RESEARCH ARTICLE

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The Predictive Significance of *Galectin 3* Expression in Patients with Unresectable Non-Small Cell Lung Cancer without *EGFR* or *ALK* Mutations Treated Platinum-Based Doublet Chemotherapy

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Abstract

Objective: The objective of our study is to evaluate the predictive value of cytoplasmic *Galectin-3* (*Gal-3*) expression in tumor cells (TCs) as well as in tumor-infiltrating immune cells (TIICs), including tumor-associated macrophages (TAM), tumor-associated neutrophils (TAN), and tumor-associated lymphocytes (TAL), in Moroccan patients with unresectable non-small cell lung cancer (NSCLC). **Methods:** This is a prospective study conducted between 2019 and 2023 on 56 Moroccan patients diagnosed with unresectable NSCLC. These patients were treated with platinum-based doublet chemotherapy and followed at the Mohammed VI Center for Cancer Treatment at CHU Ibn Rochd in Casablanca. Immunohistochemistry (IHC) was used to assess *Gal-3* (clone 9C4) expression in TCs and TIICs (TAM, TAN, and TAL). The characteristics of the patients were obtained from the patients' medical records. Statistical analysis, including Kaplan-Meier survival analysis and Cox regression, was performed using SPSS v.21. **Results:** Survival analysis across different subgroups revealed that only patients with high *Gal-3* expression in TCs, along with positive expression in TIICs or TAL, exhibited significantly improved overall survival (OS = 16.81 months, p = 0.007; OS = 15.95 months, p = 0.034) compared to other subgroups. Additionally, multivariate analysis showed that poor performance status (PS 1-2) and the presence of bone metastases were significantly associated with decreased progression-free survival (PFS) (p = 0.015 and p = 0.029, respectively). The histological type (squamous cell carcinoma) was significantly correlated with OS (p = 0.000). **Conclusion:** The concomitant expression of *Gal-3* in TCs (high expression), TIICs, and TAL may serve as a predictive biomarker for chemotherapy response in patients with unresectable NSCLC.

Keywords: Non-small cell lung cancer- *Galectin-3*- tumor-infiltrating immune cells- overall survival- chemotherapy

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Introduction

Lung cancer represents a major public health issue. In Morocco, lung cancer is the second most common cancer after breast cancer, with a prevalence of 13.9% for both sexes and up to 25.6% in men [1]. First-line treatment strategies have evolved in recent years, although platinum-based chemotherapy doublets remain the cornerstone for patients with metastatic non-small cell lung cancer (NSCLC) whose tumor cells do not exhibit *EGFR* or *ALK* oncogene addictions, nor high tumor expression of *PD-L1* (TPS < 50%) [2]. However, this therapeutic approach is associated with overall survival of 6 to 12 months and modest response rates [3, 4]. Therefore, a better understanding of the tumor microenvironment (TME) is necessary to identify the biological mechanisms limiting this therapeutic approach.

The TME represents a complex ecosystem involving interactions among immune cells, tumor cells, stromal cells, and the extracellular matrix, and can promote tumor proliferation, survival, and metastasis. It achieves immunosuppression through various mechanisms, notably the expression of galectin 3 [5], a 29 to 35 kDa glycoprotein that binds to β-galactoside [6]. Galectin 3 is predominantly found in the cytoplasm but also in the nucleus and can be secreted through non-classical secretory pathways [7, 8].

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It is expressed in various cell types such as macrophages, fibroblasts, activated T lymphocytes, and epithelial cells [9]. Galectin 3 promotes tumor aggressiveness by enhancing tumor cell proliferation through anti-apoptotic characteristics, promoting angiogenesis, enhancing tumor cell mobility, and facilitating metastatic activity [10, 11]. Moreover, an in vitro study using a NSCLC-derived cell line, conducted by Hongxing et al. (China, 2020), demonstrated that galectin 3 increases *PD-L1* expression via *STAT 3* phosphorylation. In NSCLC, high galectin 3 expression in tumor cells has been associated with tumor progression, poor prognosis, and chemoresistance [12, 13].

The question of whether tumor and immune expression of galectin 3 influences chemotherapy efficacy remains a major challenge that needs to be elucidated. The objective of our study is to evaluate the predictive significance of cytoplasmic expression of galectin 3 by tumor cells (TCs) and within tumor-infiltrating immune cells (TIICs), such as macrophages (TAM), neutrophils (TAN), and lymphocytes (TAL), in Moroccan patients with unresectable NSCLC.

Materials and Methods

Ethical Considerations

The methodological framework of our study was conducted in accordance with the principles of the Declaration of Helsinki (2000 version) and was approved by the ethics committee of the Ibn Rochd University Hospital Center (CHUIRC) in Casablanca, with approval number 03/2022. All participants provided informed consent.

Patients and Samples

This is a prospective study conducted on 56 Moroccan patients diagnosed with NSCLC who have wild-type *EGFR* and *ALK* status. These patients were treated with platinum-based doublet chemotherapy and followed at the Mohammed VI Center for Cancer Treatment at Ibn Rochd University Hospital in Casablanca (CHUIRC) from January 2019 to December 2023. Tumor samples were obtained from the tumor bank of the anatomical pathology laboratory at CHUIRC, ensuring standardized and reliable data collection. The characteristics of the patients were extracted from the patients' medical records (Table 1).

Hematoxylin and Eosin (HE) Staining

HE staining was performed on formalin-fixed, paraffin-embedded (FFPE) tumor biopsies to identify a representative tumor area for further analyses, including IHC expression of *Gal-3* and identification of TIICs. The proportion of TIICs was classified into three groups: a proportion <30% considered low, a proportion between 30 and 60% considered medium, and a proportion >60% considered high.

Immunohistochemistry (IHC) of Gal-3

The evaluation of immunohistochemical expression of *Gal-3* was conducted on FFPE tumor biopsy samples using a rabbit monoclonal anti-*Gal-3* antibody (Clone 9C4, BioSB). Two confirmed pathologists examined the slides under a light microscope (Olympus *BX43,* Magnification: x40). The cytoplasmic expression of *Gal-3* in TCs was assessed using a staining intensity scale from no staining to strong staining, rated from 0 to 3. This intensity was then multiplied by the percentage of TCs expressing *Gal-3* (ranging from 0 to 100%) to obtain a histological score (H-Score). An H-Score of 0 indicates no staining, while an H-Score of 300 represents diffusely intense staining throughout the tumor tissue [7]. With a median H-Score of 130, we defined two types of tumor expression of *Gal-3*: low expression (H-Score < 130) and high expression (H-Score ≥ 130). TIICs, TAM, TAN, and TAL were considered positive when more than 1% expressed *Gal-3* (Figure 1, A, B, and C). Human papillary thyroid carcinoma tissue was used as a positive control due to its known positivity for *Gal-3* (Figure 1, D).

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%).

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.

Treatment protocol

Different platinum-based chemotherapy doublet protocols were administered to our patients. Among them, 33.93% (N=19) received a combination of carboplatin and paclitaxel, 28.57% (N=16) were treated with carboplatin and vinorelbine, 25% (N=14) received cisplatin and vinorelbine, 7.14% (N=4) received a combination of pemetrexed and carboplatin, 3.57% (N=2) were treated with gemcitabine and cisplatin, and 1.78% (N=1) received a treatment of pemetrexed and cisplatin.

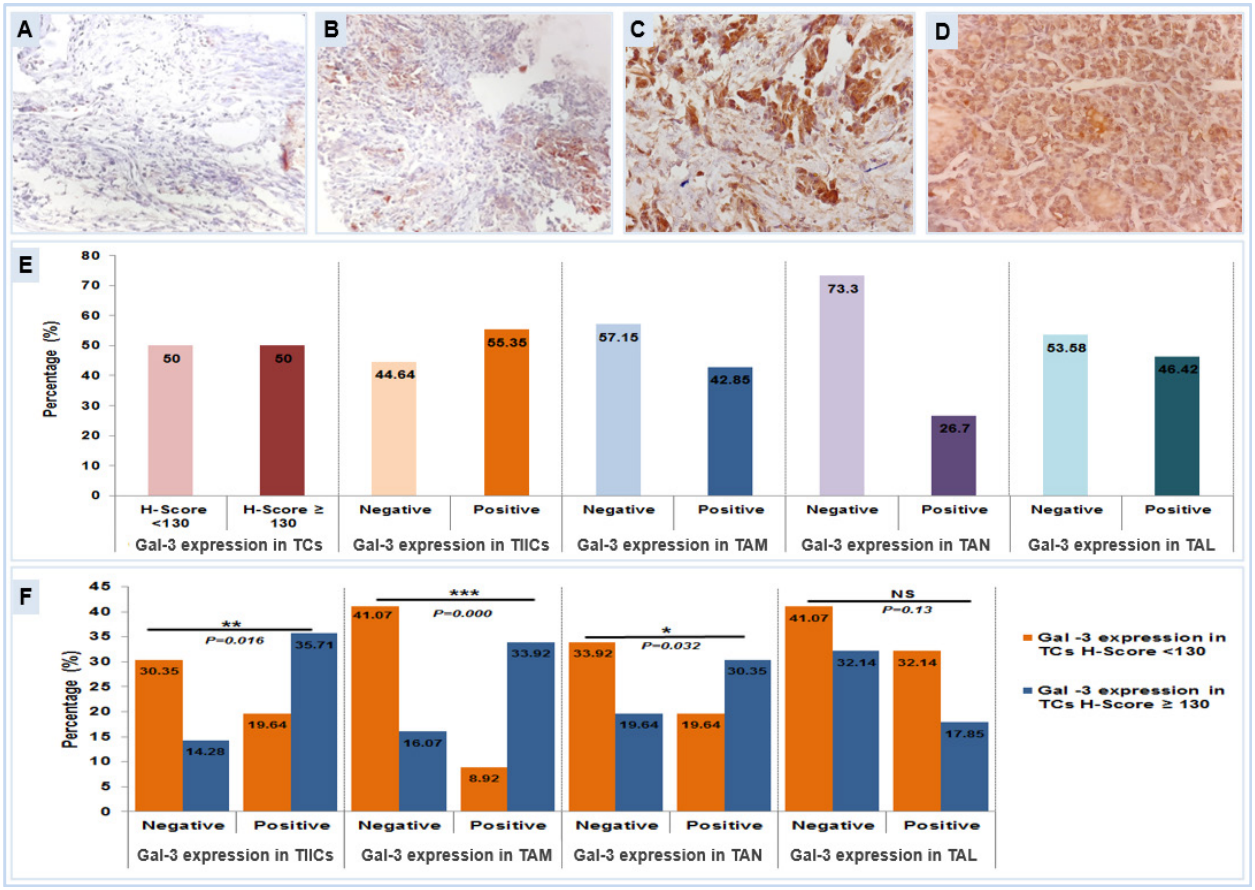

Figure 1. Tumoral and Immune Expression of *Gal-3* in NSCLC by IHC. A, B, C, Tumoral expression of *Gal-3* observed at different H-Scores (A, H-Score = 0; B, H-Score = 100; C, H-Score = 300); A, B, C, Positive or negative expression of *Gal-3* in TIICs, TAM, TAN, and TAL (A, negative expression; B, C, positive expression); D, Expression of *Gal-3* in human papillary thyroid carcinoma tissue (positive control); E, Frequency of *Gal-3* expression in TCs, TIICs, TAM, TAN, and TAL; F, Association between *Gal-3* expression in TCs and in TIICs, TAM, TAN, and TAL; *, significant difference; NS, non-significant difference.

Evaluation of Treatment Response

The effectiveness of the tumor response to treatment was evaluated by thoracic computed tomography (CT) according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. Progression-free survival (PFS) was defined from the first day of chemotherapy treatment to the day of physician-assessed disease progression or death from any cause. Overall survival (OS) was defined as the duration from chemotherapy treatment to death or the end of the study period.

Statistical Analysis

Statistical analysis was performed using SPSS software version 21. The Chi-square test was used to evaluate the association between tumor expression of *Gal-3* (Low: H-Score < 130; High: H-Score ≥ 130), clinicopathological characteristics, and *Gal-3* expression in TIICs, TAM, TAN, and TAL (Table 2, Figure 1). The Kaplan-Meier method was used to assess OS and PFS, and the log-rank test was used to calculate the significance of differences. Univariate and multivariate Cox regression analyses were also performed to explore the impact of clinical and pathological variables on PFS and OS of the patients. A P-value < 0.05 was considered statistically significant.

Results

Patient Characteristics

Table 1 shows the characteristics of the 56 patients included in this study, of whom 83.92% (N=47) were men. The median age of the patients was 67 years (ranging from 38 to 82 years), and 53.75% (N=30) were aged 67 years or older. 78.57% (N=44) of the patients had adenocarcinoma, 33.92% (N=19) had a performance status (PS) of 0, 80.35% (N=45) were smokers, and 82.14% (N=46) were diagnosed with stage IV disease. Additionally, 17.90% (N=10) of the patients had more than three metastatic organs involved (Metastatic Burden ≥ 3). Regarding the sites of metastasis, the results showed that 33.92% (N=19), 30.35% (N=17), 25% (N=14), 17.85% (N=10), 12.5% (N=7), 8.92% (N=5), and 7.14% (N=4) of the patients developed bone, contralateral lung, adrenal, pleural, brain, liver, and lymph node metastases, respectively. The molecular profile of the patients revealed that 23.21% (N = 13) exhibited high expression of *PD-L1*, no *EGFR* mutations were observed, and 1.78% (N = 1) of the patients had tumors positive for *ALK* rearrangement. The data concerning the response to chemotherapy treatment show that only 10.71% (N=6) of the patients exhibited a positive response to treatment, and 73.21%

Table 1. Characteristics of the Patients Recruited in This Study

Variables	Number (%)
Gender	
Men	47 (83.92)
Women	09 (16.07)
Sex ratio	5.2
Age at diagnosis (years)	
Median [Rank]	67 [38 -82]
< 67	26 (46.42)
≥ 67	30 (53.57)
Histological aspect	
Adenocarcinoma	44 (78.57)
Squamous Cell Carcinoma	12 (21.42)
PS	
PS 0	19 (33.93)
PS 1 -2	37(66.07)
Smoking status	
Yes	45 (80.35)
No	11 (19.64)
Stage of disease	
IIIc	10 (17.85)
IV	46 (82.14)
Metastatic burden	
< 3	46 (82.10)
≥ 3	10 (17.90)
Lymph node metastasis	
Yes	04 (07.14)
No	52 (92.85)
Liver metastasis	
Yes	05 (08.92)
No	51 (91.07)
Bone metastasis	
Yes	19 (33.92)
No	37 (66.07)
Brain metastasis	
Yes	07 (12.50)
No	49 (87.50)
Pleural metastasis	
Yes	10 (17.85)
No	46 (82.14)
Contralateral lung metastasis	
Yes	17 (30.35)
No	39 (69.64)
Adrenal metastasis	
Yes	14 (25.00)
No	42 (75.00)
Expression *PD-L1*	
TPS<1%	32 (57.14)
TPS : 1 – 49%	11 (19.64)
TPS : ≥ 50%	13 (23.21)
EGFR mutation status	
Wild type	56 (100.0)
Mutant	00 (00.00)
ALK Status	
Negative	55 (98.21)
Positive	01 (01.78)
Treatment response	
Complete response	00 (00.00)
Partial response	04 (07.14)
Stable disease	02 (03.57)
Progressive disease	50 (89.28)
Vital status	
Alive	15 (26.78)
Dead	41 (73.21)

ALK, Anaplastic lymphoma kinase; *EGFR,* Epidermal growth factor receptor; *PD-L1*, Programmed Cell Death Ligand 1

(N=41) had died.

Gal-3 Expression in TCs, TIICs, TAM, TAN, and TAL

Figure 1 shows the cytoplasmic expression of *Gal-3* with varying H-Scores of 0, 130, and 300 (Figure 1, A, B, and C), as well as the distribution of *Gal-3* expression in TCs, TIICs, TAM, TAN, and TAL (Figure 1, E). The results showed that 50% (N=28) of the patients had high *Gal-3* expression in TCs, 55.35% (N=31) in TIICs, 42.85% (N=24) in TAM, 26.70% (N=15) in TAN, and 46.42% (N=26) in TAL (Figure 1, E).

Association between Gal-3 Tumoral Expression and Patient Characteristics

The results regarding the association between *Gal-3* tumoral expression and patients' clinical and pathological characteristics are summarized in Table 2. In total, 56 patients were divided into 2 groups based on *Gal-3* tumoral expression: low expression (H-Score < 130) and high expression (H-Score ≥ 130). The latter was significantly associated with the absence of pleural metastases (P=0.036) (Table 2).

Association between Gal-3 Expression in TCs and in TIICs, TAM, TAN, and TAL

The results of simultaneous *Gal-3* expression by TCs (H-Score ≥ 130) and in TIICs, TAM, TAN, and TAL revealed the following data: 35.71% (N=20), 33.92% (N=19), 30.35% (N=17), and 17.85% (N=10), respectively (Figure 1, F). These findings highlight a significant association between high *Gal-3* expression in TCs and the presence of *Gal-3* (+) TIICs, *Gal-3* (+) TAM, and *Gal-3* (+) TAN compared to *Gal-3* (-) counterparts (35.71% vs 14.28%, p=0.016; 33.92% vs 16.07%, p=0.000; 30.35% vs 19.64%, p=0.032) (Figure 1, F).

Gal-3 Expression in TCs and its Association with Patient Survival

The impact of *Gal-3* tumoral expression (low vs

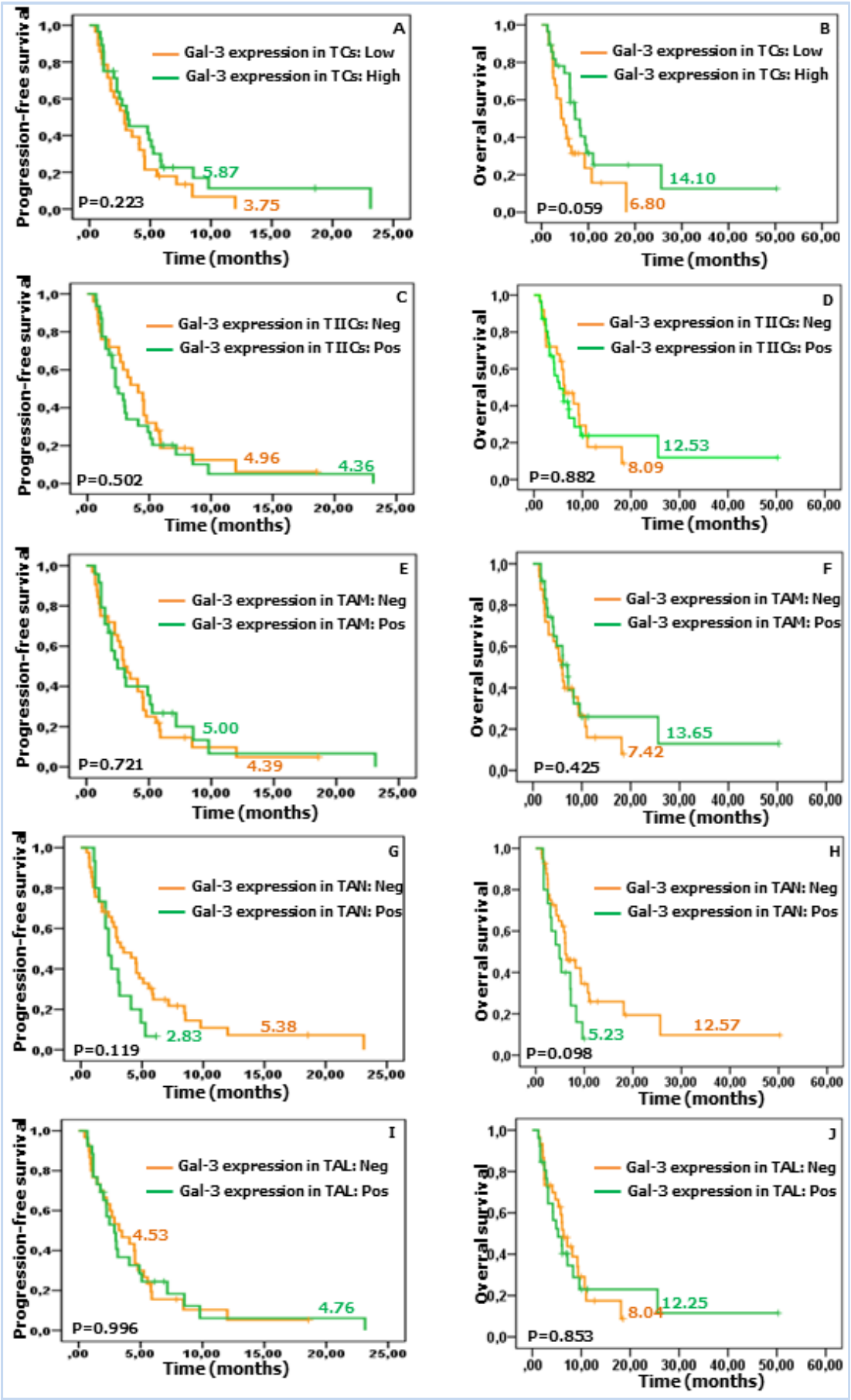

Figure 2. PFS and OS According to *Gal-3* IHC Status. A, B, *Gal-3* expression in TCs; C, D, *Gal-3* expression in TIICs; E, F, *Gal-3* expression in TAM; G, H, *Gal-3* expression in TAN; I, J, *Gal-3* expression in TAL. TCs, Tumor Cells; TIICs, Tumor-Infiltrating Immune Cells; TAM, Tumor-Associated Macrophages; TAN, Tumor-Associated Neutrophils; TAL, Tumor-Associated Lymphocytes.

Table 2. Associations between *Gal-3* Tumoral Expression andCharacteristics of Patients

Variables	Total	Gal-3 on Tumor cells N (%)		P value
	(N=56)	Low (H-Score <130)	High (H-Score ≥ 130)	
Gender				0.716
Men	47 (83.92)	23 (48.94)	24 (51.06)	
Women	09 (16.07)	05 (55.55)	04 (44.44)	
Age(years)				0.108
< 67	26 (46.42)	16 (61.54)	10 (38.46)	
≥ 67	30 (53.57)	12 (40.00)	18 (60.00)	
Histological aspect				0.515
Adenocarcinoma	44 (78.57)	23 (52.27)	21 (47.73)	
Squamous Cell Carcinoma	12 (21.42)	05 (41.67)	07 (58.33)	
PS				0.778
PS 0	19 (33.93)	10 (52.63)	09 (47.37)	
Ps 1 - 2	37(66.07)	18 (48.65)	19 (51.35)	
Smoking status				0.093
Yes	45 (80.35)	25 (55.55)	20 (44.44)	
No	11 (19.64)	03 (27.27)	08 (72.73)	
Stage of disease				0.099
IIIc	10 (17.85)	05 (50.00)	05 (50.00)	
IV	46 (82.14)	23 (50.00)	23 (50.00)	
Metastatic burden				0.778
< 3	46 (82.10)	22 (47.82)	24 (52.17)	
≥ 3	10 (17.90)	06 (60.00)	04 (40.00)	
Lymph node metastasis				0.099
Yes	04 (07.14)	02 (50.00)	02 (50.00)	
No	52 (92.85)	26 (50.00)	26 (50.00)	
Liver metastasis				0.147
Yes	05 (08.92)	04 (80.00)	01 (20.00)	
No	51 (91.07)	24 (47.06)	27 (52.94)	
Bone metastasis				0.397
Yes	19 (33.92)	08 (42.10)	11 (57.89)	
No	37 (66.07)	20 (54.05)	17 (45.94)	
Brain metastasis				0.686
Yes	07 (12.50)	03 (42.86)	04 (57.14)	
No	49 (87.50)	25 (51.02)	24 (48.98)	
Pleural metastasis				0.036*
Yes	10 (17.85)	08 (80.00)	02 (20.00)	
No	46 (82.14)	20 (43.48)	26 (56.52)	
Contralateral lung metastasis				0.771
Yes	17 (30.35)	09 (52.94)	08 (47.06)	
No	39 (69.64)	19 (48.72)	20 (51.28)	
Adrenal metastasis				0.537
Yes	14 (25.00)	06 (42.86)	08 (57.14)	
No	42 (75.00)	22 (52.38)	20 (47.62)	
Treatment response				0.388
Complete response	00 (00.00)	00 (00.00)	00 (00.00)	
Partial response	04 (07.14)	00 (00.00)	04 (100.0)	
Stable disease.	02 (03.57)	02 (100.0)	00 (00.00)	
Progressive disease	50 (89.28)	26 (52.00)	24 (48.00)	

H-score, Histological score; *PD-L1,* Programmed death-ligand 1; TIICs, tumor-infiltrating immune cells; *, Statistically significant at $p \leq 0.05$.

Table 2. Continued

Variables	Total	Gal-3 on Tumor cells N (%)		P value
	(N=56)	Low (H-Score <130)	High (H-Score ≥ 130)	
Vital status				0.365
Alive	15 (26.78)	06 (40.00)	09 (60.00)	
Dead	41 (73.21)	22 (53.65)	19 (46.34)	
PD-L1 expression				0.589
TPS <1%	32 (57.14)	17 (53.12)	15 (46.87)	
TPS ≥1%	24 (42.86)	11 (45.83)	13 (54.17)	
TIICs percentage				0.146
< 30 (1+)	17 (30.36)	11 (64.70)	06 (35.30)	
≥ 30 (2+ – 3+)	39 (69.64)	17 (43.59)	22 (56.41)	

H-score, Histological score; *PD-L1,* Programmed death-ligand 1; TIICs, tumor-infiltrating immune cells; *, Statistically significant at p ≤ 0.05.

high expression) on chemotherapy treatment response in terms of PFS and OS was studied (Figure 2). According to Kaplan-Meier analysis, the median PFS and OS were lower in patients with low *Gal-3* expression compared to those with high *Gal-3* expression. However, no significant difference was observed according to the Log-Rank test (PFS: 3.75 vs 5.87 months, p=0.223; OS: 6.80 vs 14.10 months, p=0.059), respectively.

Gal-3 Expression in TIICs, TAM, TAN, and TAL and its Association with Patient Survival

Patients were stratified based on positive and negative *Gal-3* expression in TIICs, TAM, TAN, and TAL. No significant difference was observed between *Gal-3* expression in TIICs, TAM, TAN, and TAL and PFS (TIICs - : 4.96 months vs TIICs + : 4.36 months, p=0.502; TAM - : 4.39 months vs TAM + : 5 months, p=0.721; TAN - : 2.83 months vs TAN + : 5.38 months, p=0.119; TAL - : 4.53 months vs TAL + : 4.76 months, p=0.996) and OS (TIICs - : 8.09 months vs TIICs + : 12.53 months, p=0.882; TAM - : 7.42 months vs TAM + : 13.65 months, p=0.425; TAN - : 5.23 months vs TAN + : 12.57 months, p=0.098; TAL - : 8.04 months vs TAL + : 12.25 months, p=0.853) (Figure 2).

Simultaneous Gal-3 Expression in TCs, TIICs, TAM, TAN, and TAL and its Association with Patient Survival

Our results showed that patients with high *Gal-3* expression in TCs and positive *Gal-3* expression either in TIICs or TAL had significantly prolonged OS compared to other respective subgroups (OS=16.81 months, p=0.007; OS=15.95 months, p=0.034) (Figure 3, D and H).

Univariate and Multivariate Analysis of PFS and OS

Univariate analysis of PFS and OS revealed several factors significantly associated with unfavorable PFS and OS, such as performance status (PS 1-2) (p=0.007) and presence of bone metastases (p=0.015) for PFS (Table 3), and histological type (squamous cell carcinoma) (p=0.044) and performance status (PS 1-2) (p=0.049) for OS (Table 3). However, in the multivariate analysis, some of these factors retained their significance as independent predictors of unfavorable PFS and OS, specifically performance status (PS 1-2) and presence of bone metastases (p=0.015 and p=0.029, respectively) for PFS (Table 3). Meanwhile, histological type (squamous cell carcinoma) was significantly associated with OS (p=0.000) (Table 4).

Discussion

Galectin-3 is one of the most studied galectins, highlighting its crucial role in various biological processes. Numerous reports have underscored its ability to regulate diverse functions ranging from cell proliferation and differentiation, mRNA splicing, induction of apoptosis, immune surveillance and inflammation, cell adhesion, angiogenesis, cancer progression to metastasis. The molecular mechanisms through which *Gal-3* regulates tumor invasion and metastasis are strongly influenced by the tumor microenvironment [14,15]. Its ubiquitous expression and multiple subcellular localizations confer diverse biological functions to galectin-3. Present in both the cytoplasm and nucleus, it can also be secreted via non-classical secretory pathways, demonstrating its versatility in cellular interactions [8].

In this study, our aim is to evaluate the predictive significance of cytoplasmic expression of *Gal-3* by tumor cells (TCs) and in tumor-infiltrating immune cells (TIICs), such as macrophages (TAM), neutrophils (TAN), and lymphocytes (TAL) in Moroccan patients with unresectable non-small cell lung cancer (NSCLC).

Statistical analysis revealed no significant association between *Gal-3* tumor expression (Low vs High) and clinicopathological characteristics, except for pleural metastases (p=0.036) (Table 2). A study by Saraswati Pokhare et al. (USA, 2022) revealed a significant association between *Gal-3* tumor expression and disease stage (p=0.012) on one hand, and nodal metastases (p=0.013) on the other [16].

The low expression of *Gal-3* observed in patients with pleural metastases could be attributed to *Gal-3* secretion into the extracellular milieu by TCs, which may modulate detachment of TCs from their primary site, thereby promoting migration and invasion [8]. *Gal-3* plays a crucial role in mediating homotypic aggregation of tumor cells invading blood vessels, as well as adhesion of these cells to endothelial cells, thereby maintaining them in

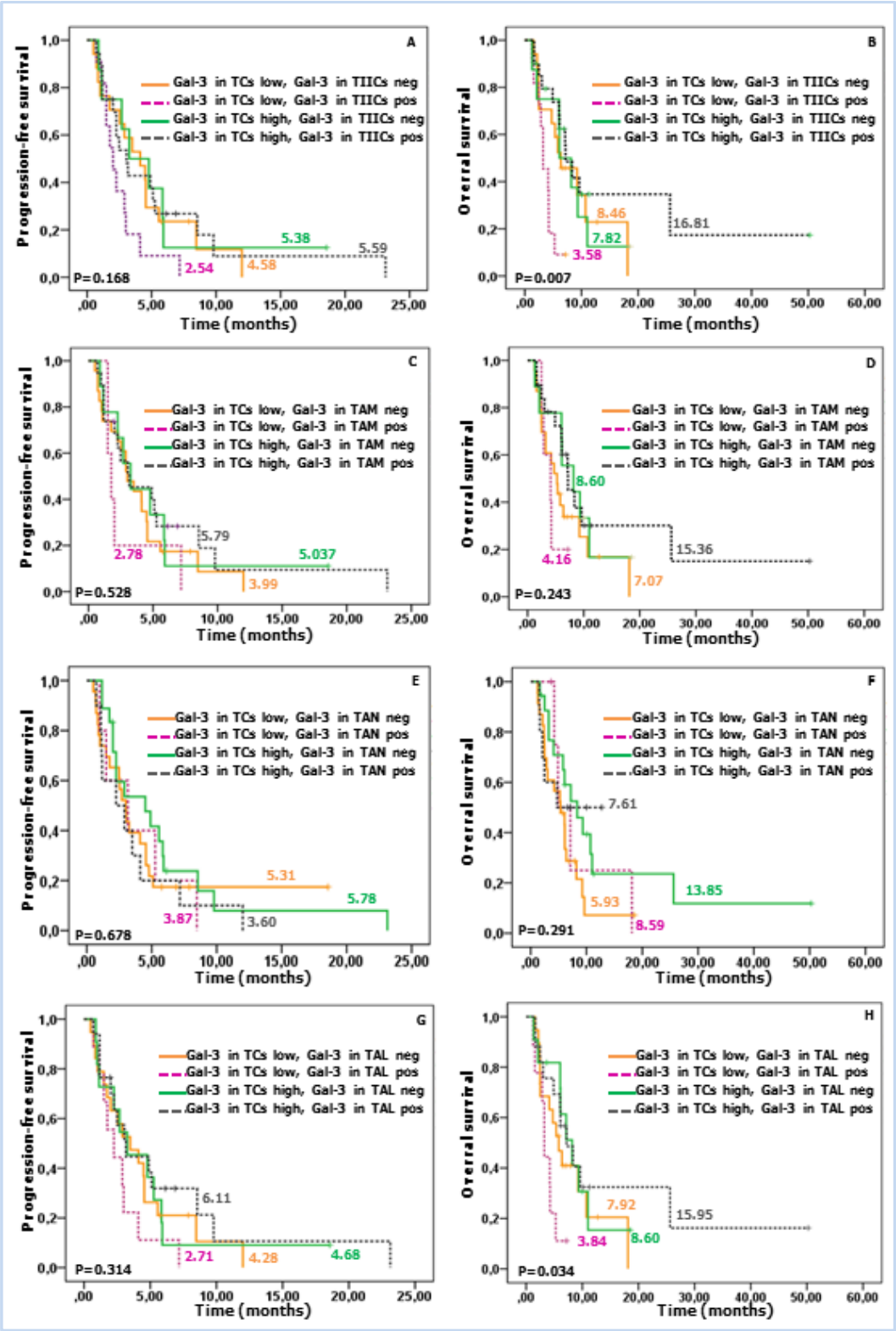

Figure 3. PFS and OS based on *Gal-3* Tumoral Expression combined with *Gal-3* expression in TIICs (A, B), TAM (C, D), TAN (E, F), and TAL (G, H) respectively. CTs, Tumor Cells; TIICs, Tumor-Infiltrating Immune Cells; TAM, Tumor-Associated Macrophages; TAN, Tumor-Associated Neutrophils; TAL, Tumor-Associated Lymphocytes.

circulation and enabling them to reach distant organ sites, notably the pleura [17-20]. However, further extensive studies are needed to fully validate these findings.

Our results also revealed a significant association between *Gal-3* expression in TCs and in TIICs, TAM, and TAN, respectively (p=0.016, p=0.000, and p=0.032) (Figure 1, F). These observations suggest that *Gal-3* expression in TIICs, TAM, and TAN could be induced by cytokines secreted by tumor cells during their proliferation. This hypothesis is supported by the fact that TCs express *Gal-3*, which in turn promotes their proliferation. According to the work of Alison Mackinnon et al. (UK, 2008), alternative activation of macrophages by IL-4 leads to accelerated biosynthesis and increased

Table 3. Analysis of Predictive Factors for PFS in Univariate and Multivariate Analysis.

Variables		Univariate analysis			Multivariate analysis		
	mPFS (months)	HR	95% CI	p-value	HR	95% CI	p-value
Gender		0.507	1.696 – 3.684	0.136		Not included	
Men vs Women (C.REF)	4.230 vs 7.022						
Age(years)		0.304	2.454 – 3.646	0.913		Not included	
< 67 (C.REF) vs ≥ 67	4.544 vs 4.757						
Histological aspect		0.996	0.738 – 4.642	0.246		Not included	
Adenocarcinoma (C.REF) vs Squamous CC	5.291 vs 3.158						
Performance Status (PS)		0.371	1.963 – 3.417	0.007*	3.706	1.286 – 10.678	0.015*
PS 0 (C.REF) vs PS 1-2	6.916 vs 3.603						
Smoking status		0.376	2.154 – 3.626	0.192		Not included	
Yes vs No (C.REF)	4.211 vs 7.864						
Stage of disease		1.376	1.854 – 7.246	0.596		Not included	
IIIc (C.REF) vs IV	4.934 vs 5.080						
Metastatic burden		0.348	1.577 – 2.943	0.663		Not included	
< 3 (C.REF) vs ≥ 3	4.750 vs 3.950						
Lymph node metastasis		0.500	1.280 – 3.240	0.946		Not included	
Yes vs No (C.REF)	3.280 vs 4.805						
Liver metastasis		0.843	0.000 – 3.134	0.071		Not included	
Yes vs No(C.REF)	2.150 vs 5.076						
Bone metastasis		0.909	1.256 – 4.821	0.015*	0.086	0.009 – 0.778	0.029*
Yes vs No (C.REF)	3.038 vs 5.426						
Brain metastasis		0.628	1.258 – 3.722	0.563		Not included	
Yes vs No (C.REF)	5.796 vs 4.431						
Pleural metastasis		2.070	0.000 – 7.557	0.692		Not included	
Yes vs No (C.REF)	3.522 vs 4.921						
Contralateral lung metastasis		1.307	0.329 – 5.451	0.242		Not included	
Yes vs No (C.REF)	8.397 vs 4.038						
Adrenal metastasis		1.721	0.000 – 5.903	0.992		Not included	
Yes vs No (C.REF)	4.516 vs 4.709						
PD-L1 expression in TCs		0.440	2.137 – 3.863	0.340		Not included	
<1% (C.REF) vs ≥1%	5.488 vs 3.561						
Gal-3 expression in TCs		0.487	2.206 – 4.114	0.223		Not included	
Low (<130) (C.REF) vs High (≥ 130)	3.755 vs 5.878						
TIICs percentage		0.450	2.007 – 3.773	0.278		Not included	
< 30% (C.REF) vs ≥ 30%	5.835 vs 4.186						
Gal-3 expression on TIICS		0.473	1.564 – 3.416	0.502		Not included	
Negative (C.REF) vs Positive	4.967 vs 4.365						
Gal-3 expression in TAM		0.810	0.903 – 4.077	0.721		Not included	
Negative (C.REF) vs Positive	4.390 vs 5.001						
Gal-3 expression in TAN		0.604	1.707 – 4.073	0.996		Not included	
Negative (C.REF) vs Positive	5.381 vs 2.838						
Gal-3 expression in TAL		0.309	1.654 – 2.866	0.119		Not included	
Negative (C.REF) vs Positive	4.539 vs 4.762						

C.REF, Category reference; mPFS, Mean progression-free survival; TAM, Tumor-associated macrophages; TAN, Tumor-associated neutrophils; TAL, Tumor-associated lymphocytes; TCs, Tumor cells; TIICs, Tumor-infiltrating immune cells; *, Statistically significant at $p \leq 0.05$.

Table 4. Analysis of Predictive Factors for OS in Univariate and Multivariate Analysis.

Variables	Univariate analysis				Multivariate analysis		
	mOS (months)	HR	95% CI	p-value	HR	95% CI	p-value
Gender		0.567	4.938 – 1.162	0.129		Not included	
Men vs Women (C.REF)	9.557 vs 12.007						
Age(years)		1.774	2.633 – 9.587	0.707		Not included	
< 67 (C.REF) vs ≥ 67	7.771 vs 10.289						
Histological aspect		0.970	2.989 – 6.791	0.044*	32.627	4.782 – 222.58	0.000*
Adenocarcinoma (C.REF) vs Squamous CC	12.867 vs 5.183						
Performance Status (PS)		0.848	3.688 – 7.012	0.049*	1.567	0.495 – 4.955	0.445
PS 0 (C.REF) vs PS 1-2	15.553 vs 6.390						
Smoking status		0.765	4.300 – 7.300	0.283		Not included	
Yes vs No (C.REF)	9.902 vs 8.886						
Stage of disease		1.119	3.857 – 8.243	0.584		Not included	
IIIc (C.REF) vs IV	13.279 vs 8.882						
Metastatic burden		2.667	0.22 – 16.680	0.263		Not included	
< 3 (C.REF) vs ≥ 3	11.450 vs 5.930						
Lymph node metastasis		0.605	2.034 – 4.406	0.413		Not included	
Yes vs No (C.REF)	4.338 vs 11.045						
Liver metastasis		1.939	0.430 – 8.030	0.730		Not included	
Yes vs No (C.REF)	5.866 vs 11.034						
Bone metastasis		3.161	0.000 – 11.546	0.173		Not included	
Yes vs No (C.REF)	6.287 vs 12.689						
Brain metastasis		0.262	2.707 – 3.733	0.884		Not included	
Yes vs No (C.REF)	7.873 vs 10.022						
Pleural metastasis		2.948	0.000 – 10.507	0.322		Not included	
Yes vs No (C.REF)	5.687 vs 11.588						
Contralateral lung metastasis		0.918	5.390 – 8.990	0.429		Not included	
Yes vs No (C.REF)	10.347 vs 11.419						
Adrenal metastasis		2.413	0.520 – 9.980	0.502		Not included	
Yes vs No (C.REF)	6.905 vs 11.300						
PD-L1 expression in TCs		1.406	2.595 – 8.105	0.191		Not included	
<1% (C.REF) vs ≥1%	10.303 vs 10.035						
Gal-3 expression in TCs		1.240	4.759 – 9.621	0.059		Not included	
Low (<130) (C.REF) vs High (≥ 130)	6.803 vs 14.102						
TIICs percentage		1.395	3.065 – 8.535	0.353		Not included	
< 30% (C.REF) vs ≥ 30%	8.827 vs 10.108						
Gal-3 expression on TIICS		1.233	2.833 – 7.667	0.882		Not included	
Negative (C.REF) vs Positive	8.092 vs 12.530						
Gal-3 expression in TAM		0.737	5.675 – 8.565	0.425		Not included	
Negative (C.REF) vs Positive	07.419 vs 13.650						
Gal-3 expression in TAN		1.504	2.301 – 8.199	0.853		Not included	
Negative (C.REF) vs Positive	12.575 vs 5.233						
Gal-3 expression in TAL		1.307	2.327 – 7.453	0.098		Not included	
Negative (C.REF) vs Positive	8.041 vs 12.250						

C.REF, Category reference; mOS, Mean overall survival; TAM, Tumor-associated macrophages; TAN, Tumor-associated neutrophils; TAL, Tumor-associated lymphocytes; TCs, Tumor cells; TIICs, Tumor-infiltrating immune cells; *, Statistically significant at $p \leq 0.05$.

secretion of *Gal-3* [21].

Finally, our study shows that chemotherapy efficacy depends on simultaneous expression of *Gal-3* in TCs and in TIICs and TAL (Figure 3). Several subgroups showed a significantly favorable response in terms of OS, especially those with high *Gal-3* expression in TCs and positive *Gal-3* expression in TIICs and TAL compared to other subgroups, respectively (OS = 16.81 months, p=0.007; OS = 15.95 months, p=0.034) (Figure 3, B and H).

A study by Seiichiro Kusuhara et al. (Japan, 2021) in patients with resectable NSCLC treated with platinum-based adjuvant chemotherapy showed that high *Gal-3* expression in TCs was significantly associated with recurrence-free survival (p=0.001) and unfavorable OS (p=0.015) [12]. Furthermore, Matthias Ilmer and his team's study (USA, 2016) in breast cancer patients with lymph node invasion revealed that those with high *Gal-3* expression in TCs had significantly better recurrence-free survival and OS than those with low *Gal-3* expression in TCs, respectively (p=0.034, p=0.019) [22].

The role of *Gal-3* in lymphocytes is predominantly determined by its localization. Extracellular presence of *Gal-3*, secreted by TCs, blocks NK cell functions, thus favoring tumor evasion from the host immune system and facilitating its growth [23]. Additionally, it triggers apoptosis of CD8+ T lymphocytes and negatively modulates the expression of the T cell receptor (TCR). It has also been observed that extracellular *Gal-3* binds to lymphocyte activation gene 3 (LAG-3) on CD8 T lymphocytes, potentially leading to suppression of their function. These mechanisms could partly explain the results shown in Figure 3H regarding the group *Gal-3* in TCs low, *Gal-3* in TAL neg and the group of patients *Gal-3* in TCs low, *Gal-3* in TAL pos (Figure 3, H). Thus, it is conceivable that TCs with low expression of *Gal-3* secrete this protein into the tumor microenvironment, thereby inhibiting NK cell activities and promoting survival of T lymphocytes. In contrast, intracellular *Gal-3* seems to play a crucial role in apoptosis inhibition and is involved in promoting cell growth, as well as enhancing TCR signaling (Figure 3, H) [24-26]. This could explain our results regarding the group of patients *Gal-3* high in TCs, *Gal-3* in TAL pos (Figure 3, H). Therefore, it is plausible that TCs with high expression of *Gal-3* retain this protein intracellularly.

The difference in OS between different patients 'groups highlights the potentially critical role of *Gal-3* expression in TAL in the prognosis of NSCLC patients. Due to the crucial role of the TME in promoting tumor progression and metastasis, modulation of the TME to reduce immunosuppression and promote immune system activation has become a major focus in cancer immunotherapy. *Gal-3*, heavily involved in enhancing tumor growth, metastasis, and immune suppression, has emerged as a promising target for this therapeutic approach [5]. The use of *Gal-3* inhibitors in the group of patients *Gal-3* in TCs low, *Gal-3* in TAL pos or the combination of *Gal-3* inhibitors with T cell agonists for the group of patients *Gal-3* in TCs low, *Gal-3* in TAL neg, or even the use of T cell agonists for the group of patients *Gal-3* in TCs high, *Gal-3* in TAL neg, could potentially enhance anti-tumor immunity and promote tumor regression.

Moreover, a study by L. Vuong and colleagues (UK, 2019) demonstrated that the *Gal-3* inhibitor (GB 1107) effectively reduced lung adenocarcinoma growth in both humans and mice, while blocking metastasis in a xenograft model [9]. Findings from the study by H. Zhang and his team (China, 2021) also highlighted the benefits of administering a *Gal-3* inhibitor, by enhancing T cell infiltration and granzyme B release in tumors [13]. Lastly, research conducted on murine tumor models (sarcoma, breast carcinoma, and prostate adenocarcinoma) by E. Sturgillun and colleagues (USA, 2020) on *Gal-3* inhibition with belapectin combined with anti-OX40 treatment reprograms the tumor microenvironment to promote anti-tumor immunity, significantly improving survival in tumor-bearing mice through a mechanism dependent on CD8+ T lymphocytes. Additionally, this therapy increases the density of CD8+ T lymphocytes in tumors and reduces the frequency and proliferation of FOXP3+ CD4+ regulatory T lymphocytes [27]. These various studies underscore the growing importance of therapies targeting *Gal-3* in cancer treatment, opening new avenues in the fight against this disease. Our study has certain limitations that should be highlighted to enrich the critical analysis and guide future research. First, the relatively small sample size of 56 NSCLC patients treated with platinum-based chemotherapy may limit the generalizability of the results, requiring studies on larger cohorts to strengthen their robustness. Additionally, the evaluation of *Gal-3* expression by immunohistochemistry, a semi-quantitative method, may be subject to inter-observer variability; the use of complementary techniques, such as quantitative PCR or Western blot, could refine these measurements. Furthermore, the absence of a control group that did not receive chemotherapy prevents us from making a direct comparison of the impact of *Gal-3* expression on survival. Similarly, although our study identified an association between *Gal-3* expression and treatment response, the underlying biological mechanisms remain unclear, warranting further in vitro and in vivo investigations. Moreover, as some patients may have benefited from additional therapeutic approaches, the potential impact of these adjuvant treatments should be considered to refine our conclusions. To overcome these limitations, prospective multicenter studies on larger populations and an in-depth exploration of the interactions between *Gal-3* and the tumor microenvironment could open new avenues for improving NSCLC management.

In conclusion, our study highlights several aspects of *Gal-3* expression in NSCLC. Firstly, high *Gal-3* expression is significantly correlated with absence of pleural metastases, suggesting its potential as a biomarker for predicting metastatic risk in these patients. Furthermore, significantly higher co-expression of *Gal-3* on TCs in the presence of various components of the TME, including TIICs, TAM, and TAN, underscores the potential role of *Gal-3* in modulating interactions between TCs and immune components of the microenvironment, with important implications for tumor progression and treatment response. Finally, our results show

significant differences in OS among patients based on *Gal-3* expression on TCs and different components of the TME. Patients with high *Gal-3* expression on TCs associated with positive *Gal-3* expression on TIICs or TAL have significantly prolonged OS compared to other subgroups, highlighting the predictive importance of *Gal-3* in NSCLC.

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. Abderahman Mellouki: Formal analysis, Data collection. Fathi sofia and Charkaoui Meryeme: Formal analysis, Visualization. Acharki Abdelkader, Benchakroun Nadia and Sahraoui Souha: 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.

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).

Conflict of Interest

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

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