIIA (T4, N0/N1, M0) + cancer has spread to the ipsilateral lymph nodes around the carina or mediastinum
IIIC	T3, N3, M0 T4, N3, M0	The tumour has one/more following features: - Any above description in T3 IIB (T3, N0, M0) or T4 IIIA - (T4, N0/N1, M0) + cancer has spread to lymph nodes in any undescribed parts of the body
IV	Any T. N, M1	Cancer can be any size or even without growing into the lymph node, but has one or more following features: - Cancer has spread to the other lung - Cancer has pleural (fluid around the lung) / pericardial (fluid around the heart) effusion - Cancer has spread to organs outside of the lung

These are 5-years Survival Rates (Based on SEER and TNM 9th Edition)[40]

- Stage I: 68–92%
- Stage II: 53–60%
- Stage III: 13–36%
- Stage IV: <10%

1.4 Molecular Pathogenesis of NSCLC

NSCLC development is driven by specific genetic alterations, often referred to as oncogenic drivers. These mutations tend to be mutually exclusive, meaning that a tumor usually relies on one primary signaling pathway for growth and survival, which makes them ideal targets for therapy.[41]

1- Driver Gene Mutations

- EGFR (Epidermal Growth Factor Receptor).
- KRAS(Kirsten rat sarcoma viral oncogene homolog).
- ALK(anaplastic lymphoma kinase) and ROS1(ROS proto oncogen1)Rearrangements.
- BRAF (B-rapidly accelerated fibrosarcoma), MET (mesenchymal epithelial transition factor proto-oncogen), RET (Rearranged during transfection proto-oncogene), HER2 (Human epidermal growth factor 2 receptor).[42]

2- Tumor Suppressor Gene Inactivation

Mutations in genes such as TP53, STK11, KEAP1, and NFE2L2 contribute to genomic

instability, altered metabolism, chemoresistance, and poor prognosis.[43]

3- Epigenetic and Non-Coding RNA Alterations

Abnormal DNA methylation, histone modifications, and microRNA dysregulation can promote tumor progression, metastasis, and treatment resistance.[44]

4- Tumor Microenvironment and Immune Evasion

Non-small cell lung cancer (NSCLC) often escapes immune control by exploiting mechanisms within its tumor microenvironment (TME). A central immune evasion strategy is the overexpression of PD-L1 (Programmed Death Ligand-1) on tumor or stromal cells, which suppresses T cell activity by engaging the PD-1 receptor on T lymphocytes. Immune checkpoint inhibitors targeting PD-1/PD-L1 have become a critical therapeutic strategy in NSCLC.[45]

The tumor microenvironment comprises not only cancer cells but also immune cells (T cells, macrophages, dendritic cells, and regulatory T cells), fibroblasts, blood vessels, and extracellular matrix. These components interact dynamically, shaping immune responses.[46]

Tumors can "reprogram" their microenvironment to become immunosuppressive—for example, by recruiting regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), or producing suppressive cytokines.[47]

Within this environment, upregulation of PD-L1 acts as a molecular "brake" on anti-tumor immunity, allowing tumor cells to survive and proliferate despite immune presence.[48]

1.4.1 Mechanism of PD-L1 Upregulation and Its Effects

- **Induction by Interferon-gamma (IFN-γ):** One of the main inducers of PD-L1 is IFN-γ, secreted by activated T cells or NK cells in response to tumor antigens. IFN-γ signals through the JAK/STAT/IRF1 pathway to increase transcription of the CD274 gene (which encodes PD-L1).[49]
- **Oncogenic signaling and micro environmental stress:** PD-L1 expression can also be upregulated by cancer cell–intrinsic pathways, such as activation of **EGFR**, **MYC**, or hypoxia-inducible factors (HIF1) under low oxygen conditions.[50]
- **Post-transcriptional and post-translational regulation:** PD-L1 levels and stability are also affected by RNA-binding proteins, microRNAs, glycosylation, and protein degradation pathways. For example, glycosylation of PD-L1 can mask epitopes and

affect antibody binding in immunohistochemistry assays.[51]

When PD-L1 binds to PD-1 on T cells, it sends an inhibitory signal that Reduces T cell proliferation and cytokine secretion, Induces T cell exhaustion or apoptosis Dampens cytotoxic (killer) function of $CD8^+$ T cells.

Thus, even when tumor-specific T cells are present, PD-L1/PD-1 interactions can prevent effective tumor cell killing.[52]

1.4.2 Clinical Relevance and Immune Checkpoint Inhibitors

- Drugs targeting the PD-1/PD-L1 pathway (e.g. pembrolizumab, nivolumab, atezolizumab, durvalumab) block this inhibitory interaction, reactivating T cell responses against tumor cells.[53]
- In NSCLC, PD-L1 expression (typically assessed by immunohistochemistry) is used as a predictive biomarker—patients whose tumors express higher PD-L1 are more likely to respond to checkpoint inhibitors.[54]
- However, not all PD-L1–positive patients respond, and some PD-L1–negative patients do respond, indicating that PD-L1 is an imperfect biomarker.[55]

1.4.3 Resistance and Challenges

- **Primary and acquired resistance:** Even in tumors with high PD-L1, ~30-40% of patients may not respond or eventually develop resistance.[56]

- **Mechanisms of resistance** may include:
  - Loss or downregulation of antigen presentation machinery (e.g. reduced MHC class I)
  - Activation of alternative immune checkpoint pathways (e.g. TIM-3, LAG-3)
  - Development of an “immune-cold” microenvironment (few T cells can infiltrate)
  - Presence of exosomal PD-L1, which can bind and suppress T cells at a distance and contribute to systemic immune suppression.[57]

- **Dynamic changes in PD-L1 expression:** Treatments such as chemotherapy, radiotherapy, or targeted agents can alter PD-L1 levels over time, complicating use of a single measurement.[58]

1.4.5 Future Directions and Combination Strategies

- Combining checkpoint inhibitors with chemotherapy, radiotherapy, targeted therapies, or

other immunotherapies (e.g. anti-CTLA-4) is a promising approach to overcome resistance.[59]

- Better biomarkers are needed (e.g. composite biomarkers, gene signatures) to more accurately predict who will benefit.
- Targeting exosome biogenesis or removing exosomal PD-L1 may be an emerging strategy to reduce immune suppression.[60]

2.1 Patients

2.1.1 Study design: This study is retrospective study that was done at the laboratory of Al-Sader Teaching hospital in Basrah during the period from June 2025 to the end of December 2025 after approval of research ethics committee.

2.1.2 Study group: A total 47 patients newly diagnosed with non-small cell lung cancer the age range from 42 to 88 years.

2.1.3 Inclusion criteria: the patients diagnosed with non-small cell lung cancer based on histopathological diagnosis.

2.1.4 Exclusion criteria: Patients who had carcinoma other than non-small cell lung carcinoma, Patients who had primary and metastatic carcinoma other than lung cancer, histological grade and stage not include in this study.

2.2.Materials

2.2.1. Instruments and equipment: The equipment used in processing and Staining of immunohistochemistry was displayed in the following box.

Table 2.1: instrument and equipment.

Equipment & Instruments	Microtome
	Drying oven
	Pressure cooker
Microscopic slides (positively charged)	Water bath
Cover slip	Timer
Light microscope	Glass staining jar
Slide holder	Slide marker pen
Tissue paper	Rubber gloves

2.2.2. Chemicals: The chemicals used to obtain paraffin-embedded sections for IHC staining are displayed in the following box:

Table 2.2: chemicals.

Chemicals
Ethanol (different concentration absolute, 70%, 90%)
Xylene
Distilled water
Tap water
DPX(Dibutylphthalate Polystyrene Xylene)

Table 2.3: Immunohistochemical material.

PDL1 IHC 22C3
Peroxidase-blocking reagent
Monoclonal mouse anti-PDL1 ,clone 22C3
Linker , anti –mouse
Visualization reagent –HRP
DAB+substrate buffer
DAB + chromogen
DAB enhancer
Envision flex target retrieval solution low PH(50x)
Control slide

2.3 METHODS

Reviewed H&E-stained slides and FFPE blocks from newly diagnosed NSCLC cases sent to the AL-Sader Teaching Hospital laboratory and private laboratory (Each patient's histopathological report confirmed the diagnosis of NSCLC). The immunohistochemical biomarker (PDL1) were evaluated using fully automated stainer.

2.3.1. Immunohistochemistry staining protocol

It includes the following steps:

1. **Tissue Preparation:** Sections of 3-µm thickness are cut from selected FFPE NSCLC blocks using a microtome, The Sections are then placed on salinized microscope slides and incubated in an oven to ensure tissue adhesion.
2. **Deparaffinization and Rehydration:** Slides are immersed in xylene for 5 minutes (with two changes) to remove paraffin and Rehydration Slides are passed through a graded ethanol series (100% ethanol Twice, 95% ethanol twice, and 75% ethanol) for 5 minutes each, followed by Rinsing in running tap water.
3. **Antigen Retrieval:** Slides are placed in a pressure cooker filled with target antigen retrieval buffer and heated for 15 minutes at a boiling temperature. Afterward, allow the slides to cool for 20 minutes and rinse them in distilled water for 2 minutes.
4. **Load Slides into Auto stainer**: Transfer the prepared slides into the Auto stainer(PT –

link), Then Automated Staining Protocol performed in auto stainer as programmed. The Dako Auto stainer performs the following steps:

a. **Peroxidase Blocking:-** Apply Peroxidase-Blocking Reagent to block endogenous peroxidase activity. - Incubate for 5–10 minutes, then rinse with wash buffer.
b. **Primary Antibody Application:-** Apply the PD-L1 primary antibody (e.g., clone 22C3). - Incubate for 20–30 minutes. - Rinse slides with wash buffer.
c. **Secondary Antibody (Detection Reagent):** Apply the EnVision FLEX+ HRP Detection Reagent (Horseradish Peroxidase) and incubate for 20 minutes then Rinse with wash buffer. 2 A Appendix (A)
d. **DAB Chromogen Development**: Apply the DAB chromogen solution. Incubate for 5–10 minutes until desired staining intensity is achieved. Rinse slides thoroughly with wash buffer.
e. **Counterstaining**: Apply hematoxylin to stain nuclei. Incubate for 2–3 minutes, rinse, and dip in a bluing reagent.
f. **Dehydration**: Dehydrate slides through graded alcohol and xylene.
g. **Mounting**: Non-aqueous, permanent mounting medium is required

NOTE: the processing of IHC was done in automated way in both AL-Sader teaching hospital and private laboratory.

2.4 Evaluation

The stained slides examined under a microscope: The expression of PDL1 was assessed and scored according to NSCLC – PD-L1 expression determined by Tumor Proportion Score (TPS).

$$TPS(\%) = \frac{PD-L1\ staining\ cells\ (tumer\ cells)}{Total\ of\ vaible\ tumer\ cells} \times 100$$

All viable tumor cells on the entire tissue section must be evaluated and included in the PD-L1 scoring assessment. A minimum of 100 viable tumor cells must be present in the PD-L1 stained slide for the specimen to be considered adequate for PD-L1 evaluation. Slide evaluation should be performed by a pathologist using a light microscope. For evaluation of the immunohistochemical staining and scoring, an objective of 40x magnification is appropriate. Any perceptible membrane staining of tumor cells should be included in the scoring. PD-L1 protein expression is determined by using TPS, which is the percentage of viable tumor cells showing partial or complete membrane staining at any intensity. Score

partial or complete cell membrane staining (≥ 1+) that is perceived distinct from cytoplasmic staining. Cytoplasmic staining should be considered non-specific staining and is excluded in the assessment of staining intensity. Normal cells and tumor-associated immune cells such as infiltrating lymphocytes or macrophages should not be included in the scoring for the determination of PD-L1 expression.

Table 2.4: tumor proportion score.

Tumor proportion score			
PD-L1expression level	TPS<1 %	TPS≥1%	TPS≥50%
PD-L1expression status	No PD-L1 expression	PD-L1 expression	High PD-L1 expression

Student t-test was used to compare means between two groups. Pearson Chi-Square test and Fisher Exact test were used to find the association between categorical variables. A P-value of ≤ 0.05 was considered as significant.

RESULTS

3.1 Clinicopathological analysis

Among the 47 cases included in this study, males constituted the majority with 32 cases (68.1%), while female accounted for 15 cases (31.9%), as illustrated in Table 3.1

Table 3.1: Sex distribution of the study sample.

Sex	Frequency	Percent
Male	32	68.1%
Female	15	31.9%
Total	47	100.0%

The majority of patients were between 61–70 years (38.3%), followed by those aged 71 years or older (31.9%). Patients aged 51–60 years accounted for 19.1%, while only 10.6% of the cases were 50 years or younger. This indicates that most patients were in the older age groups.

Table 3.2: Age distribution of the study sample.

Age (Year)	Frequency	Percent
50 or younger	5	10.6%
51-60	9	19.1%
61-70	18	38.3%
71 or older	15	31.9%
Total	47	100.0%

adenocarcinoma was the predominant histological subtype, representing 70.2% of the cases,

whereas squamous cell carcinoma accounted for 29.8%. This finding is in line with current global trends reporting adenocarcinoma as the most frequent type of non-small cell lung carcinoma.

Table 3.3: Distribution of cancer according to type.

Cancer type	Frequency	Percent
Adenocarcinoma	33	70.2%
Squamous cell carcinoma	14	29.8%
Total	47	100.0%

Table 3.4 shows that 29 patients (61.7%) expressed PD-L1, while 18 patients (38.3%) were negative. This relatively high prevalence of PD-L1 positivity suggests a potential role for immunotherapy in a substantial proportion of the study population.

Table 3.4: Distribution of the study sample according to the presence of PDL1.

PDL1	Frequency	Percent
Negative	18	38.3%
Positive	29	61.7%
Total	47	100.0%

Among the 29 PD-L1–positive cases, low expression was more common (82.8%), whereas high expression was detected in only 17.2% of cases (Table 3.5).

Table 3.5: Distribution of the study sample according to the level of expression.

Level of expression	Frequency	Percent
Low expression	24	82.8%
High expression	5	17.2%
Total	29	100.0%

3.6 Table demonstrates the relationship between PD-L1 expression and sex. PD-L1 positivity was observed in 56.3% of males and 73.3% of females. However, the difference was not statistically significant (p = 0.261).

Table 3.6: The association between PDL1 and sex.

		Sex		Total	P-value*
		Male	Female		
PDL1	Negative	14	4	18	0.261
		43.8%	26.7%	38.3%	
	Positive	18	11	29	
		56.3%	73.3%	61.7%	
Total		32	15	47	
		100.0%	100.0%	100.0%	

* Pearson Chi-Square

As illustrated in Table3.7, PD-L1 positivity increased across older age categories, being most frequent among patients aged 61–70 years (61.1%) and 71 years or older (66.7%). Nonetheless, the association was not statistically significant (p = 0.795)

Table 3.7: The association between PDL1 and age.

		Age (Year)				Total	P-value*
		50 or younger	51-60	61-70	71 or older		
PDL1	Negative	3	3	7	5	18	0.795
		60.0%	33.3%	38.9%	33.3%	38.3%	
	Positive	2	6	11	10	29	
		40.0%	66.7%	61.1%	66.7%	61.7%	
Total		5	9	18	15	47	
		100.0%	100.0%	100.0%	100.0%	100.0%	

* Fisher's Exact Test

Table 3.8 reveals that PD-L1 positivity was slightly more frequent in adenocarcinoma (63.6%) compared to squamous cell carcinoma (57.1%). However, this difference was not statistically significant (p = 0.675).

Table 3.8: The association between PDL1 and cancer type.

		Cancer type		Total	P-value*
		Adenocarcinoma	Squamous cell carcinoma		
PDL1	Negative	12	6	18	0.675
		36.4%	42.9%	38.3%	
	Positive	21	8	29	
		63.6%	57.1%	61.7%	
Total		33	14	47	
		100.0%	100.0%	100.0%	

* Pearson Chi-Square

Table (3.9) shows that there was no any significant statistical association between the level of expression and sex (P=1.000).

Table 3.9: The association between the level of expression and sex.

		Level of expression		Total	P-value*
		Low expression	High expression		
Sex	Male	15	3	18	1.000
		62.5%	60.0%	62.1%	
	Female	9	2	11	
		37.5%	40.0%	37.9%	

Total	24	5	29	
	100.0%	100.0%	100.0%	

* Fisher's Exact Test

The level of expression was not associated significantly to age (P=0.162), Table (3.10).

Table 3.10: The association between the level of expression and age.

		Level of expression		Total	P-value*
		Low expression	High expression		
Age (Year)	50 or younger	1	1	2	0.162
		4.2%	20.0%	6.9%	
	51-60	5	1	6	
		20.8%	20.0%	20.7%	
	61-70	8	3	11	
		33.3%	60.0%	37.9%	
	71 or older	10	0	10	
		41.7%	0.0%	34.5%	
Total		24	5	29	
		100.0%	100.0%	100.0%	

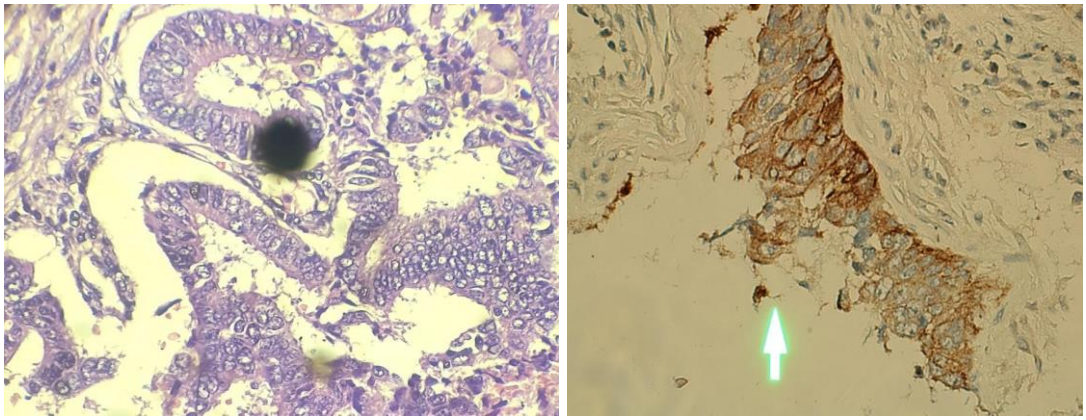
* Fisher's Exact Test

There was a significant statistical association between the level of expression and type of cancer, (P=0.013). Low expression was significantly associated with adenocarcinoma, while high expression was associated with squamous cell carcinoma, Table (3.11).

Table 3.11: The association between the level of expression and cancer type.

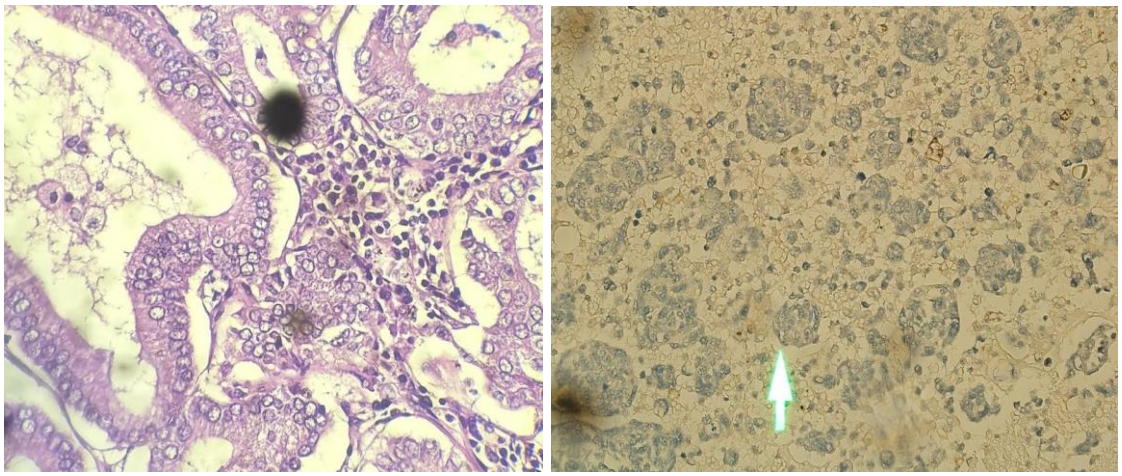
		Level of expression		Total	P-value*
		Low expression	High expression		
Cancer type	Adenocarcinoma	20	1	21	0.013
		83.3%	20.0%	72.4%	
	Squamous cell carcinoma	4	4	8	
		16.7%	80.0%	27.6%	
Total		24	5	29	
		100.0%	100.0%	100.0%	

* Fisher's Exact Test



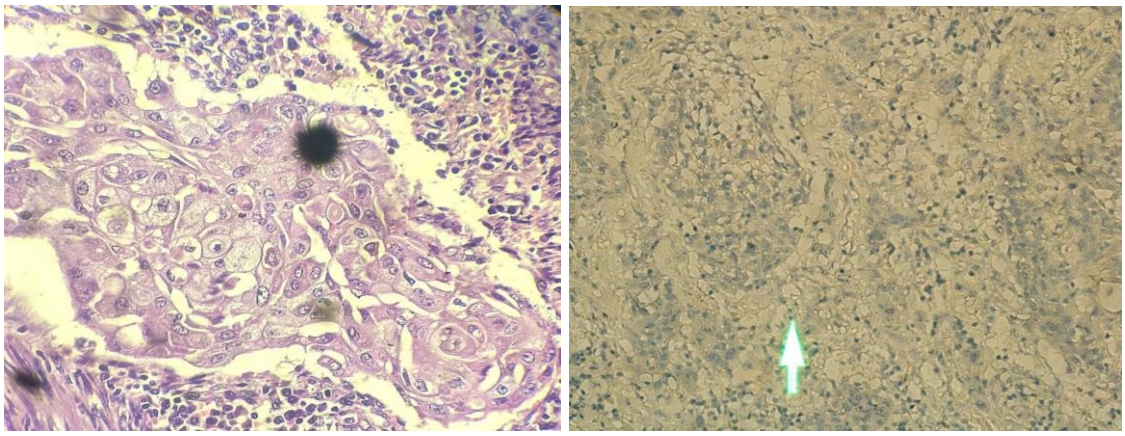
A. **B.**

Figure 3.1 Adenocarcinoma by H &E (A)(400x), with positive PDL1 stain and High expression >50 % (B)(400x).



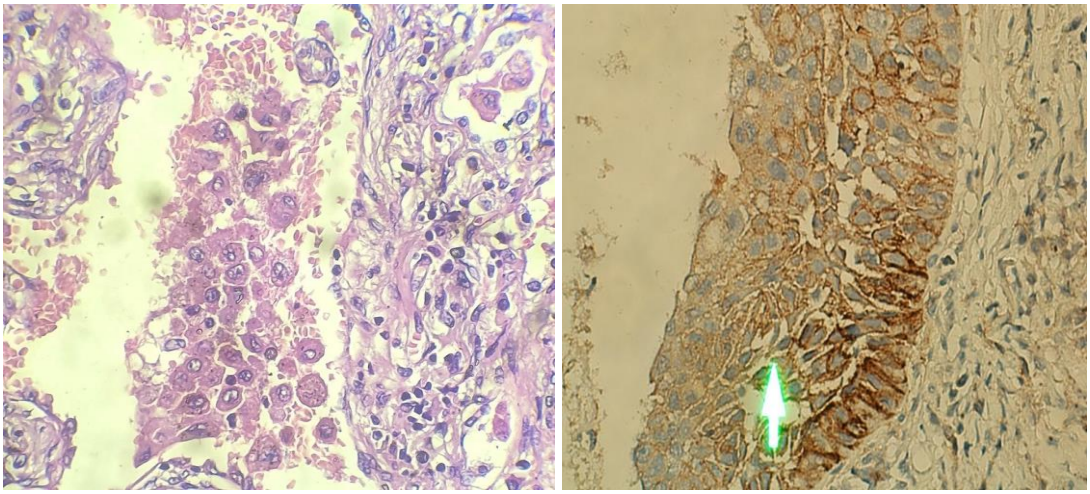
A. **B.**

Figure 3.2: Adenocarcinoma by H&E (A)(400x) and negative PDL1 stain (B)(100x).



A. **B.**

Figure 3.3 Squamous cell carcinoma in H&E (A)(400x), with negative PDL1 expression (B)(400x).



A. **B.**

Figure 3.4: Squamous cell carcinoma in H&E (A)(400x), with positive PDL1 stain and high expression > 50% (B)(400x).

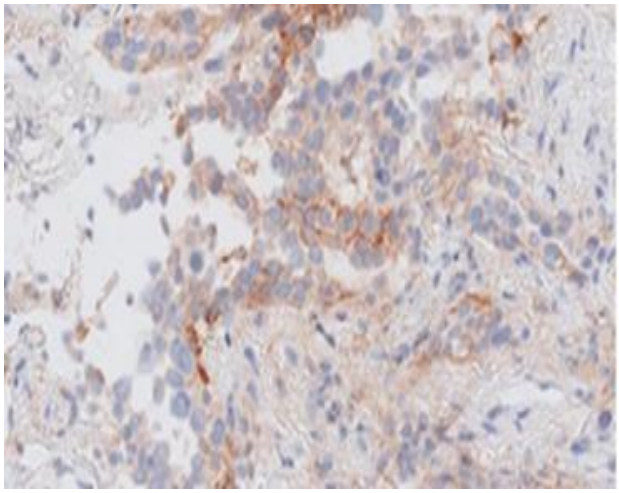


Figure 3.5: Adenocarcinoma with positive PDL1 low expression < 50%(100x).

4.1 DISCUSSION

Recent studies revealed that lung tumors develop multiple pathways to escape immune system so that they can develop and progress. Prolonged T cell receptor stimulation causes an upregulation of PD-L1 expression. PD-L1 is expressed by tumor cells as a consequence of inflammatory cytokines and oncogenic signaling pathways, thus inhibiting TCR-mediated positive signaling, resulting in decreased proliferation, reduced cytokine secretion, and reduced survival of T cells, hence escaping the immune response.

This study presents a review of 47 cases with non-small cell lung carcinoma with evaluation of PDL1 immunohistochemical expression in correlation with various parameters include age, gender, tumor type and level of expression.

In the present study, the percentage of positive PD-L1 expression was 61.7%, which lies within the previously reported range of PD-L1 expression in non-small cell lung carcinoma (NSCLC) (13%–75%). This relatively high rate of PD-L1 positivity compared with some earlier studies, such as those by **Manish Kumar et al**. (33.6%), **Dhawan et al**. (44%), and **Saif et al**. (40%), may be attributed to differences in several factors, including sample size, patient demographics, tumor histological subtypes, antibodies used, as well as variations in scoring systems like CPS(combined positive score), IC(immune cell score)and TC (tumor cell score) and interpretation criteria. Nevertheless, the findings of the current study are consistent with the general observation that a substantial proportion of NSCLC cases exhibit PD-L1 positivity, supporting the role of PD-L1 as an important biomarker for immunotherapy eligibility. The age of patients in this study ranged from 42 to 88 years, and no statistically significant correlation was found between age and PD-L1 expression (P = 0.795), although higher positivity was observed among older patients. This result is consistent with the findings of **Manish Kumar et al.**[61] and **Saez de Gordoa et al.**[62] Regarding gender distribution, the study included 32 male and 15 female patients, with no significant association between gender and PD-L1 expression (P = 0.261). This finding agrees with **Saez de Gordoa et al**., but contrasts with the results of **Manish Kumar et al**. and **Kilaru et al**,[63] who reported male predominance, possibly related to environmental factors influencing epigenetic expression.[62] Concerning tumor type, the study comprised 33 cases of adenocarcinoma and 14 cases of squamous cell carcinoma (SCC), of which 21 adenocarcinoma cases and 8 SCC cases showed positive PD-L1 expression. There was no statistically significant relationship between tumor type and PD-L1 immunohistochemical expression (P = 0.675). This finding is consistent with Jain et al., but differs from the results reported by **Ye et al.,**[65] **Saez de Gordoa et al**., and **Fu et al**.,[64] who demonstrated lower PD-L1 expression in adenocarcinoma. With regard to the level of PD-L1 expression,[63] among the 29 positive cases, 82.8% exhibited low expression, while 17.2% showed high expression. These findings are comparable to those reported in an Indian study by **Manish Kumar et al**.,[61] who found that 67.6% of PD-L1-positive patients demonstrated high expression (>50%), whereas 32.3% showed low expression (<50%). Furthermore, analysis of the association between the level of PD-L1 expression and clinicopathological parameters revealed a statistically significant relationship between expression level and tumor type (P = 0.013), with low expression being significantly associated with adenocarcinoma and high expression with squamous cell carcinoma this is due to tumor microenvironment in which SCC strongly associated with smoking which lead to high mutational burden and massive

immune response while adenocarcinoma its cold tumor with less T cell infiltration This finding is contrary to the results reported by **Hacihasanoglu et al**. in Turkey but is in agreement with **Saez de Gordoa et al.,**[62] in Spain. In contrast, no significant association was observed between the level of PD-L1 expression and patient age or gender, which is consistent with the findings of both **Hacihasanoglu et al**. and **Saez de Gordoa et al.**[62]

Several limitations associated with the present study

First: the relatively small sample size may have limited the statistical power to detect subtle but clinically relevant associations. Larger, multi-center studies are needed to validate these findings and ensure their generalizability.

Second: the analysis was restricted to sex, age, and cancer type; other potentially important clinicopathological variables such as tumor stage, grade, smoking status, and molecular alterations were not included. These factors may have a significant influence on PD-L1 expression and should be addressed in future research.

Third: study was limited to small biopsies; we did not examine surgical biopsies. material might not accurately reflect the full picture of the entire tumor, especially if it is a tumor with mixed growth patterns.

Forth: The data were collected from a single institution, which may limit the generalizability of the findings.

5.1 CONCLUSION

1- This study demonstrated a statistically significant association between PD-L1 expression levels and histological subtype, with higher expression more frequently observed in squamous cell carcinoma, whereas lower expression was more common in adenocarcinoma.
2- In contrast, no statistically significant association was identified between PD-L1 expression and patients' demographic variables, including age and sex.
3- These results suggest that PD-L1 expression is more likely to be driven by tumor-intrinsic factors rather than basic demographic or histological characteristics.
4- The direct evaluation of PD-L1 remains crucial for guiding immunotherapy decisions, and further large-scale studies are needed to better define its clinical role.

5.2 Recommendations

1. Since PD-L1 expression was not significantly associated with demographic or histological factors, clinicians should rely on direct immunohistochemical assessment rather than patient characteristics when considering immunotherapy eligibility.
2. Future studies should include larger and more diverse cohorts to increase statistical power and generalizability of findings. Multi-center collaborations may help to overcome the limitations of small sample sizes.
3. Further research should investigate the relationship between PD-L1 expression and other important clinicopathological or molecular factors, such as tumor stage, smoking status, genetic mutations, and immune microenvironment characteristics.
4. To improve patient selection for immunotherapy, PD-L1 expression should be studied in combination with additional biomarkers such as tumor mutational burden (TMB) and microsatellite instability (MSI).
5. Longitudinal studies: Prospective designs including follow-up and survival analysis would help to establish the prognostic value of the biomarker.

ACKNOWLEDGMENT

First, all praise and thanks to Allah, for granting me strength and perseverance, without Allah mercy, none of this would be possible.

Then, I would like to express my heartfelt gratitude toward my supervisors **consultant. Dr.Sura ALnamil** for her support, and guidance during the period of this work.

All thanks to **Dr. saad abdulbaqi** for his great help and support.

All thanks for my family for their help and encouragement, patience and great understanding. Lastly, I offer my gratitude and regards to all of those who helped me in any aspect during the completion of the study.

REFERENCES

1. Mok TS, Wu YL, Kudaba I, Kowalski DM, Cho BC, Turna HZ, et al. Pembrolizumab versus chemotherapy for advanced non-small-cell lung cancer with PD-L1 expression (KEYNOTE-042): a randomised, open-label, controlled, phase 3 trial. The Lancet., 2019; 393(10183): 1819-1830. Available from: https: //doi.org/10.1016/S0140-6736(18)32409-7

2. National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology: Non-Small Cell Lung Cancer. Version 1.2025. Plymouth Meeting, PA: NCCN; 2024.Available from: https: //www.nccn.org/guidelines/category_1/lung_cancer [Accessed 4 Feb 2026].
3. Han Y, Liu D, Li L. PD-1/PD-L1 pathway: current researches in cancer immunotherapy J Hematol Oncol., 2020; 13(1): 94 Available from: https: //doi.org/10.1186/s13045-020-00929-5
4. awelczyk K, Piotrowska A, Ciesielska U, Jurek T, Dziegiel P. Role of PD-L1 expression in non-small cell lung cancer and prospects for its prognostic significance. Theor Biol Med Model, 2019; 16(1): 2 Available from: https: //doi.org/10.1186/s12976-019-0098-6
5. Yang H, Wu Z, Wang J, Li S. PD-L1 expression and its association with clinicopathological features in surgically resected non-small cell lung cancer. J Cancer Res Clin Oncol., 2025; 151(1): 42-56 Available from: https: //pmc.ncbi.nlm.nih.gov/articles/PMC12234839/
6. Karakas Y, Yalcin S, Karakoyun-Celik N, Ercelep O. The relationship between clinicopathological parameters and PD-L1 expression level in advanced stage non-small cell lung cancer. Turk J Med Sci., 2022; 52(3): 612-619. Available from: https: //pubmed.ncbi.nlm.nih.gov/35785877/
7. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin., 2024; 74(3): 229-263. Available from: https: //doi.org/10.3322/caac.21834
8. International Agency for Research on Cancer (IARC). Iraq Source: Globocan 2022. Lyon,France: World Health Organization; 2024 Available from: https: //gco.iarc.who.int/media/globocan/factsheets/populations/368-iraq-fact-sheet.pdf
9. Iraqi Cancer Board. Cancer Registry of Iraq Annual Report 2022. Baghdad, Iraq: Ministry of Health; 2024. Available from: https: //moh.gov.iq/upload/2024_03_31_11983087032.pdf
10. Siegel RL, Giaquinto AN, Jemal A. Cancer statistics, 2024. CA Cancer J Clin. 2024; 74(1): 12-49. Available from: https: //doi.org/10.3322/caac.21820
11. Hang B, Wang P, Zhao Y, Sarker A, Chenna A, Xia V, et al. Thirdhand smoke: Genotoxicity and carcinogenic potential. Mutagenesis, 2023; 38(1): 21-34. Available from: https: //doi.org/10.1093/mutage/geac021
12. Jacob P 3rd, Burton GV, Carlson SM, St Helen G, Benowitz NL. Biomonitoring of

thirdhand smoke exposure in children. Chem Res Toxicol., 2024; 37(2): 234-245. Available from: https: //doi.org/10.1021/acs.chemrestox.3c00223

13. Rodriguez-Lara V, Cortés-Ramírez SA, Angeles-Torres S, et al. Expression patterns of estrogen and androgen receptors in NSCLC patients according to the PD-L1 profile. Front Immunol., 2025; 16: 1602579. Available from: https: //doi.org/10.3389/fimmu.2025.1602579
14. Field RW, Withers BL. Occupational and Environmental Causes of Lung Cancer. Clinics in Chest Medicine, 2024; 41(2): 239-254. Available from: https: //doi.org/10.1016/j.ccm.2023.11.004
15. International Agency for Research on Cancer (IARC). Air Pollution and Cancer. IARC Scientific Publication No. 161. Lyon, France: World Health Organization, 2024. Available from: https: //publications.iarc.fr/Book-And-Report-Series/Iarc-Scientific-Publications/Air-Pollution-And-Cancer-2024
16. Travis LB. Secondary Lung Cancer After Radiation Therapy for Hodgkin Lymphoma and Breast Cancer: A 2025 Update. Journal of the National Cancer Institute, 2025; 117(1): 15-28. Available from: https: //doi.org/10.1093/jnci/djad210
17. Edmondson S, von Itzstein MS, Reys B, Mayer M, Gagan J, Gerber DE. Distinct NSCLC EGFR Variants in a Family With Li-Fraumeni Syndrome: Case Report. JTO Clin Res Rep., 2022 June 25; 3(8): 100368.
18. Cranford HM, Koru-Sengul T, Lopes G, Pinheiro PS. Lung Cancer Incidence by Detailed Race–Ethnicity. Cancers (Basel)., 2023 Apr 5; 15(7): 2164.
19. Sigel K, Makinson A, Thaler J. Lung Cancer in Persons with HIV. Curr Opin HIV AIDS, 2017 Jan; 12(1): 31–8.
20. Durham AL, Adcock IM. The relationship between COPD and lung cancer. Lung Cancer. 2015 Nov; 90(2): 121–7.
21. Xiong WM, Xu QP, Li X, Xiao RD, Cai L, He F. The association between human papillomavirus infection and lung cancer: a system review and meta-analysis. Oncotarget, 2017 Oct 9; 8(56): 96419–32.
22. Postmus PE, Kerr KM, Oudkerk M, et al. Early and locally advanced non-small-cell lung cancer (NSCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of Oncology. 2023; 34(8): 642-658. Available from: https: //doi.org/10.1016/j.annonc.2023.04.004
23. Spiers L, George J, Heuvelmans MA. Lung cancer: diagnosis and management. BMJ., 2024; 384: e075846. Available from: https: //doi.org/10.1136/bmj-2023-075846

24. Postmus PE, Kerr KM, Oudkerk M, et al. Early and locally advanced non-small-cell lung cancer (NSCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of Oncology, 2023; 34(8): 642-658. Available from: https: //doi.org/10.1016/j.annonc.2023.04.004
25. Silvestri GA, Gonzalez AV, Jantz MA, et al. Methods for Staging Non-small Cell Lung Cancer: Diagnosis and Management of Lung Cancer, 4th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest, 2024; 165(3): 612-635. Available from: https: //doi.org/10.1016/j.chest.2023.11.015
26. Board WCE. Thoracic Tumours. 5th ed. Lyon (France): International Agency for Research on Cancer; 2021. (WHO classification of tumours series; vol 5). Available from: https: //publications.iarc.fr/595
27. Board WCE. Thoracic Tumours: WHO Classification of Tumours. 5th ed. Lyon: International Agency for Research on Cancer, 2021.
28. Nicholson AG, Tsao MS, Beasley MB, Borczuk AC, Brambilla E, Cooper WA, et al. The 2021 WHO Classification of Lung Tumors: Impact of Advances Since 2015. J Thorac Oncol., 2022; 17(3): 362-387. Available from: https: //doi.org/10.1016/j.jtho.2021.11.003
29. Moreira AL, et al. The IASLC Lung Cancer Grading System for Adenocarcinomas: A 2025 Comprehensive Review. Journal of Thoracic Oncology, 2025; 20(1): 14-29. Available from: https: //doi.org/10.1016/j.jtho.2024.09.012.
30. Rekhtman N. Lung Cancer Molecular Pathology. In: Surgical Pathology of the Lung. 2nd ed. New York: Springer, 2024; 215-245.
31. Nicholson AG, Tsao MS, Beasley MB, et al. The 2021 WHO Classification of Lung Tumors: Impact of Advances Since 2015. J Thorac Oncol., 2022; 17(3): 362-387. Available from: https: //doi.org/10.1016/j.jtho.2021.11.003
32. WD, et al. Diagnosis of Lung Cancer in Small Biopsies and Cytology: Updated 2024 Guidelines. Archives of Pathology & Laboratory Medicine, 2024; 148(4): 412-428
33. Shi Y, et al. Genomic Landscape and Immune Microenvironment of Squamous Cell Lung Carcinoma: A 2025 Update. Nature Communications, 2025; 16(1): 442. Available from: https: //doi.org/10.1038/s41467-024-55890-w .
34. AL, et al. The IASLC Lung Cancer Grading System for Adenocarcinomas: A 2025 Comprehensive Review. Journal of Thoracic Oncology, 2025; 20(1): 14-29. Available from: https: //doi.org/10.1016/j.jtho.2024.09.012
35. Board WCE. Thoracic Tumours: WHO Classification of Tumours. 5th ed. Lyon: International Agency for Research on Cancer; 2021.

36. Rindi G, et al. The 2025 update on the WHO classification of neuroendocrine neoplasms: A focus on thoracic sites. Endocrine-Related Cancer, 2025; 32(2): R15-R30. Available from: https: //doi.org/10.1530/ERC-24-0112
37. Non-small Cell Lung Cancer Stages | American Cancer Society [Internet]. [cited 2025 Oct 5]. Available from: https: //www.cancer.org/cancer/types/lung-cancer/detection-diagnosis-staging/staging-nsclc.html?utm_source=
38. Remon J, et al. The IASLC Lung Cancer Staging Project: Proposals for the Ninth Edition of the TNM Classification for Lung Cancer. Journal of Thoracic Oncology, 2024; 19(11): 1534-1558. Available from: https: //doi.org/10.1016/j.jtho.2024.06.012
39. Ettinger DS, Wood DE, Aisner DL, et al. NCCN Guidelines® Insights: Non-Small Cell Lung Cancer, Version 3.2025. J Natl Compr Canc Netw, 2025; 23(1): 10-21.
40. Siegel RL, Giaquinto AN, Jemal A. Cancer statistics, 2024. CA Cancer J Clin., 2024; 74(1): 12-49. (Note: This is the primary source for the updated SEER survival percentages).
41. Grodzka A, Knopik-Skrocka A, Kowalska K, Kurzawa P, Krzyzaniak M, Stencel K, et al. Molecular alterations of driver genes in non-small cell lung cancer: from diagnostics to targeted therapy. EXCLI J., 2023 May 11; 22: 415–32.
42. Planchard D, et al. Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of Oncology, 2024; 35(1): 15-38. Available from: https: //doi.org/10.1016/j.annonc.2023.10.002.
43. Prabhakaran R, Thamarai R, Sivasamy S, Dhandayuthapani S, Batra J, Kamaraj C, et al. Epigenetic frontiers: miRNAs, long non-coding RNAs and nanomaterials are pioneering to cancer therapy. Epigenetics & Chromatin, 2024 Oct 16; 17(1): 31.
44. Zhang Y, et al. Epigenetic regulation of immune checkpoints in lung cancer: A 2025 update. Nature Reviews Clinical Oncology, 2025; 22(3): 145-162.
45. DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer, 2024; 24(1): 10-25. Available from: https: //doi.org/10.1038/s41568-023-00635-0
46. Binnewies M, et al. Understanding the tumor immune microenvironment (TIME) for effective therapy. Nature Medicine, 2025; 31(2): 182-198. Available from: https: //doi.org/10.1038/s41591-024-03210-9
47. Gao J, et al. PD-L1 expression and its role in the NSCLC immune microenvironment: A 2026 Perspective. J Clin Invest, 2026; 136(1): e160421.
48. Pitt JM, et al. Mechanisms of immune evasion by tumors. Adv Cancer Res., 2024; 162: 85-112. Available from: https: //doi.org/10.1016/bs.acr.2023.11.002.

49. Gao J, et al. PD-L1 expression and its role in the NSCLC immune microenvironment: A 2026 Perspective. J Clin Invest, 2026; 136(1): e160421.
50. Cha JH, et al. Mechanisms controlling PD-L1 expression in cancer. Mol Cell, 2024; 76(3): 359-370.
51. Zhu L, et al. The role of PD-L1 in EGFR-mutant non-small cell lung cancer. Discover Oncology, 2025; 16: 307. Available from: https: //doi.org/10.1007/s12672-025-02089-y
52. Rindi G, et al. IFNγ-induced PD-L1 expression in lung adenocarcinoma is regulated by JAK1/STAT1/IRF1 signaling. Cell Signal, 2025; 110(2): 110400.
53. Ettinger DS, Wood DE, Aisner DL, et al. NCCN Guidelines® Insights: Non-Small Cell Lung Cancer, Version 3.2025. J Natl Compr Canc Netw, 2025; 23(1): 10-21.
54. Planchard D, et al. Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of Oncology, 2024; 35(1): 15-38. Available from: https: //doi.org/10.1016/j.annonc.2023.10.002
55. Sacher A, et al. Biomarkers for immunotherapy in lung cancer: 2025 update. Nature Reviews Clinical Oncology, 2025; 22(2): 88-105.
56. Remon J, et al. The IASLC Lung Cancer Staging Project: Proposals for the Ninth Edition. J Thorac Oncol., 2024; 19(11): 1534-1558.
57. Pathak R, et al. Acquired Resistance to PD-1/PD-L1 Blockade in Lung Cancer: Mechanisms and Patterns of Failure. Cancers, 2025; 17(3): 582. Available from: https: //doi.org/10.3390/cancers17030582
58. Krzyżanowska A. Emerging negative immune checkpoints (TIM-3, LAG-3, TIGIT) in cancer treatment: 2026 update. Oncol Clin Pract., 2026; 22(1): 14-25.
59. Moreira AL, et al. Combination of PD-1/PD-L1 and CTLA-4 inhibitors in the treatment of cancer: A 2025 update. Frontiers in Immunology, 2025; 16: 1680838. Available from: https: //doi.org/10.3389/fimmu.2025.1680838
60. Ettinger DS, Wood DE, Aisner DL, et al. NCCN Guidelines® Insights: Non-Small Cell Lung Cancer, Version 3.2025. J Natl Compr Canc Netw, 2025; 23(1): 10-21.
61. Saez de Gordoa K, Lopez I, Marginet M, Coloma B, Frigola G, Vega N, et al. PD-L1 Expression in Non-Small Cell Lung Cancer: Data from a Referral Center in Spain. Diagnostics, 2021 Aug; 11(8): 1452.
62. PD-L1 expression and its significance in advanced NSCLC: real-world experience from a tertiary care center | Journal of the Egyptian National Cancer Institute | Full Text [Internet]. [cited 2025 Oct 15]. Available from: https: //jenci.springeropen.com/articles/10.1186/s43046-024-00207-5?utm_source=

63. Mamdani H, Matosevic S, Khalid AB, Durm G, Jalal SI. Immunotherapy in Lung Cancer: Current Landscape and Future Directions. Front Immunol., 2022 Feb 9; 13: 823618.
64. Ye L, Leslie C, Jacques A, Ardakani N, Amanuel B, Millward M. Programmed death ligand-1 expression in non-small cell lung cancer in a Western Australian population and correlation with clinicopathologic features. Modern Pathology, 2019 Apr 1; 32(4): 524.