



Prognostic Significance of Programmed Cell Death Ligand 1 (PD-L1), CD8+ Tumor-Infiltrating Lymphocytes and p53 in Non-Small Cell Lung Cancer: An Immunohistochemical Study

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ABSTRACT

Objective: Programmed cell death ligand-1 interacts with the immune receptors on the surface of CD8+ tumor infiltrating lymphocytes and PD-1, thereby blocking its anti-tumor activity. Therapeutics suppression of this interaction will show a promise in the treatment of non-small cell lung cancer by restoring the functional anti-tumor T-cell activity. We aimed to evaluate the association between the immunohistochemical expression of PD-L1, stromal CD8+ tumor infiltrating lymphocytes and p53 with the clinicopathological characteristics, response to chemotherapy, progression-free-survival, and overall survival.

Material and Method: We examined the immunohistochemical expression of PD-L1, stromal CD8+ TILs, and p53 expression in 50 patients with advanced stage (III&IV) non-small cell lung cancer.

Results: PD-L1 was expressed in 56% of the studied cases. PD-L1 expression was related to unfavorable response to the therapy without significant difference. PD-L1 expression was significantly associated with disease progression, poor progression-free-survival & overall survival. CD8+ TILs were high in 32% of the cases. Tumors with high CD8+ TILs showed a partial response to therapy and had a better progression-free-survival and overall survival. p53 expressed in 82% of the studied cases. There was a significant negative association between PD-L1 and CD8+ TILs ($p=0.009$), while a non-significant association was found between p53 and PD-L1 ($p=0.183$).

Conclusion: PD-L1 overexpression is an unfavorable prognostic marker, while the high CD8 + TILs is a good prognostic marker in non-small cell lung cancer. PD-L1 immunohistochemical assessment may be used for the selection of patients legible for treatment with anti-PD-L1 therapy.

Key Words: PD-L1, p53, Tumor infiltrating lymphocytes, Non-small cell lung cancer, Immunohistochemistry.

INTRODUCTION

Lung cancer ranks the first in the incidence and mortality rates amongst all malignancies worldwide. Non-small cell lung cancer (NSCLC) constitutes 80% - 85% of all diagnosed cases and more than 70% of NSCLC are diagnosed as an advanced or late disease (1).

Recognition of tumor antigens by T cells leads to activation of anti-tumor immune reaction mainly by cytotoxic CD8+ tumor infiltrating lymphocytes (TILs), but malignant cells may have many pathways to evade the immunological destruction. PD-1 is a co-stimulatory molecule on T-cells that inhibits T cell activation. PD-1, which is a 288-amino acid cell-surface protein, can bind two ligands, PD-L1 and PD-L2, which negatively control the immune response. Tumor cells express PD-L1 can inhibit T cell-mediated immune response and can progress to distant metastasis (2,3).

CD8 marker is expressed on cytotoxic T cells, natural killer, and dendritic cells. CD8+ T cells are a major component of the cell-mediated immune system against malignant cells. CD8+ T cells undergo differentiation to effector cytotoxic T cells. Then the effector CD8+ cells undergo apoptosis leaving memory cells when a pathogenic response is resolved. Memory T cells and cytotoxic T cells are associated with a more favorable prognosis in NSCLC (4).

PD-L1 over-expression has been proved to be a poor prognostic marker in many human cancers (5). PD-1/PD-L1 interaction suppresses CD8+ TILs survival and proliferation and leads to apoptosis of tumor-infiltrating lymphocytes. PD-L1 over-expression in tumor cells in a syngeneic transplant model of tumor cells makes them evade from cytotoxic TILs (6).

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Blockage of PD-1/PD-L1 interaction with monoclonal antibodies will restore T cell activity in the microenvironment of the tumor and has been stated to result in an effective anti-tumor therapeutic effect in clinical trials and may have promising activity in many malignancies (7).

Inactivation of p53, which is a tumor suppressor gene present on the short arm of chromosome 17 (17p13), is a constant feature of most of the human malignancies that often characterized by aggressive behavior, poor overall survival, and treatment failure (8).

The standard treatment of advanced NSCLC according to the tumor stage was limited to radiotherapy, chemotherapy or both. Recently, targeted molecular therapies have replaced the traditional therapeutic methods for patients whose cancers express certain genetic alterations. Attempts to provide immunotherapy in lung cancers were accelerated and now the development of therapeutics targeting PD-1 and PD-L1 has developed. An immunotherapy will be stated as the third line in the treatment of advanced lung cancer (7, 9).

In this study, we aimed to evaluate the association between the immunohistochemical expression of programmed cell death ligand 1 (PD-L1), stromal CD8+ TILs and p53 with clinicopathological characteristics, response to chemotherapy, progression free-survival (PFS) and overall survival (OS) in patients with NSCLC.

MATERIAL and METHODS

Patients: This is an analytic cross-sectional study, which was conducted in Clinical oncology, Chest and Pathology Departments, Zagazig University Hospitals, in the period from January 2014 to August 2016. The study was carried out on 50 patients with advanced stage (III&IV) NSCLC (10).

Patients were subjected to the followings; 1-Thorough medical history 2- Full clinical examination 3- Laboratory investigations, including the following: (a) Complete blood count, (b) Kidney function tests (serum urea level and creatinine), (c) Liver function tests, and (d) PT, PTT & INR. 4- Plain chest X-ray: the postero-anterior view was done to all patients by X-ray machine (ROTALiX SRT 32, Philips, Italy), the chest radiographs showed pulmonary nodule, mass, infiltrate, mediastinal widening, atelectasis, hilar enlargement or presented by pleural effusion. 5- Computed tomography (C.T): conventional chest C.T was done in all cases, where CT-guided transthoracic core tissue biopsy was done for peripherally located lesions. Three-four biopsies are likely adequate in this situation.

6- Bronchoscopy: patients who had radiologically central lesion were subjected to FOB (Pentax FB15TV, Philips, Tokyo, Japan). Flexible bronchoscopy was performed with a fiberoptic scope through transnasal route under topical anesthesia (2% lignocaine). Oxygenation was monitored throughout the procedure with pulse oximetry (11).

Patients received platinum-based doublet chemotherapy (carboplatin and gemcitabine). The clinical response to chemotherapy was evaluated using computed tomography (CT). Clinical follow-up data were taken in all cases. Patient follow-up information was obtained from hospital records or patient contact. The study complied with the guidelines of institutional review board of Zagazig university hospitals.

Immunohistochemical Staining: All tissues were fixed at 10% neutral formaldehyde solution. Then, dehydration and paraffin embedding were done, followed by deparaffinization in xylene and rehydration in descending series of alcohols. For antigen retrieval, 10 mM citrate buffer (pH 6.0) at the microwave for 20 minutes was used. Endogenous peroxidase activity was blocked by 3% hydrogen peroxide for 10 min. After repeat washing in PBS, the slides were incubated with primary mouse monoclonal antibodies directed against anti-PD-L1 (sc293425, Santa Cruz Biotechnology, Santa Cruz, CA) and mouse anti-human p53 (DO-7, Dako, 1:100) and Anti-CD8 (clone C8/144B, Dako, ready to use). Binding site of primary antibodies was visualized by using the Dako EnVision™ kit (Dako, Copenhagen, Denmark). Then, the sections were counterstained with Mayer's hematoxylin. Negative controls were obtained by omission of the primary antibody. The tonsils, placenta and colon cancer tissues were used as positive control for CD8+ TILs, PD-L1 and p53 respectively.

Immunohistochemistry: PD-L1 was assessed as negative, weak positive which was defined as a membranous or cytoplasmic PD-L1 expression in 1% to 49% of tumor cells, and PD-L1 strong positive was defined as expression in ≥50% of tumor cells at higher power magnification. In this work, negative and weak positive stain was considered as a negative group (11). p53 nuclear stain in more than 5% of malignant cells was considered a positive immunoreactivity and its expression was evaluated as follow: p53-negative (≤ 5%), low p53 (5% to 50%), and high p53 (> 50%) (12). The percentages of CD8+ TILs compared with the total amount of nucleated cells in the stroma were evaluated; low density: ≤25%; intermediate density: ≥25% to ≤50%; high density: >50% (13).

Statistical Analysis: Continuous variables were expressed as the mean ± SD & median (range), and the categorical

variables were expressed as a number (percentage). Continuous variables were checked for normality by using the Shapiro-Wilk test. Independent samples Student's t-test was used to compare between two groups of normally distributed variables. One Way ANOVA test was used to compare between more than two groups of normally distributed variables, while Kruskal-Wallis H test was used for non-normally distributed variables. Percent of categorical variables were compared using Pearson's Chi-square test or Fisher's exact test when was appropriate. The trend of change in the distribution of relative frequencies of ordinal data was compared using the chi - square test for trend. PFS was calculated as the time from the assessment of response to treatment (baseline) to progression or the most recent follow-up in which no progression. Overall Survival (OS) was calculated as the time from diagnosis to death or the most recent follow-up contact (censored). Stratification of PFS and OS was done according to the

immunohistochemical markers. These time-to-event distributions were estimated using the method of Kaplan-Meier plot and compared using two-sided exact log-rank test. A p-value <0.05 was considered significant. All statistics were performed using SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA) and MedCalc windows (MedCalc Software bvba 13, Ostend, Belgium).

RESULTS

Patients and the Clinicopathological Parameters: Our study included 50 patients; 82% of them were males, with an age ranged from 25 to 77 years (mean: 55.56 ± 14.18). Thirty cases (60%) were diagnosed as adenocarcinoma and 20 (40%) cases as squamous cell carcinomas. Nineteen cases were moderately differentiated (grade II) and 31 were poorly differentiated (grade III). Tumors were classified as stage III (n =29), and stage IV (n =21). Half of the patients gave a partial response and 24 patients survive more than 9 months (Table I).

Table I: Clinicopathological parameters, immunohistochemical markers and outcome of 50 patients with NSCLC

Characteristics	Number	%	Characteristics	Number	%
Age (year)			CD8+ TILs		
Mean \pm SD	55.56 ± 14.18		Low	28	56%
Median (Range)	55 (25 - 77)		Intermediate	6	12%
< 65years	35	70%	High	16	32%
\geq 65years	15	30%	Response		
Sex			PD	10	20%
Male	41	82%	SD	15	30%
Female	9	18%	PR	25	50%
Pathology			NR	25	50%
SCC	20	40%	OAR	25	50%
Adenocarcinoma	30	60%	Follow-up (months)		
Grade			Mean \pm SD	10.60 ± 4.58	
Grade II	19	38%	Median (Range)	10 (5 - 19)	
Grade III	31	62%	Progression		
Stage			Within 6 months	22	55%
Stage III	29	58%	After 6 months	18	45%
Stage IV	21	42%	Survival		
PD-L1			< 9 months	26	52%
Negative	22	44%	> 9 months	24	48%
Positive	28	56%			
p53					
Negative	8	16%			
Low	17	34%			
High	25	50%			

Continuous variables were expressed as mean \pm SD & median (range); categorical variables were expressed as number (percentage). **PD** means progressive disease, **SD**: stable disease, **PR**: partial response, **NR**: no response, **OAR**: overall response.

Association Between PD-L1 Expression and the Clinicopathological Parameters: PD-L1 immunoreactivity was detected as membranous and/or cytoplasmic stain in the malignant cells in 56% of NSCLC cases, but the adjacent paratumor tissues didn't express. (Figure 1A, B). Regarding histopathology, PD-L1 was overexpressed in adenocarcinoma variant (60% of cases) more than squamous cell carcinoma (50% of cases). There was a significant difference between PD-L1 expression as regards the stage ($p < 0.001$) but not with the age, sex, pathological type and tumor grade. There was a negative correlation between PD-L1 immunoreactivity and CD8+ TILs ($p = 0.009$) (Table II).

Association Between p53 Expression and the Clinicopathological Parameters: p53 immunoreactivity was detected as a nuclear stain (Figure 2A,B). The

percentage of p53 positive cases in NSCLC was 82%. p53 was overexpressed in adenocarcinoma (93.3% of cases) more than squamous cell carcinoma (70% of cases) ($p = 0.05$). There was a non-significant difference between p53 expression in NSCLC as regards age, sex, pathological type, tumor grade and stage. No significant association was found between p53 and PD-L1 expression in NSCLC ($p = 0.183$) (Table II).

Association Between CD8+ TILs Expression and the Clinicopathological Parameters: High tumor-infiltrating CD8+ TILs was observed in the stroma of 32% of the cases, while intermediate CD8+TILs density was found in 12% of the cases (Figure 3A, B). There was a significant difference between CD8+ TILs expression as regards tumor stage ($p < 0.001$) but no difference with the age, sex, pathological type and tumor grade was observed (Table III).

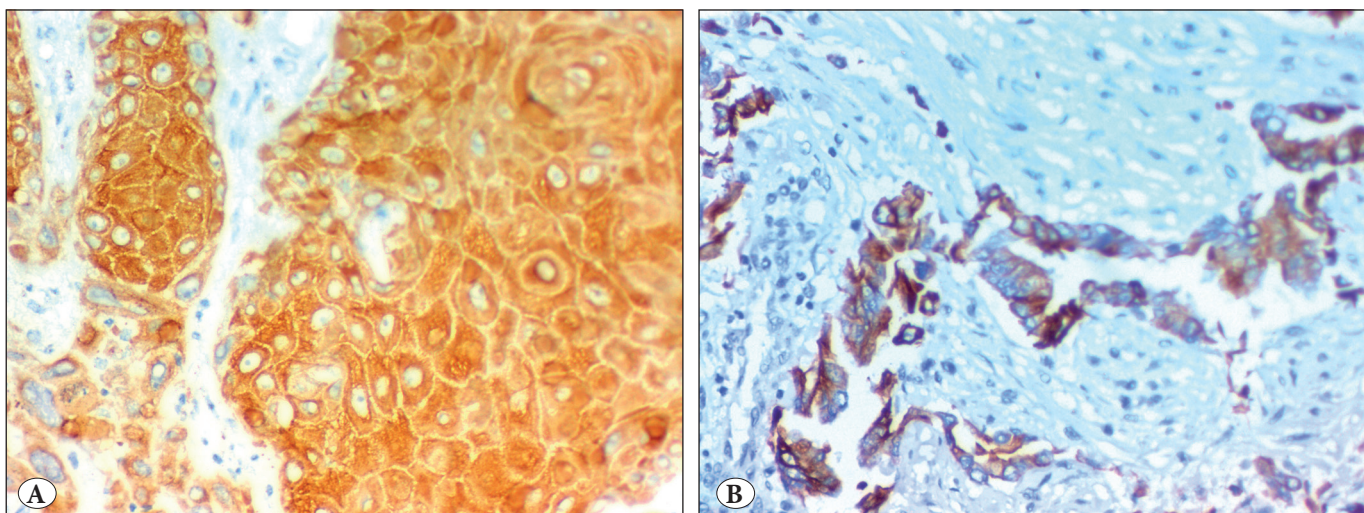


Figure 1: Representative immunohistochemical staining results for PD-L1. A) PD-L1 positive cytoplasmic stain in lung squamous cell carcinoma (PD-L1; x400). B) PD-L1 positive membranous and cytoplasmic stain in lung adenocarcinoma (PD-L1; x400).

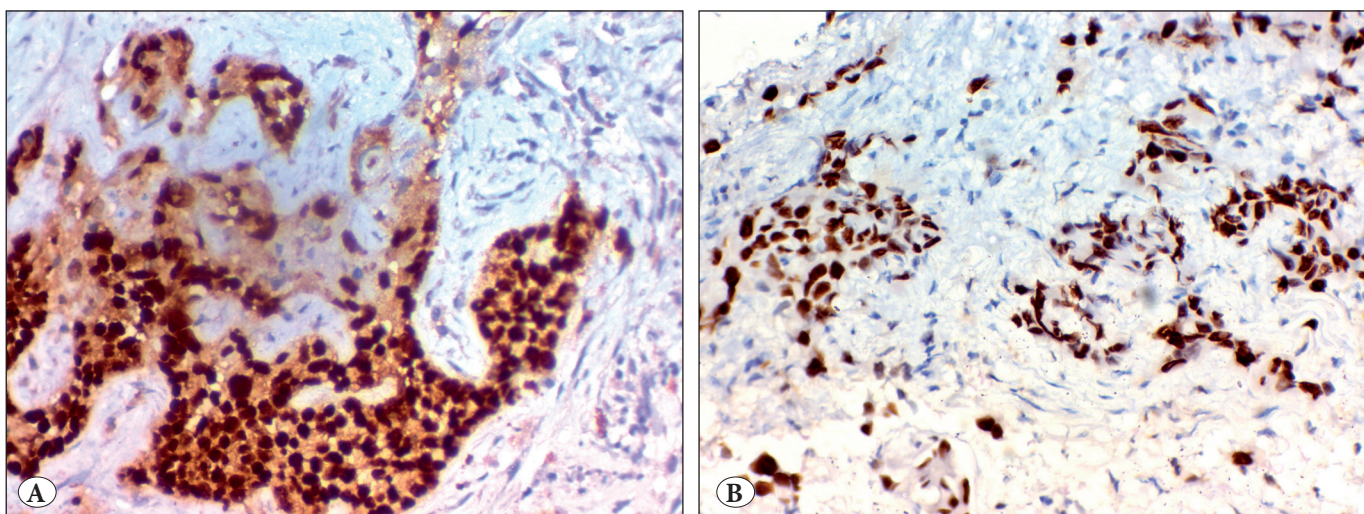


Figure 2: Representative immunohistochemical staining results for p53: A) High nuclear p53 expression in lung squamous cell carcinoma (p53; x400). B) High nuclear p53 expression in lung adenocarcinoma (p53; x400).

Association Between PD-L1, p53 and CD8+TILs Expression and Patient Outcome: PD-L1 expressing NSCLC was significantly associated with disease progression ($p=0.001$). In addition, Progressive disease expressed high p53 more than stable disease ($p=0.014$). PD-L1-positive NSCLC were related to unfavorable response to therapy, but without significant difference ($p=0.269$) (Table IV). Patients with high CD8+ TILs gave a partial response to therapy ($p = 0.022$) (Table V).

Kaplan-Meier survival curve analysis revealed a significant association between PD-L1 with shorter PFS and poor OS ($p=0.001$, $p=0.011$ respectively), while CD8+ TILs had a better PFS and OS ($p=0.001$ for each). However, no significant relationship was found between p53 and PFS or OS ($p=0.470$, $p=0.186$) (Figure 4A-C, 5A-C).

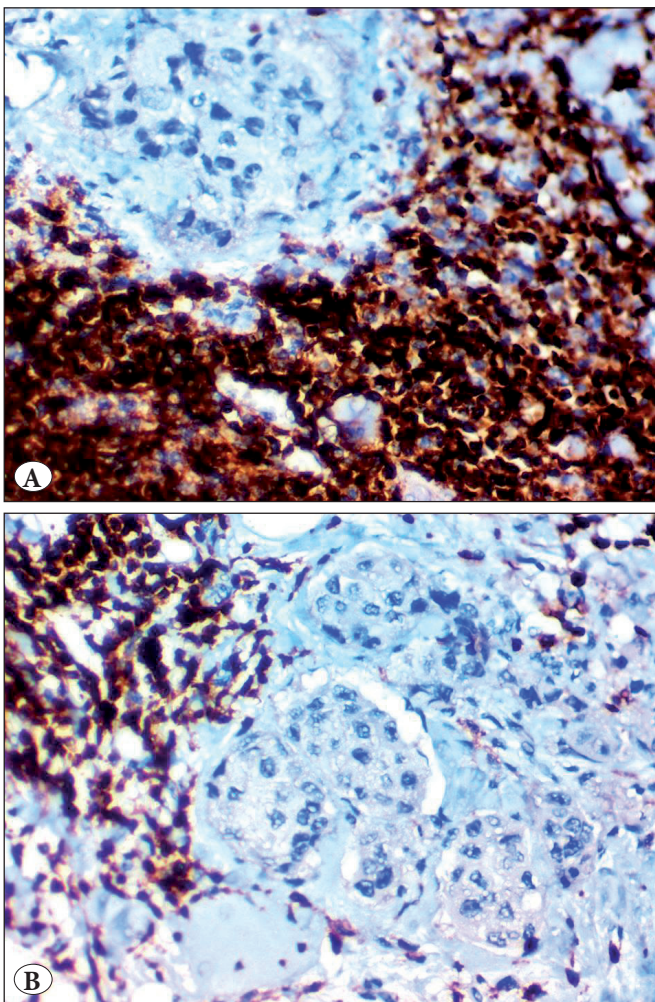


Figure 3: Representative immunohistochemical staining results for CD8+TILs. **A)** High density of CD8+ TILs in lung squamous cell carcinoma (CD8; x400). **B)** Intermediate density of CD8+ TILs in lung squamous cell carcinoma (CD8; x400). **TILs:** Tumor infiltrating lymphocytes

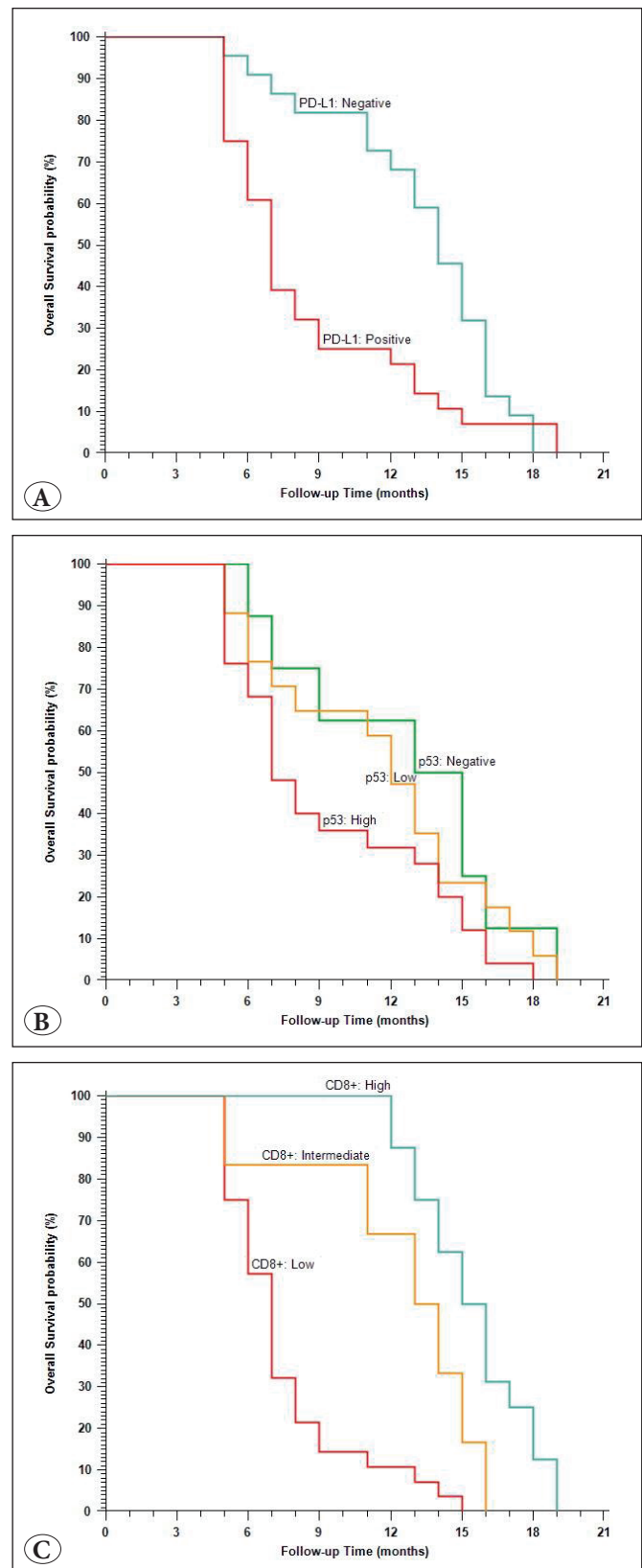


Figure 4: Kaplan-Meier Plot of overall survival: **A)** Stratified by PD-L1 IHC staining. **B)** Stratified by p53 IHC staining. **C)** Stratified by CD8+ TILs IHC staining. (IHC: Immunohistochemical)

Table II: Association between clinicopathological parameters and immunohistochemical staining of PD-L1 and p53 in NSCLC

Characteristics	All (N=50)			PD-L1			p-value	p53			p-value		
	No.	(%)		No.	(%)	Positive (N=28)		No.	(%)	Negative (N=8)		Low (N=17)	High (N=25)
Age (years)													
Mean ± SD	55.56	±14.18	57.27	±12.89	54.21	±15.21	0.455*	51	±13.61	58.11	±14.46	55.28 ±14.33	0.447 [†]
Median (Range)	55	(25-77)	57	(27-75)	53.50	(25-77)		51	(27-69)	62	(35-75)	55 (25-77)	
< 65years	35	(70%)	16	(45.7%)	19	(54.3%)	0.709‡	7	(20%)	10	(28.6%)	18 (51.4%)	0.329‡
≥ 65years	15	(30%)	6	(40%)	9	(60)		1	(6.7%)	7	(46.7%)	7 (46.7%)	
Sex													
Male	41	(82%)	20	(48.8%)	21	(51.2%)	0.266‡	8	(19.5%)	14	(34.1%)	19 (46.3%)	0.306‡
Female	9	(18%)	2	(22.2%)	7	(77.8%)		0	(0%)	3	(33.3%)	6 (66.7%)	
Pathology													
SCC	20	(40%)	10	(50%)	10	(50%)	0.485‡	6	(30%)	4	(20%)	10 (50%)	0.050
Adenocarcinoma	30	(60%)	12	(40%)	18	(60%)		2	(6.7%)	13	(43.3%)	15 (50%)	
Grade													
Grade II	19	(38%)	10	(52.6%)	9	(47.4%)	0.336‡	3	(15.8%)	7	(36.8%)	9 (47.4%)	0.944‡
Grade III	31	(62%)	12	(38.7%)	19	(61.3%)		5	(16.1%)	10	(32.3%)	16 (51.6%)	
Stage													
Stage III	29	(58%)	20	(69%)	9	(31%)	<0.001‡	5	(17.2%)	12	(41.4%)	12 (41.4%)	0.333‡
Stage IV	21	(42%)	2	(9.5%)	19	(90.5%)		3	(14.3%)	5	(23.8%)	13 (61.9%)	
PD-L1													
Negative	22	(44%)						5	(22.7%)	8	(36.4%)	9 (40.9%)	0.401‡
Positive	28	(56%)						3	(10.7%)	9	(32.1%)	16 (57.1%)	
p53													
Negative	8	(16%)	5	(62.5%)	3	(37.5%)							
Low	17	(34%)	8	(47.1%)	9	(52.9%)	0.183\$						
High	25	(50%)	9	(36%)	16	(64%)							
CD8+													
Low	28	(56%)	7	(25%)	21	(75%)		4	(14.3%)	7	(25%)	17 (60.7%)	
Intermediate	6	(12%)	4	(66.7%)	2	(33.3%)	0.004\$	1	(16.7%)	1	(16.7%)	4 (66.7%)	0.097\$
High	16	(32%)	11	(68.8%)	5	(31.3%)		3	(18.8%)	9	(56.3%)	4 (25%)	

Categorical variables were expressed as number (percentage), continuous variables were expressed as mean \pm SD & median (range); *Independent samples Student's t test; ‡ Chi-square test; § Chi-square test for trend; p<0.05 is significant.

DISCUSSION

Five-year overall survival rate of NSCLC is 15% of all stages (1) and less than 5% for metastatic NSCLC (14), so there is an urgent need to develop other therapeutic modalities to improve outcome of NSCLC especially late stages. Programmed cell death-ligand 1 (PD-L1) is a costimulatory molecule B7 family expressed on the surface of antigen-presenting cells and acts as a negative immune regulatory molecule. It inhibits T cell activity, increases T-cells apoptosis, decreases tumor immunity and lastly leads to tumor immune escape (15).

Immunotherapy has promising results to improve the outcome of several solid tumors. Therapies targeting the programmed cell death receptor PD-1 and its ligand PD-L1 have revealed promising effects. However, in lung cancer,

PD-L1 diagnostic, prognostic and predictive values remain to need more studies. Previous studies (16-19) showed that NSCLC over-expressed PD-L1 has ranged from 19% to 100%. However, in this study, the percentage of PDL1-positive cases in NSCLC was 56% and the expression increased with the more advanced stage. In addition, PDL1 reaction was more with adenocarcinoma histology variant, but without a statistically significant difference. On the other hand, one study stated that the PD-L1 expression was associated with adenocarcinoma (17) and the other reported it to be associated with squamous cell carcinoma variant (20). This difference and the wide range of immunohistochemical expression may be related to different antibodies used, genetic, environmental factors and sample size.

Table III: Association between clinicopathological parameters and immunohistochemical staining for CD8+ TILs in NSCLC.

Characteristics	All (N=50)		CD8+TILs						p-value
			Low (N=28)		Intermediate (N=6)		High (N=16)		
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	
Age (years)									
Mean ± SD	55.56	±14.18	55.67	±15.05	61.50	±8.43	53.12	±14.30	0.475*
Median (Range)	55	(25-77)	56	(25-77)	63	(51-74)	54	(27-75)	
< 65years	35	(70%)	18	(51.4%)	5	(14.3%)	12	(34.3%)	0.567‡
≥ 65years	15	(30%)	10	(66.7%)	1	(6.7%)	4	(26.7%)	
Sex									
Male	41	(82%)	21	(51.2%)	5	(12.2%)	15	(36.6%)	0.296‡
Female	9	(18%)	7	(77.8%)	1	(11.1%)	1	(11.1%)	
Pathology									
SCC	20	(40%)	8	(40%)	3	(15%)	9	(45%)	0.171‡
Adenocarcinoma	30	(60%)	20	(66.7%)	3	(10%)	7	(23.3%)	
Grade									
Grade II	19	(38%)	9	(47.4%)	3	(15.8%)	7	(36.8%)	0.607‡
Grade III	31	(62%)	19	(61.3%)	3	(9.7%)	9	(29%)	
Stage									
Stage III	29	(58%)	8	(27.6%)	5	(17.2%)	16	(55.2%)	<0.001‡
Stage IV	21	(42%)	20	(95.2%)	1	(4.8%)	0	(0%)	
PD-L1									
Negative	22	(44%)	7	(31.8%)	4	(18.2%)	11	(50%)	0.009‡
Positive	28	(56%)	21	(75%)	2	(7.1%)	5	(17.9%)	
p53									
Negative	8	(16%)	4	(50%)	1	(12.5%)	3	(37.5%)	0.097§
Low	17	(34%)	7	(41.2%)	1	(5.9%)	9	(52.9%)	
High	25	(50%)	17	(68%)	4	(16%)	4	(16%)	

Categorical variables were expressed as number (percentage), continuous variables were expressed as mean \pm SD & median (range); *One way ANOVA test; ‡ Chi-square test; § Chi-square test for trend; $p < 0.05$ is significant.

In this work, PD-L1 positive NSCLC were significantly related to a poor outcome and more progressive disease. In contrast, no correlation was found between PD-L1 tumor expression and other clinicopathological parameters except tumor stage. So, we stated that PD-L1 plays a role in disease progression and detecting PD-L1 immunoreactivity on NSCLC tissues may evaluate patient's outcome. Previous studies revealed controversy in the prognostic value of PD-L1 for NSCLC. There are some studies suggesting a negative prognostic role (17, 21-23), while others did not find any prognostic value (24, 25). However, other studies reported improved patient outcomes (20, 26). This discrepancy may be due to different PD-L1 immunohistochemistry assays, using variable detection antibodies and different cutoff. Clinical trials investigating the efficacy of concomitant treatment with anti-PD-L1 and chemotherapy may give better significant results. Also, a significant association between an improved overall survival and PDL1 immunoreactivity was observed in breast cancer, colorectal carcinoma and metastatic melanoma (27-29).

Malignant cells expressing PD-L1 will interact with the negative signal generating immune receptor on the surface of CD8+T cells and PD-1, thereby blocking anti-tumor activity. Therapeutics suppression of this interaction will show promise in the treatment of many cancers by restoring functional antitumor T-cell activity (30). Our result also showed a negative correlation between PD-L1 and CD8+ TILs. Thus, PD-L1 may function as a negative regulator of T-cell-mediated antitumor immunity. Consistent with Chen et al. (31) study, in which PD-L1 blockade has been shown to stimulate anti-tumoral T-cell responses against PD-L1-expressing tumor cells.

CD8+T cells are considered to be an indication of the host immune response to malignant cells. Increased CD8+ TILs have been associated with a better prognosis in many solid neoplasms, including colorectal, melanoma and triple-negative breast carcinoma (4). This study showed that CD8+ TILs have a significant prognostic effect, as the patients with high CD8+ TILs gave a partial response to therapy and had a better survival more than those with low CD8+ TILs; this goes with Donnem et al. (13) who found that the stromal CD8+ TILs density was a strong independent prognostic factor for PFS and OS.

There is increasing evidence that PD-L1 plays a major role in peripheral tolerance (32). This study addressed a negative correlation between PD-L1 and CD+8 TILs. This consistent with Iwai et al. (6) study which stated that the PD-1/PD-L1 interaction suppresses CD8+ TILs as PD-L1 over-expression in tumor cells in a syngeneic transplant model of tumor cells makes them evade from cytotoxic TILs.

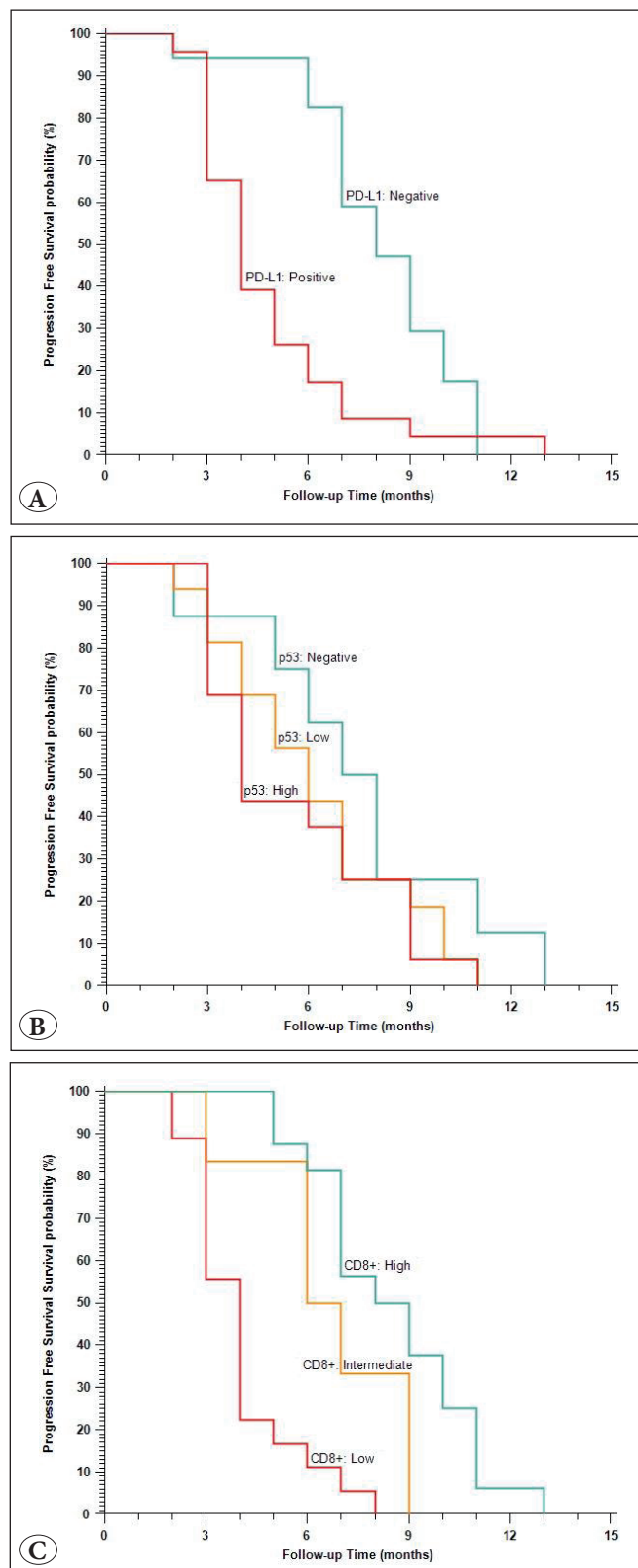


Figure 5: Kaplan-Meier Plot of progression free survival. A) Stratified by PD-L1 IHC staining; B) Stratified by p53 IHC staining; C) Stratified by CD8+ TILs IHC staining. (IHC: Immunohistochemical).

Table IV: Association between immunohistochemical staining of PD-L1 & p53 and patients outcome in NSCLC

Outcome	All (N=50)			PD-L1			p-value	p53			p-value		
	No.	(%)	No.	Negative (N=24)	Positive (N=26)	No.		(%)	No.	Low (N=17)		High (N=25)	
Response													
PD	10	(20%)	5	(22.7%)	5	(17.9%)		0	(0%)	1	(5.9%)	9	(36%)
SD	15	(30%)	4	(18.2%)	11	(39.3%)	0.269‡	1	(12.5%)	9	(52.9%)	5	(20%)
PR	25	(50%)	13	(59.1%)	12	(42.9%)		7	(87.5%)	7	(41.2%)	11	(44%)
NR	25	(50%)	9	(40.9%)	16	(57.1%)	0.254‡	1	(12.5%)	10	(58.8%)	14	(56%)
OAR	25	(50%)	13	(59.1%)	12	(42.9%)		7	(87.5%)	7	(41.2%)	11	(44%)
Progression													
Within 6 months	22	(55%)	3	(17.6%)	19	(82.6%)	<0.001‡	3	(37.5%)	9	(56.3%)	10	(62.5%)
After 6 months	18	(45%)	14	(82.4%)	4	(17.4%)		5	(62.5%)	7	(43.8%)	6	(37.5%)
Progression Free Survival													
Median PFS	6 months	8 months	4 months	0.001†	7.5 months	6 months	4 months	7.5 months	6 months	4 months	0.470†		
HR (95%CI)	----	0.382 (0.195 - 0.746)			----	1.550 (0.631 - 3.809)	1.796 (0.728 - 4.427)				0.361*		
6 months PFS	45%	82.4%	17.4%		62.5%	43.7%	37.5%						
12 months PFS	2.5%	0%	4%		12%	0%	0%						
Survival													
< 9 months	26	(52%)	5	(22.7%)	21	(75%)	<0.001‡	3	(37.5%)	6	(35.3%)	17	(68%)
> 9 months	24	(48%)	17	(77.3%)	7	(25%)		5	(62.5%)	11	(64.7%)	8	(32%)
Overall Survival													
Median OS	9 months	14 months	7 months	0.011†	13 months	12 months	7 months	13 months	12 months	7 months	0.186†		
HR (95%CI)	----	0.503 (0.279 - 0.906)			----	1.242 (0.534-2.893)	1.842 (0.813- 4.170)				0.164 *		
6 months OS	74%	90.9%	60.7%		87.5%	76.5%	68%						
12 months OS	42%	68.2%	21.4%		62.5%	47.1%	32%						
18 months OS	4%	0%	7%		12.5%	5.9%	0%						

Categorical variables were expressed as number (percentage); ‡ Chi-square test; § Chi-square test for trend; † Log rank test; p<0.05 is significant. * Breslow test; negative group was taken as reference for p53.

Table V: Association between immunohistochemical staining of CD8+ TILs and patients outcome in NSCLC

Outcome	All (N=50)		CD8+TILs						p-value
			Low (N=28)		Intermediate (N=6)		High (N=16)		
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	
Response									
PD	10	(20%)	10	(35.7%)	0	(0%)	0	(0%)	0.022§
SD	15	(30%)	6	(21.4%)	3	(50%)	6	(37.5%)	
PR	25	(50%)	12	(42.9%)	3	(50%)	10	(62.5%)	
NR	25	(50%)	16	(57.1%)	3	(50%)	6	(37.5%)	0.217§
OAR	25	(50%)	12	(42.9%)	3	(50%)	10	(62.5%)	
Progression									
Within 6 months	22	(55%)	16	(88.9%)	3	(50%)	3	(18.8%)	<0.001§
After 6 months	18	(45%)	2	(11.1%)	3	(50%)	13	(81.3%)	
Progression Free Survival									
Median PFS	6 months		4 months		6 months		8 months		<0.001†
HR (95%CI)					0.311 (0.115 - 0.840)		0.146 (0.061 - 0.350)		<0.001*
6 months PFS	45%		11.1%		50%		81.3%		
12 months PFS	2.5%		0%		0%		6.3%		
Survival									
< 9 months	26	(52%)	25	(89.3%)	1	(16.7%)	0	(0%)	<0.001§
> 9 months	24	(48%)	3	(10.7%)	5	(83.3%)	16	(100%)	
Overall Survival									
Median OS	9 months		7 months		13 months		15 months		<0.001†
HR (95%CI)					0.288 (0.113 - 0.737)		0.122 (0.053 - 0.279)		<0.001*
6 months OS	74%		57.1%		83.3%		100%		
12 months OS	42%		10.7%		66.7%		87.5%		
18 months OS	4%		0%		0%		12.5%		

Categorical variables were expressed as number (percentage); § Chi-square test for trend; † Log rank test; * Breslow test; low group was taken as reference for CD+ TILs. p<0.05 is significant.

A p53 gene mutation inhibits the tumor suppressor gene and enables malignant cells to behave more aggressive and spread to different sites. This genetic defect is detected in more than 50% of the resected NSCLCs (33). However, in this study p53 was over-expressed in 82% of cases as all our cases were at an advanced stage. To test if PD-L1 correlates with the activation of apoptotic pathways, we analyzed the relationship between it and p53 expression in NSCLC. However the association was non-significant, this was in contrast to Kan and Dong (34), who hypothesized that the PD-L1 expression is significantly related to p53 protein expression in hepatocellular carcinoma.

In conclusion, a large proportion of advanced NSCLC cases have a positive PD-L1 immunoreactivity as reported by previous studies, so they may be potentially responsive to PD-L1 immunotherapy. Immunohistochemical expression of stromal CD8+ TILs had a favorable prognostic impact

in NSCLC in contrast to PD-L1 over-expression. Further studies to confirm our observations and to assess the predictive value of anti-PD-L1 immunotherapy in NSCLC are necessary.

CONFLICT of INTEREST

The authors declare that they have no conflict of interest.

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