RESEARCH ARTICLE

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Immunotherapy and *PD-L1* Tumor Expression in Moroccan Non-Small Cell Lung Cancer Patients with Various Metastasis

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Abstract

Inroduction: The question of whether tumor expression of *PD-L1* and the presence of distant metastasis could influence the efficacy of immunotherapy represents a major challenge and needs to be further elucidated. The aim of this study is to evaluate the predictive significance of tumor expression of PD-L1 as well as the number and site of metastasis in non-small cell lung cancer (NSCLC) among Moroccan patients treated with immunotherapy.**Material and Methods:** Between January 2019 and February 2023, we recruited Moroccan patients with metastatic NSCLC. All were treated with immunotherapy, either as monotherapy or in combination with chemotherapy. Immunohistochemistry was used to assess *PD-L1* (clone 22C3) and ALK (clone D5F3) status. EGFR status was established by qPCR. Tumor *PD-L1* expression was classified into 2 levels: TPS <1% (negative expression) and TPS ≥1% (positive expression). Statistical analysis was performed using SPSS Statistics V.21 software. **Results:** The median age of patients (N=40) was 67 years (39- 92 years) and the sex ratio was 9. Disease dissemination revealed that 22.5% (N=9) of patients had a metastatic burden ≥ 3 (MB≥3). As for the sites of metastasis, the results showed that 20% (N=8), 10% (N=4), 42.5% (N=17), 22.5% (N=9), 27.5% (N=11), 45% (N=18) and 27.5% (N=11) of patients had developed lymph node, liver, bone, brain, pleural, contralateral lung and adrenal metastasis respectively. Positive *PD-L1* expression was significantly associated with shorter overall survival (OS = 17.19 vs. 28.85 months, p=0.01). High metastatic burden (MB ≥ 3) was associated with lower objective response rate (ORR), shorter progression-free survival (PFS), and reduced OS, respectively (ORR = 0 vs. 58.06%, p=0.002; PFS = 10.23 vs. 25.27 months, p=0.001; and OS = 11.60 vs. 27.91 months, p=0.003). Only the presence of osseous metastasis was significantly associated with lower ORR, shorter PFS, and OS compared to other metastatic locations (ORR = 5.88 vs. 73.9%, p=0.000; PFS = 10.72 vs. 31.33 months, p=0.000; and OS = 11.39 vs. 36.17 months, p=0.000). Finally, the presence of hepatic metastasis was significantly associated with shorter PFS (10.75 months) compared to those without hepatic metastasis (22.53 months) (p=0.046). Finally, the results of the multivariate analysis revealed that the presence of bone metastasis was strongly correlated with a significant decrease in progression-free survival (p=0.001) as well as overall survival (p=0.002). **Conclusion:** Our results suggest that tumor expression of *PD-L1* and metastatic burden should play a significant role in predicting the response to immunotherapy. Furthermore, it is important to note that the presence of osseous and hepatic metastasis could negatively influence the clinical outcomes of immunotherapy.

Keywords: Non-small cell lung cancer- immunotherapy- *PD-L1*- metastatic burden- site of metastasis

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Introduction

Lung cancer is one of the most prevalent malignant diseases [1] and the leading cause of cancer-related deaths worldwide [2]. In Morocco, lung cancer is the second most common cancer, with a prevalence of 13.9 % for both sexes and up to 25.6 % in males [3]. Consequently, non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancer cases [4]. Most patients are diagnosed at an advanced stage of the disease [5]. In NSCLC, the spread of metastasis is a major concern [2] as they can affect various organs such as the bones, brain, and liver. Indeed, bone metastasis account for approximately 34% of cases, while brain and liver metastasis affect nearly 39% and 20% of NSCLC patients, respectively [6]. Furthermore, studies have shown that different types of metastasis have distinct prognostic value in NSCLC patients, especially liver and multi- organ metastasis, which are associated with an increased risk of mortality [7]. Emerging therapeutic approaches have improved

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the prognosis of NSCLC patients [8] and among them, immunotherapy using immune checkpoint inhibitors (ICIs), specifically anti-PD-1 (Pembrolizumab) or anti-*PD-L1* (Atezolizumab), has transformed the treatment landscape for advanced or metastatic NSCLC patients [9] and has become a powerful therapeutic strategy [10, 11]. Despite the survival benefits offered by ICIs, it remains challenging to identify patients who will fully benefit from them. Currently, *PD-L1* expression is the only clinically validated biomarker to identify patients most likely to respond to immunotherapy [12]. However, certain clinical factors, such as performance status, the number of affected metastatic organs (metastatic burden), and the site of metastasis, have also emerged as potential predictors of immunotherapy efficacy [13, 14]. The results of the Keynote-189 study suggest that patients with brain metastasis should benefit from immunotherapy, contrary to other studies such as Keynote 024, which reached the opposite conclusion [6]. The use of immunotherapy as a treatment, especially in NSCLC patients with different *PD-L1* statuses and distant metastasis localized in specific organs such as the liver, brain, and bones, remains a complex issue to address. Therefore, the aim of this study is to evaluate the predictive significance of tumor *PD-L1* expression as well as the number and site of metastasis in Moroccan NSCLC patients treated with immunotherapy.

Materials and Methods

Ethical Consideration

This study was approved by the local ethics committee of Ibn Rochd University Hospital (CHU) in Casablanca (Approval number: 03/2022). All patients provided informed consent before participating in the study. Thus, the protocol of our study adheres to the principles outlined in the Helsinki Declaration.

Patients

Between January 2019 and February 2023, 40 Moroccan patients with metastatic NSCLC were recruited from two different institutions: the Mohammed VI Center for Cancer Treatment at Ibn Rochd University Hospital in Casablanca and the Ryad Oncology Clinic in Casablanca. Eligible participants for our study must meet the following predefined criteria: age ≥ 18 years, presenting histologically confirmed NSCLC, having been treated with immunotherapy alone or in combination with chemotherapy, and having clinical and pathological data available. Exclusion criteria concern patients with other types of lung cancer or those under chemotherapy alone. The patients' characteristics are cited in Table 1.

Outcome Assessment

The assessment of response to immunotherapy was based on iRECIST criteria (Immunotherapy Response Evaluation Criteria in Solid Tumors, Version 1.1). Specific organ responses included complete responses (CR), partial responses (PR), stable disease (SD), and progressive disease (PD). The objective response rate (ORR) corresponds to the ratio of patients who achieved CR, PR, and SD to the total. Progression-free survival (PFS) was defined from the first day of ICI treatment until the day of disease progression assessment by the physician or death, regardless of the cause. Overall survival (OS) corresponds to the duration of immunotherapy treatment (deceased or alive).

Expression of PD-L1

Tumor expression of *PD-L1* was assessed from formalin-fixed paraffin-embedded (FFPE) tumor samples using the 22C3 pharmDX test on the Dako Link 48 platform. Tumor cells showing partial or total membranous staining were considered positive. Thus, the tumor expression of *PD-L1* was evaluated using the tumor proportion score (TPS), defined as the percentage of positive *PD-L1* tumor cells (TC+) relative to the total number of TC. Based on *PD-L1* expression, tumor cells were classified into three groups: negative expression (TPS < 1%), low expression (TPS from 1 to 49%), and high expression (TPS ≥ 50%) [15].

EGFR Test

Molecular alterations of EGFR were detected using real-time polymerase chain reaction (qPCR) with the cobas® mutation test. This test identifies various mutations within EGFR exons from FFPE tissues. Specific mutations targeted include those in exon 18 (G719A, G719C, and G719S), exon 19, exon 20 (S768I, T790M), and exon 21 (L858R and L861Q). The results revealed the presence either absence of specific EGFR gene mutations in the tested samples.

ALK Status

ALK translocation testing was performed using immunohistochemistry (IHC) with a rabbit monoclonal anti-ALK antibody (Clone D5F3, Ventana, Roche). A positive result is characterized by intense granular cytoplasmic staining observed within tumor cells.

Statistical Analysis

Statistical analysis was conducted using SPSS version 21 statistical software. The chi-square test was used to analyze the difference in the objective response rate (ORR) between subgroups. The Kaplan-Meier method was used to assess overall survival (OS) and progression-free survival (PFS), and the log-rank test was used to determine the significance of differences. We also performed univariate and multivariate Cox regression analysis to explore the impact of clinical variables on patients' PFS and OS. A P-value <0.05 was considered statistically significant.

Results

Patient Characteristics

Table 1 presents the characteristics of the 40 patients included in this study. Among these patients, 90% (N=36) were men with a sex ratio of 9. The median age of the patients was 67 years (range 39 to 92 years), with 55% (N=22) aged ≥ 67 years. 95% (N=38) of the patients had adenocarcinoma, 76.5% (N=27) had a performance status (PS) of 0, and 85% (N=34) were smokers. Additionally, 22.5% (N=9) of the patients had more than 3 affected

Table 1. Characteristics of the Patients Recruited in This Study

Variables	Number (%)
Gender	
Men	36 (80.5)
Women	4 (19.4)
Sex ratio	9
Age at diagnosis (years)	
Median [Rank]	67 [39-92]
< 67	18 (45)
≥ 67	22 (55)
Histological aspect	
Adenocarcinoma	38 (95)
Squamous cell carcinoma	2 (5)
Performance status (PS)	
PS 0	27 (67.5)
PS 1- 2	13(32.5)
Smoking status	
Current/ Former	34 (85)
Never	06 (15)
Metastatic burden	
<3	31(77.5)
≥3	9 (22.5)
lymph node metastasis	
No	32 (80)
Yes	08 (20)
Liver metastasis	
No	36 (90)
Yes	04 (10)
Bone metastasis	
No	23 (57.5)
Yes	17 (42.5)
Brain metastasis	
No	31 (77.5)
Yes	09 (22.5)
Pleural metastasis	
No	29 (72.5)
Yes	11 (27.5)
Contralateral lung metastasis	
No	22 (55)
Yes	18 (45)
Adrenal metastasis	
No	29 (72.5)
Yes	11 (27.5)
Expression PD-L1	
TPS<1%	16 (40)
TPS1-49%	12 (30)
TPS ≥ 50%	12 (30)
EGFR mutation status	
Wild type	38 (95)
Mutant	02 (05)
ALK Status	
Negative	40 (100)
Positive	00 (00)
Treatment strategy	
Atezolizumab alone	02 (05)
Pembrolizumab alone	06 (15)
Atezolizumab + chemotherapy	20 (50)
Pembrolizumab + chemotherapy	12 (30)
Treatment response	
CR	07 (17.5)
PR	05 (12.5)
SD	06 (15)
PD	22 (55)
Vital status	
Vivant	21 (52.5)
Cancer-related deaths	15 (37.5)
Non-cancer deaths	04 (10)

ALK, Anaplastic lymphoma kinase; CR, Complete response; EGFR, Epidermal growth factor receptor; PD, Progressive disease; *PD-L1,* Programmed Cell Death Ligand 1; PR, Partial response; SD, Stable disease.

metastatic organs (Metastatic Burden ≥ 3). Regarding metastatic sites, the results showed that 20% (N=8), 10% (N=4), 42.5% (N=17), 22.5% (N=9), 27.5% (N=11), 45% (N=18), and 27.5% (N=11) of the patients had developed lymph nodes, liver, bone, brain, pleura, contralateral lung, and adrenal glands metastasis, respectively. The molecular profile of the patients revealed that 30% (N=12) had high *PD-L1* expression, 5% (N=2) had tumors with EGFR mutation, while no ALK rearrangement was observed. Various therapeutic protocols were used for our patients. 5% (N=2) received Atezolizumab alone, 15% (N=6) received Pembrolizumab alone, 50% (N=20) received Atezolizumab combined with chemotherapy, and 30% (N=12) received Pembrolizumab combined with chemotherapy. The results of immunotherapy treatment response revealed that 17.5% (N=7) of the patients achieved complete recovery (CR), 12.5% (N=5) had partial response (PR), 15% (N=6) maintained stable disease (SD), and 47.5% (N=9) experienced disease progression (PD). The vital status of the patients showed that 52.5% (N=21) were still alive, while 37.5% (N=15) of the deaths were attributed to cancer. It is worth noting that 10% (N=4) of the deaths were due to other causes unrelated to cancer, including two patients who died from SARS-CoV2 infection, one from meningitis, and one from heart failure.

Results of PD-L1 tumor expression and response to immunotherapy

Figure 1 depicts the results of *PD-L1* expression and response to immunotherapy based on ORR (Figure 1, A), PFS (Figure 1, B), and OS (Figure 1, C). The results reveal a significant difference in overall survival duration

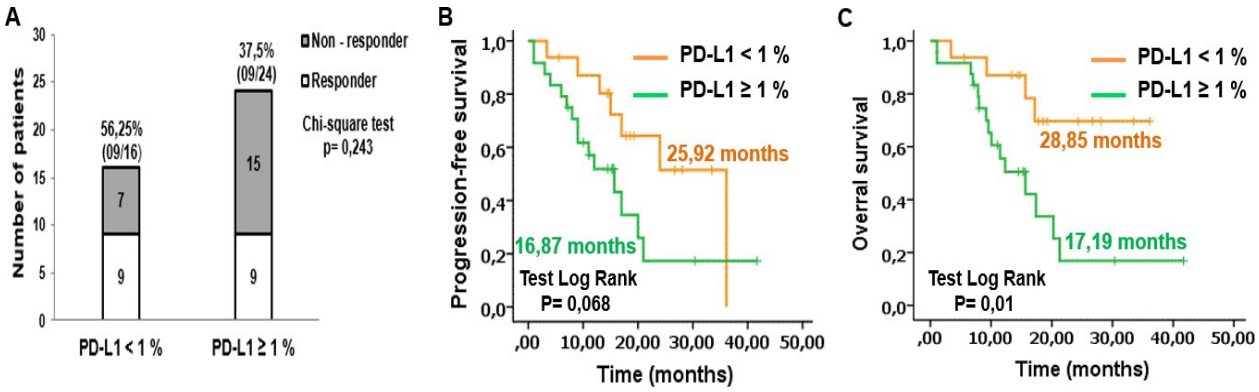

Figure 1. ORR (A), PFS (B), and OS (C) as a Function of Response to Immunotherapy Treatment. *PD-L1*: Programmed Cell Death Ligand 1.

between patients with negative *PD-L1* expression (28.85 months) and those with positive expression (17.19 months) (p=0.01).

The results concerning the metastatic burden, metastatic site, and response to immunotherapy

We classified patients into two groups based on the number of affected metastatic organs (MB <3 and

Table 2. Predictive Factors of Progression-Free Survival (PFS) in Univariate and Multivariate Analysis

Variables	Univariate survival analysis of PFS			Multivariate survival analysis of PFS	
	mPFS (months)	HR:95% CI	P-value	HR:95% CI	P-value
Gender			0.517		
Men VS Women (C.REF)	23.47 VS 11.33	2.90: 17.77 – 29.18		Not included	
Age at diagnosis (years)			0.459		
< 67 (C.REF) VS ≥ 67	20.58 VS 22.01	3.70: 14.76 – 29.27		Not included	
Histological aspect			0.277		
Adc (C.REF) VS SCC				Not included	
Performance status (PS)			0.277		
0 (C.REF) VS 1-2	25.17 VS 13.34	3.59: 06.29 – 20.38		Not included	
Smoking status			0.709		
Current/ Former VS Never (C.REF)	23.68 VS 17.79	3.05: 17.69 – 29.67		Not included	
Metastatic burden			0.001*		0.785
<3 (C.REF) VS ≥3	25.27 VS 10.23	2.35 :07.01 – 16.25		1.16: 0.38 – 3.60	
Lymph node metastasis			0.231		
No (C.REF) VS Yes	22.54 VS 14.54	2.58: 9.48 – 19.60		Not included	
Liver metastasis			0.046*		0.803
No (C.REF) VS Yes	22.53 VS 10.75	3.79: 3.31 – 18.18		0.85: 0.23 - 3,03	
Bone metastasis			0.000*		0.001*
No (C.REF) VS Yes	31.33 VS 10.72	1.62: 8.30 – 14.65		0.12: 0.03 – 0.43	
Brain metastasis			0.533		
No (C.REF) VS Yes	22.01 VS 12.49	1.80: 9.45 – 16.53		Not included	
Pleural metastasis			0.724		
No (C.REF) VS Yes	19.35 VS 20.13	5.50: 10.44 - 32,03		Not included	
Contralateral lung metastasis			0.144		
No (C.REF) VS Yes	18.03 VS 22.28	2.51: 19.43 – 29.28		Not included	
Adrenal metastasis			0.875		
No (C.REF) VS Yes	22.05 VS 19.71	4.38: 14.86 – 32.05		Not included	
Expression PD-L1			0.068		
TPS<1% (C.REF) VS TPS ≥ 1%	25.92 VS 16.87	3.45: 11.09 – 24.64		Not included	
EGFR mutation status			0.432		
Wild type (C.REF) VS Mutant	23.97 VS 16.00	5.00: 06.20 – 25.80		Not included	

mOS, Mean overall survival; *PD-L1*, Programmed death-ligand 1; EGFR, Epidermal growth factor receptor; CI, Confidence interval; HR, Hazard ratio; C.REF, Category reference; ADC, Adenocarcinoma; SCC, Squamous cell carcinoma; *, Statistically significant at p ≤ 0.05.

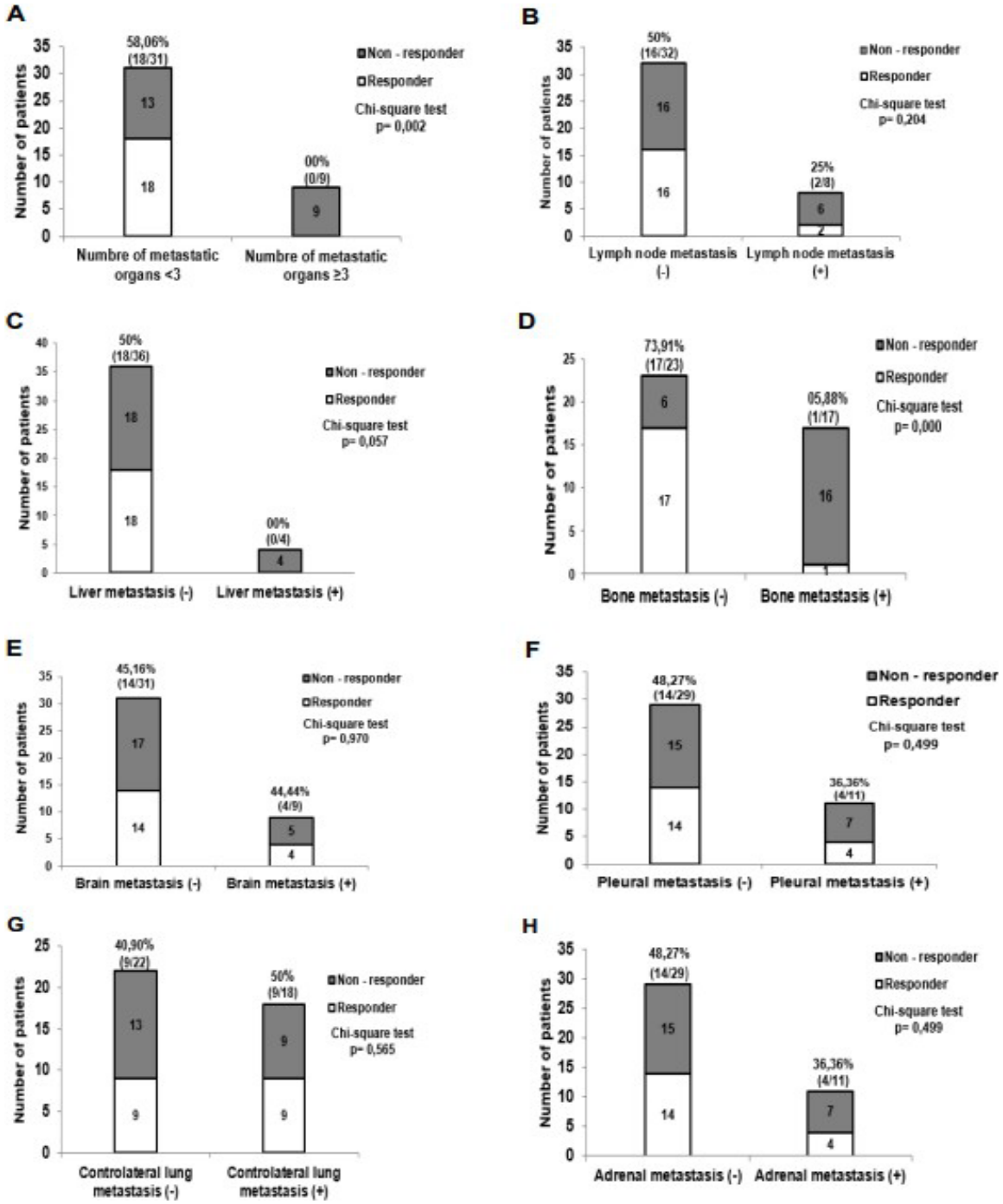

Figure 2. ORR as a Function of Metastatic Burden and Metastatic Sites. A: Metastatic burden; B: Lymph node metastasis; C: Hepatic metastasis; D: Bone metastasis; E: Brain metastasis; F: Pleural metastasis; G: Contralateral pulmonary metastasis; H: Adrenal metastasis.

MB ≥3). The results show a significant association between MB and ORR (MB<3: ORR=58.06; MB≥3: ORR=0%; p=0.002), PFS (MB<3: PFS=25.27 months; MB≥3: PFS=10.23 months; p=0.001), and OS (MB<3: OS=27.91 months; MB≥3: OS=11.60 months; p=0.003) (Figure 2, A; Figure 3, A; Figure 4, A).Indeed, patients with bone metastasis have a significantly lower ORR to immunotherapy and reduced PFS and OS compared to those without bone metastasis (ORR: 5.88% vs 73.91%, p=0.000; PFS: 10.72 vs 31.33 months, p=0.000; OS: 11.39 vs 36.17 months, p=0.000) (Figure 2, D; Figure 3, D; Figure 4, D). Finally, PFS was significantly reduced in the presence of hepatic metastasis compared to the absence of hepatic metastasis (PFS: 10.75 vs 22.53 months, p=0.046) (Figure 3, C).

Study of PD-L1 Tumor Expression Based on Metastatic Burden and Sites

Our study reveals significant differences in ORR, PFS, and OS among patients based on their *PD-L1* tumor expression, metastatic burden, and site. Consequently, certain patients exhibited significantly shorter ORR, lower PFS, and reduced OS (Figure 4, A, B, and C).

Table 3. Predictive Factors of Overall Survival (OS) in Univariate and Multivariate Analysis.

Variables	Univariate survival analysis of OS			Multivariate survival analysis of OS	
	mOS (months)	HR:95% CI	P-value	HR:95% CI	P-value
Gender			0.181		
Men VS Women (C.REF)	24.61 VS 12.45	2.97: 18.78 – 30.45		Not included	
Age at diagnosis (years)			0.505		
< 67 (C.REF) VS ≥ 67	20.88 VS 21.97	3.64: 14.83 – 29.12		Not included	
Histological aspect			0.997		
ADC (C.REF) VS SCC	23.29 VS 15.73	8,72: 00.00 – 32.84		Not included	
Performance status (PS)			0.000*		0.047*
0 (C.REF) VS 1-2	26.45 VS 12.76	2.71: 21.13 – 31.77		2.96: 1.01 – 8.676	
Smoking status			0.748		
Current/ Former VS Never (C.REF)	23.78 VS 16.05	3.04: 17.82 – 29.74		Not included	
Metastatic burden			0.003*		0.438
<3 (C.REF) VS ≥3	27.91 VS 11.60	2.14: 7.39 – 15.81		0.81: 0.47 - 1,37	
Lymph node metastasis			0.792		
No (C.REF) VS Yes	23.95 VS 16.13	2.41: 11.40 – 20.86		Not included	
Liver metastasis			0.259		
No (C.REF) VS Yes	24.75 VS 14.04	4.05: 6.09 – 21.98		Not included	
Bone metastasis			0.000*		0.002*
No (C.REF) VS Yes	36.17 VS 11.39	1.42: 1.42 – 14.18		0.08: 0.01 – 0.38	
Brain metastasis			0.533		
No (C.REF) VS Yes	24.87 VS 12.94	1.42:10.15 VS 15.7		Not included	
Pleural metastasis			0.858		
No (C.REF) VS Yes	20.28 VS 24.36	5.56: 13.45 – 35.27		Not included	
Contralateral lung metastasis			0.067		
No (C.REF) VS Yes	19.05 VS 23.95	2,71: 18.63 – 29.26		Not included	
Adrenal metastasis			0.498		
No (C.REF) VS Yes	25.18 VS 18.81	3.67: 11.60 – 26.02		Not included	
Expression PD-L1			0.010*		0.915
TPS<1% (C.REF) VS TPS ≥ 1%	28.85 vs 17.19	3.04: 11.23-23.161		1.07: 0.29 – 3.85	
EGFR mutation status			0.524		
Wild type (C.REF) VS Mutant	24.38 VS 16.38	4.92: 6.73 – 26.03		Not included	

mOS, Mean overall survival; *PD-L1*, Programmed death-ligand 1; EGFR, Epidermal growth factor receptor; CI, Confidence interval; HR, Hazard ratio; C.REF, Category reference; ADC, Adenocarcinoma; SCC, Squamous cell carcinoma; *, Statistically significant at $p \leq 0.05$.

Univariate and multivariate overall survival analysis

In our study, we thoroughly examined factors significantly associated with unfavorable progression of PFS and OS. Univariate analysis revealed several significant variables related to unfavorable PFS and OS. Among these, metastatic burden (≥3) (p=0.001) and the presence of hepatic and osseous metastasis (p=0.046, p=0.000 respectively) were particularly notable for PFS (Table 2). Regarding OS, performance status (PS 1-2) (p=0.000), metastatic burden (≥3) (p=0.003), osseous metastasis (p=0.000), and tumor expression of *PD-L1* (TPS ≥ 1%) (p=0.010) were identified as significant factors (Table 3). However, in multivariate analysis, some of these factors retained their importance as independent predictors of unfavorable PFS and OS. Specifically, the presence of osseous metastasis (p=0.001) remained a significant predictor for PFS (Table 2), while performance status (PS 1-2) (p=0.047) and the presence of osseous metastasis (p=0.002) remained important predictive factors for OS (Table 3).

Discussion

The objective of our study was to assess the predictive value of *PD-L1* tumor expression and the burden and sites of metastasis in patients with non-small cell lung cancer (NSCLC) treated with immunotherapy. A significant difference in overall survival (OS) duration was observed between patients with negative *PD-L1* expression (28.85 months) and patients with positive expression (17.19 months) (p=0.01). These results are in line with those reported by Kaiyan Chen et al [16] in NSCLC patients (OS: *PD-L1* <1% = 29.3 months, *PD-L1* ≥1% = 15.20 months, p=0.0006). Additionally, another study conducted by Si-yan Liu et al. [17] found a significant difference in OS between *PD-L1* (+) patients (OS=78.6 months) and

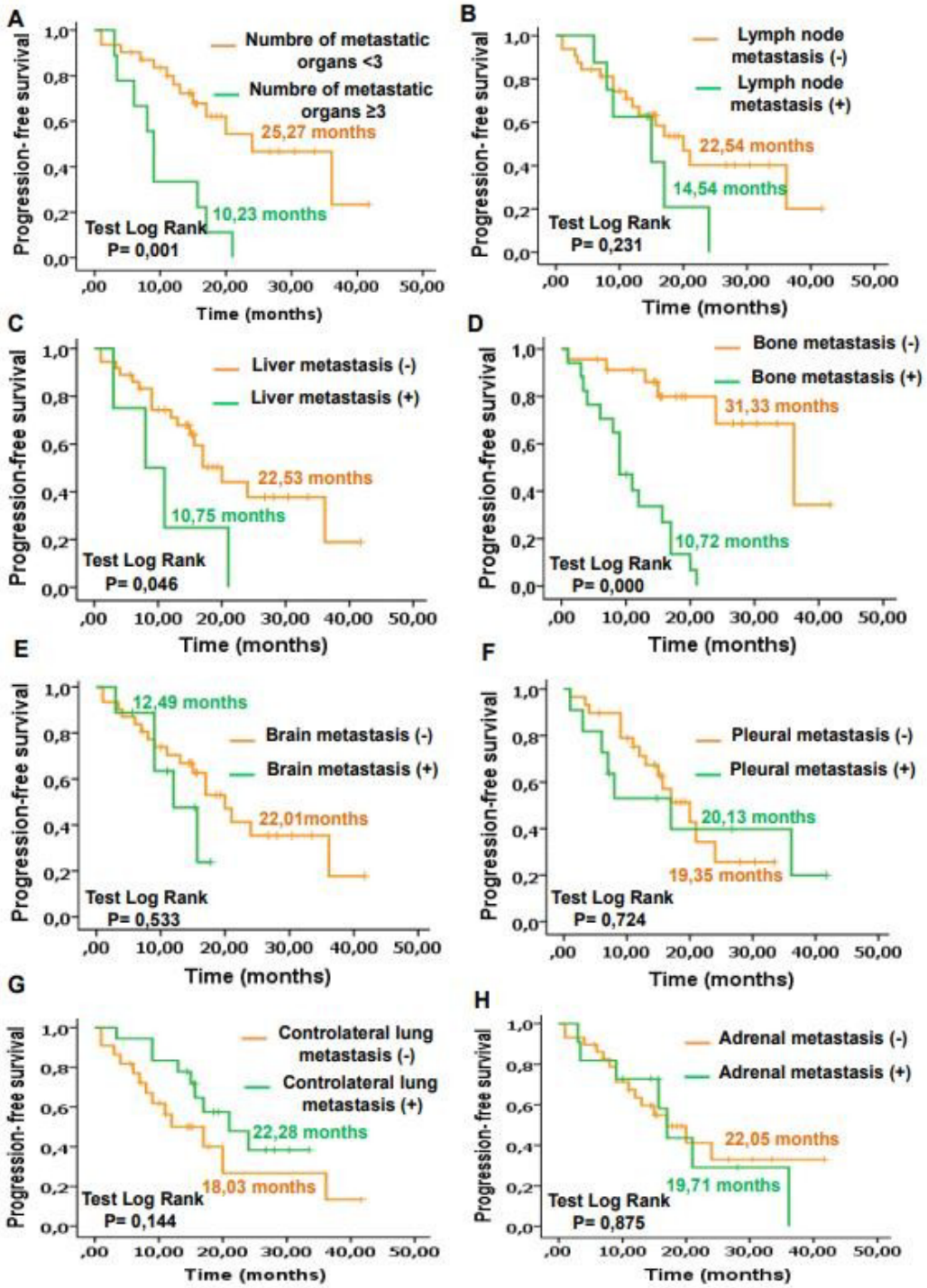

Figure 3. PFS as a Function of Metastatic Burden and Metastatic Sites. A, Metastatic burden; B, Lymph node metastasis; C, Hepatic metastasis; D, Bone metastasis; E, Brain metastasis; F, Pleural metastasis; G, Contralateral pulmonary metastasis; H, Adrenal metastasis.

PD-L1 (-) patients (OS=93.4 months) (p=0.0005). Our results should be interpreted considering several factors that may influence the final interpretation, including the type of analyzed specimen. In our study, we worked with tissue biopsies since the patients were inoperable at the time of diagnosis. It is worth noting that *PD-L1* IHC tests can yield false negatives due to the intratumoral heterogeneity of *PD-L1* expression, as emphasized in the recommendations from the PATTERN group of thoracic pathologists [18]. Furthermore, treatment resistance, the presence of other oncogenic drivers, the burden, and the metastatic site must be taken into consideration. Moreover, concerning NSCLC treatments, immunotherapy represents a promising strategy aimed at mobilizing the immune system to recognize and potentially eliminate tumor cells, thus prolonging patient survival.

However, some patients develop resistance to treatment, whether it's primary resistance (lack of initial response or clinical benefit) or acquired resistance (alterations in the INF-γ signaling pathway, loss of effector

Table 4. (A) ORR , (B) PFS , and (C) OS According to Tumor *PD-L1* Expression, Metastatic Burden, and Sites. [a]P: Chi-square test; [b]P: Log-rank test.

Metastatic organs"	*PD-L1* < 1%			*PD-L1* ≥ 1%			P value	*PD-L1* < 1%	*PD-L1* ≥ 1%	P value	*PD-L1* < 1%	*PD-L1* ≥ 1%	P value
	No. Of Patients			No. Of Patients				mPFS (Months)			mOS (Months)		
	R	NR	ORR (%)	ORR (%)	R	NR							
Metastatic burden							0.019			0.002			0.000
< 3	9	5	64.28	52.94	9	8		28.84	22.08		32.19	20.96	
≥ 3	0	2	0	0	0	7		06.19	11.38		06.32	13.30	
Lymph node							0.267			0.150			0.057
No	8	4	66.67	40	8	12		29.31	17.74		31.23	17.37	
Yes	1	3	25.00	25.00	1	3		16.87	12.00		17.32	14.70	
Liver							0.046			0.058			0.033
No	11	5	68.70	50.00	10	10		25.92	18.43		28.85	17.91	
Yes	-	-	-	0	0	4		-	10.75		-	14.04	
Bone							0.002			0.000			0.000
No	10	3	76.90	80.00	8	2		30.07	34.16		33.85	33.84	
Yes	1	2	33.00	14.28	2	12		09.79	10.21		09.94	11.71	
Brain							0.664			0.262			0.058
No	7	6	53.84	38.89	7	11		26.45	17.94		29.57	18.24	
Yes	2	1	66.67	33.33	2	4		13.39	11.63		13.52	12.40	
Pleural							0.516			0.180			0.049
No	7	6	53.84	43.75	7	9		22.30	16.28		11.37	11.89	
Yes	2	1	66.67	25.00	2	6		36.13	13.24		25.87	07.99	
Controlateral lung							0.075			0.017			0.012
No	4	0	100	33.00	6	12		19.32	11.04		12.72	11.69	
Yes	7	5	58.33	66.67	4	2		15.99	17.83		18.64	17.93	
Adrenal							0.310			0.232			0.058
No	8	4	66.67	35.29	6	11		25.49	17.29		29.21	18.48	
Yes	1	3	25.00	42.85	3	4		21.16	15.76		21.23	15.38	

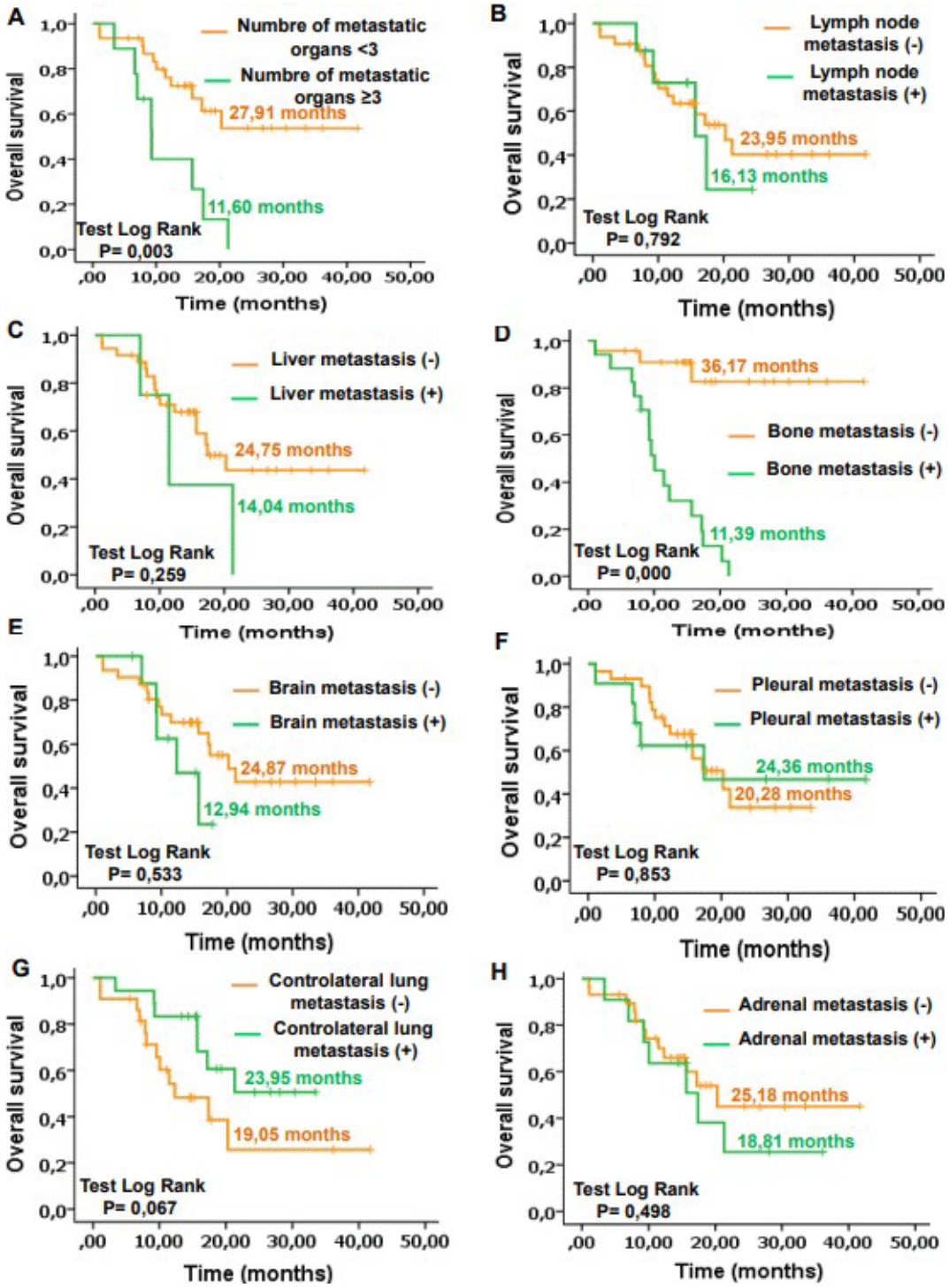

Figure 4. OS as a Function of Metastatic Burden and Metastatic Sites. A, Metastatic burden; B, Lymph node metastasis; C, Hepatic metastasis; D, Bone metastasis; E, Brain metastasis; F, Pleural metastasis; G, Contralateral pulmonary metastasis; H, Adrenal metastasis.

function of T lymphocytes, and upregulation of alternative immune checkpoint receptors). This occurs when tumor progression occurs after a median progression-free survival of 4 to 10 months [19, 20-21, 22]. Thus, among the 19 deceased patients, 9 may have acquired resistance to immunotherapy (PFS > 4 months, 3 with *PD-L1* (+) and 6 with *PD-L1* (-)). As for primary resistance to immunotherapy, 4 out of 19 deceased patients had the following profile (PFS < 4 months, adenocarcinoma NSCLC, EGFR-, ALK-, and smokers). This suggests that this resistance may be due to rare EGFR mutations, as observed in the study by How-wen Ko (Taoyuan, 2022), which demonstrated a positive relationship between smoking and the frequency of these rare EGFR mutations, reinforcing this hypothesis [23]. Mutations in the HER-2 gene can also lead to tumor progression and patient death, which could be the case for one of the 19 deceased patients in our study who presented (PFS < 4 months, adenocarcinoma NSCLC, non-smoker, EGFR-, ALK-, and *PD-L1*+). According to Mathieu Chevalier et al.

[24] , 1 to 3% of NSCLC patients had HER-2 mutations, mainly observed in cases of adenocarcinoma, especially in women and non-smokers. Additionally, among the 19 studied patients, 2 of them had activating EGFR mutations, which may have contributed to their death. Finally, it is important to highlight that among the 19 deceased patients, 11 had a metastatic burden ≥ 2, and 15 had bone metastasis. These results confirm the significant association we found in our study between metastatic burden and immunotherapy effectiveness (Figure 2, A; Figure 3, A; Figue 4, A). These observations align with the results of a study conducted by Jiayi Deng et al. [25] in NSCLC patients, which showed a significant decrease in PFS with increasing metastatic burden (metastatic burden ≥3: PFS=4.4 months, metastatic burden=2: PFS=14.4 months, metastatic burden=1: PFS=25.2 months, p=0.0052).

Our results also revealed significantly unfavorable objective response rate (ORR), progression-free survival (PFS), and overall survival (OS) compared to patients without bone metastasis (ORR=17.7% vs. 78.3%, p=0.000; PFS=10.72 vs. 31.33 months, p=0.000; OS=11.39 vs. 36.17 months, p=0.000) (Figure 2, D; Figure 3, D; Figure 4, D). These results can be explained by the fact that the bone marrow exhibits notable immune vulnerability due to various factors, including the presence of immature and inhibitory immune cells, a high proportion of regulatory T cells (Tregs), and myeloid-derived suppressor cells (MDSCs). This immune vulnerability in the bone poses a major challenge in the treatment of bone metastasis and requires specific therapeutic approaches to target the immune microenvironment in this area [26]. Furthermore, patients with liver metastasis had significantly shorter progression-free survival than those without liver metastasis (PFS: 10.75 vs. 22.53 months, p=0.046). The study conducted by Meng Qiao et al. [27] demonstrated that liver metastasis were associated with shorter progression-free survival and overall survival compared to no liver metastasis (PFS: 2.3 vs. 5 months, p <0.001; OS: 9.8 vs. 23.5 months, p = 0.031) [27].

The negative impact of liver metastasis on immunotherapy effectiveness can be attributed to several factors

Induction of hepatic immune tolerance (elimination of tumor-specific CD8+ T lymphocytes through Fas-FasL pathway-induced apoptosis by macrophages) [28].

Tumor progression induced by hepatocytes, sinusoidal cells, and Kupffer cells, producing angiogenesis factors (VEGF) [14]. Finally, our study shows that the effectiveness of immunotherapy depends on the tumor *PD-L1* status, metastatic burden, and site (Table 4). Indeed, *PD-L1* expression differs between the primary site and metastatic sites, which have distinct genetic and immunological profiles. It is worth noting that a study conducted by Sarah B. Goldberg et al (USA, 2020) in the context of a phase 2 clinical trial showed the efficacy of pembrolizumab in treating NSCLC patients with brain metastasis and *PD-L1* expression (*PD-L1* ≥1%), highlighting pembrolizumab's activity in brain metastasis of NSCLC with *PD-L1* expression ≥1% [29].

In conclusion, our study highlights the importance of immunotherapy in the management of NSCLC while emphasizing the complexity of treatment responses. Our results suggest that tumor expression of *PD-L1* and MB play a significant role in predicting the response to immunotherapy. A MB ≥ 3 appears to be a major risk factor, as well as the presence of bone and hepatic metastasis. Finally, immunotherapy remains a promising strategy, provided it is combined with the customization of care for NSCLC patients based on biomarkers and clinical characteristics to enhance treatment efficacy.

Author Contribution Statement

The authors of this article have made significant contributions to the design, data collection, analysis, and manuscript writing. Their individual contributions are as follows:

Aazzane Oussama: Writing - original version, Conceptualization, Methodology, Data collection. Fathi sofia: Formal analysis, Visualization. Charkaoui Meryem: Formal analysis, Data collection. Acharki Abdelkader, Sahraoui Souha and Benchakroun Nadia: Investigation and Revision. Fellah Hassan and Karkouri Mehdi: Investigation, Supervision, Conceptualization, Methodology, Validation, Revision and Editing.

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Compliance and Thesis Approval

We also declare that the thesis of Oussama Aazzane is currently undergoing defense at the Faculty of Medicine and Pharmacy, Hassan II University of Casablanca.

Conflict of Interest

We declare that there are no conflicts of interest to disclose.

Ethics Approval and Participant Consent

This study was approved by the local ethics committee of Ibn Rochd University Hospital (CHU) in Casablanca (Approval number: 03/2022). All patients provided informed consent before participating in the study. Thus, the protocol of our study adheres to the principles outlined in the Helsinki Declaration.

Availability of data

The data used in this research is available upon request from the authors (Oussama Aazzane, Hassan Fellah and Mehdi Karkouri).

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