



## PD-L1 testing and clinical management of newly diagnosed metastatic non-small cell lung cancer in Spain: MOREL study

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**Aim:** To describe the clinical management and PD-L1 testing of patients with newly diagnosed stage IV non-small cell lung cancer (NSCLC) without driver mutations in Spain. **Methods:** Multicenter, retrospective study. **Results:** Among 297 evaluated patients, 89.2% received systemic treatment for stage IV disease, of whom 53.6% received platinum doublet therapy, 26.8% immunotherapy as monotherapy and 14.7% immunotherapy + chemotherapy, with 9.4% receiving treatment as part of a clinical trial. Treatment was initiated 1 month after histological diagnosis, with PD-L1 test results available in most cases (92.6%). PD-L1 testing was performed in 287 patients, 95.1% by in-house tests, mostly with the 22C3 pharmDx assay. The factor most strongly associated with treatment selection was, as expected, the expression of PD-L1. **Conclusion:** PD-L1 testing is implemented in clinical practice and seems to guide treatment decisions in patients with NSCLC in Spain.

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Lung cancer is the leading cause of cancer deaths worldwide, with 1.8 million deaths globally in 2020 [1]. The incidence and mortality rate of non-small cell lung cancer (NSCLC) in Spain are 61.2 and 47.6 per 100,000 inhabitants, respectively [2,3]. NSCLC is the most common type of lung cancer, accounting for 84% of all lung cancer diagnoses in general. Most patients with NSCLC are diagnosed with an advanced, unresectable disease. Histological diagnosis and molecular testing for individual patient management must often be made on small biopsy and/or cytology samples [4], which is a challenge from the point of view of clinical practice.

Among several driver genetic alterations found in NSCLC, *EGFR* and *BRAF* mutations as well as *ALK* and *ROS1* rearrangements are successfully targetable with approved drugs. Identification of such biomarkers can determine the selection of the most appropriate individualized therapy for this disease [5,6]. However, most patients with NSCLC do not harbor these oncogenic drivers, and for these patients, treatment options have been limited to platinum-based combination therapy for the last two decades [7].

Immune checkpoint inhibitors are approved immunotherapy strategies in cancer. These agents target immune pathways that intensify the body's ability to recognize and destroy tumor cells. PD-1 is a cell surface receptor on

certain lymphocytes and plays a critical role in tumor immune escape [8]. PD-L1, the main ligand of PD-1, is upregulated in several types of tumors, including NSCLC. PD-L1 triggers negative costimulatory signals and binds PD-1 to decrease cellular immune responses by inducing T-cell apoptosis [9,10]. Four PD-1/PD-L1 antibodies (pembrolizumab, atezolizumab, nivolumab and cemiplimab) are currently approved for the treatment of metastatic NSCLC, both as monotherapy and in combination with other treatments [11]. The KEYNOTE-024 study showed that pembrolizumab is more effective than standard platinum doublet chemotherapy in patients with untreated, advanced NSCLC with high expression of PD-L1 [12]. Likewise, IMPOWER 110 and EMPOWER-Lung 1 studies demonstrated the superiority of atezolizumab or cemiplimab versus platinum doublet chemotherapy [13,14]. In addition, the results of KEYNOTE-042 and CheckMate227 (nivolumab + ipilimumab) demonstrated the benefit of immunotherapy versus platinum doublets in tumors with low PD-L1 expression [15,16]. Furthermore, results from KEYNOTE-189, KEYNOTE-407, IMpower 150, IMpower 130 and CheckMate9LA showed better outcomes in all PD-L1 subgroups receiving immunotherapy in combination with platinum doublet chemotherapy and therefore extended the benefit to patients with non-PD-L1-expressing tumors [17–21].

Nonetheless, responsiveness to anti-PD-1/PD-L1 inhibitors may be predicted by the expression of PD-L1 on tumor cells and/or tumor-infiltrating immune cells. Indeed, PD-L1 expression testing is recommended as a part of the diagnostic evaluation of patients with advanced NSCLC in order to select treatment with anti-PD-1/PD-L1 immunotherapy [22]. The current landscape of commercially available PD-L1 diagnostic tests includes several antibody-based platforms (DAKO 22C3, DAKO 28-8, VENTANA SP263 and VENTANA SP142), with different cutoffs for PD-L1 positivity. The experience and expertise among pathology laboratories evaluating PD-L1 is also variable [23].

In Spain, there is a lag period for access to new therapies and new indications after EMA approval. In most institutions, the delay ranges between 9 and 26 months, related to price and reimbursement negotiation processes [24]. This fact could have an influence on treatment selection.

The aims of the present retrospective study are to analyze the clinical management of newly diagnosed stage IV NSCLC patients as well as to characterize and describe how PD-L1 expression testing is performed in different centers in Spain.

## Patients & methods

### Study design

This was a multicenter, observational, retrospective, longitudinal follow-up study that described patterns of clinical management of newly diagnosed stage IV NSCLC patients with no prior therapy and neither tumor-activating *EGFR* or *BRAF* mutations nor *ALK* or *ROS1* rearrangements. The study aimed to describe how PD-L1 expression was determined and to what extent the results influenced treatment.

### Patients

The study enrolled men and women  $\geq 18$  years old, who were diagnosed with stage IV NSCLC from 1 January to 31 December 2019 in ten Spanish centers. Patients suitable for definitive local treatment were not included in the study. Identified patients were enrolled in the study and their medical history data were collected retrospectively from diagnosis of metastatic disease until the day of inclusion in the study. Therefore the observation period occurred before the inclusion of subjects.

### Study aims

The primary objectives of the study were to describe patterns of clinical management of newly diagnosed stage IV NSCLC patients, with neither tumor-activating *EGFR* or *BRAF* mutations nor *ALK* or *ROS1* rearrangements, and to characterize and describe how PD-L1 expression testing is performed in ten representative centers in Spain.

The secondary objectives were to describe the clinical management of first-line stage IV NSCLC patients with different clinicopathological characteristics; the clinical management of these patients based on PD-L1 status; the differences in turnaround timelines based on the type of institution where PD-L1 testing was performed (in-house vs centralized laboratory); and the correlation of PD-L1 expression with patient demographics and clinicopathological characteristics in order to identify independent factors to guide treatment decisions in the first-line setting.

The exploratory objectives included the description of PD-L1 tumor expression status in the study population and PD-L1 tumor expression status according to the diagnostic method and tumor sample used.

## Variables analyzed

### *Baseline demographic & clinical characteristics*

The demographic and clinical data collected were: age, gender and smoking habits; description of Eastern Cooperative Oncology Group (ECOG) status at the time of treatment commencement for stage IV NSCLC; stage IV NSCLC status (M1a, M1b, M1c); description of histological subtype of NSCLC (squamous or nonsquamous); description of sites of metastases (brain, bone, liver, lung, adrenal glands, other); description, number and percentage of patients with autoimmune diseases (Crohn's disease, ulcerative colitis, psoriasis, myasthenia gravis, rheumatoid arthritis, Guillain–Barré syndrome, hemolytic anemia, other); description of clinical history of an autoimmune disease (active or inactive at the time of diagnosis of the stage IV NSCLC); number and percentage of patients with viral infections (HIV, hepatitis B, hepatitis C, other); number and percentage of comorbidities (renal impairment, hepatic impairment, cardiac and lung disease, others).

### *Treatment description*

The data collected were: number and percentage of patients who received systemic treatment, and description of treatments received; number and percentage of patients who received other treatments, and description of treatments received; number and percentage of patients who received corticosteroid treatments within 1 week before starting cancer treatment; and description of doses received.

### *PD-L1 testing*

The data collected were: number and percentage of patients with diagnosis of biomarkers for NSCLC; description of reasons why the biomarker analysis was not performed (sample not available, not enough sample, other); description of institutions that performed the PD-L1 testing for NSCLC (in-house, centralized laboratory); description of biomarkers performed and results; description of material used for PD-L1 testing (biopsy/cytology); description of biopsy (site and method); description of PD-L1 antibody and platform used; time elapsed between sample collection and PD-L1 testing request; time elapsed until PD-L1 test results; number and percentage of PD-L1 expression (<1, 1–49 and  $\geq 50\%$ , as well as not evaluable PD-L1); number and percentage of PD-L1 positivity according to the diagnostic method; and tumor sample and antibody used.

PD-L1 expression was assessed according to the specific protocols detailed by manufacturers [25–28]. Tumor proportion score – that is, the percentage of viable tumor cells showing partial or complete membrane staining at any intensity – was assessed for 22C3, 28–8 and SP263. For SP142, the ‘tumor cells’ score was defined as the percentage of tumor cells with the presence of PD-L1 membrane staining of any intensity. In addition, the ‘tumor-infiltrating immune cells’ score was defined as the proportion of tumor area, including associated intratumoral and contiguous peritumoral stroma, occupied by PD-L1-staining immune cells of any intensity.

## Statistical considerations

Ten representative Spanish sites, selected based on their experience in managing stage IV NSCLC, participated in this study. Data from approximately 300 patients with newly diagnosed stage IV NSCLC with no tumor-activating *EGFR* or *BRAF* mutation nor *ALK* or *ROS1* rearrangements were collected in order to allow us to describe patterns of clinical management in our population. For any characteristic to be considered relevant, it had to occur in at least 5% of patients with 95% CI and a precision of  $\pm 2.5\%$ . Given the descriptive nature of the study, mean, median, standard deviation (SD), 25th and 75th percentiles, minimum and maximum and mean 95% CIs were calculated for continuous variables, and absolute and relative frequencies for categorical variables (missing values were not considered for the calculation of percentages). The absolute frequency of missing data for each variable was described separately (due to the rounding of the values, the partial sum might not match with its total sum).

Comparisons between groups were performed by means of parametric (Student *t* test) or non-parametric tests (Mann–Whitney U test or Kruskal–Wallis test), based on the characteristics of the study variables, for continuous variables. For categorical variables, the  $\chi$ -squared test or Fisher's exact test were applied. Multivariate analyses were performed by means of a logistic regression (inputting into the model all factors with  $p < 0.10$  in a univariate analysis) in order to study the factors related to PD-L1 status and factors related to the most frequent systemic treatments (platinum doublet chemotherapy, immunotherapy monotherapy and platinum doublet–immunotherapy).

Statistical significance was indicated by  $p < 0.05$ .

All statistical analyses were performed using the SAS (version 9.4) statistical package (SAS Institute, Inc., NC, USA).

## Results

### Patient characteristics

From January 2020 to July 2020, 308 patients diagnosed with stage IV NSCLC between 1 January and 31 December 2019 were included in the study. Of those, 297 met all the inclusion criteria and none of the exclusion criteria. The median age was 67 years (range: 37–89), 79.8% of the patients were aged  $\leq 75$  years, and 77.1% of patients were male. Half of the patients were former smokers (50.2%), 38.7% were current smokers, and 10.1% never smoked. In smoker patients, the mean number of years' smoking was 42.8 (SD: 10.8) and the number of cigarettes per day was 24.8 (SD: 13.1). In former smokers, the mean of years' smoking was 37.3 (SD: 12.6) and the number of cigarettes per day was 30.2 (SD: 15.2). The percentages of patients with ECOG score 0, 1 or  $\geq 2$  were 27.8, 46.4 and 25.8%, respectively. A total of 17.8% of patients had squamous NSCLC and 82.2% nonsquamous NSCLC. The main sites of metastases were lung, bone and brain (42.4, 39.1 and 21.9%, respectively). More than half of the patients (58.2%) reported comorbidities, with cardiac disorders being the most frequently reported (21.2%), followed by respiratory (14.5%), vascular (14.5%) and metabolic and nutritional disorders (13.1%). Renal and urinary disorders were reported in 4.7% of patients. At the time of diagnosis of the stage IV NSCLC, there were five patients with autoimmune disease (four of them with active disease) and seven with a viral infection (hepatitis B, hepatitis C or HIV (Table 1).

### Treatment description

A total of 265 patients (89.2%) received systemic treatment. The median time from histological diagnosis to the beginning of systemic treatment was 31 days (25th/75th percentiles: 22/43), and in most cases PD-L1 testing results were available at that time (92.6%). Of those, 25 patients (9.4%) participated in a clinical trial. With regard to the treatment received, 117 patients (44.2%) received immunotherapy as first-line treatment: 71 (26.8%) patients in monotherapy, 39 (14.7%) in combination with platinum doublets, six (2.3%) combined with another immunotherapy drug and one (0.4%) in combination with an antiangiogenic agent. In addition, 142 patients (53.6%) received platinum doublet chemotherapy, five (1.9%) received mono-chemotherapy and one received a combination with an antiangiogenic agent (0.4%) (Supplementary Table 1). Almost one-third of all patients (28.3%) received complementary treatments for stage IV disease in the form of radiotherapy (79 patients; 94.0%) or surgery (8 patients; 9.5%). Nearly half of the patients (49.1%) received systemic corticosteroids 1 week before or during the starting of cancer treatment, and more than two-thirds of them (70.4%) as standard of care for their chemotherapy treatment. Dexamethasone was the most frequent corticosteroid (82.1%) in patients who received it for any reason different than chemotherapy treatment, followed by prednisone (17.9%).

### Characterization & description of PD-L1 expression

Biomarker analysis was performed in 287 patients (96.6%), including PD-L1 testing in all patients. In most cases the biopsy was obtained from the primary tumor (65%). With respect to the diagnostic method, 72.3% of the samples were from core/bronchial biopsy, 14.9% from cytology, 9% were surgical resection specimens, 2.7% were lymph node biopsies and 1.1% were obtained from biopsy of pleural or soft tissue metastasis. Of the cases diagnosed by cytology, 82.1% had a cell block while the remaining samples were processed as cytological smears (17.9%). Most of the institutions performed PD-L1 testing in-house (95.1%), with the remaining 4.9% of tests done in a centralized laboratory. The most common assay used to assess PD-L1 was 22C3 pharmDx (54.7%), followed by 28-8 pharmDx (17.8%), SP263 pharmDx (13.2%) and SP263 laboratory-developed test (13.6%); SP142 antibody was only used in one patient. Tonsil tissue was used as a control in 64.8% of the cases, followed by cell lines (14%), lung tissue (14%) and placenta tissue (6.6%). A total of 58.9% of the patients showed PD-L1 expression  $\geq 1\%$  (26.8% with values of 1–49%, and 32.1% with values  $\geq 50\%$ ) and 40.1% were PD-L1 negative (PD-L1 levels of  $< 1\%$ ). PD-L1 was not evaluable in three patients due to sample unsuitability (Table 2).

### Clinical management regarding clinicopathological characteristics

No significant differences were observed in the pathological diagnostic variables (site of biopsy, method of diagnosis, sample used or cytology format) with regards to sex, smoking history, ECOG status or histopathological subtype. Only age was statistically associated with the type of tissue acquisition ( $p = 0.0297$ ): in patients aged  $> 75$  years, biopsies from the primary tumor were more frequent compared with younger patients (77.2 vs 61.8%, respectively). The percentage of biopsies in primary sites was higher for brain metastases (79.7%) than for bone or liver metastases (62.4 and 55.8%, respectively).

Table 1. Baseline demographic and clinical characteristics.

Characteristic	n (%)
<b>Total patients</b>	<b>297</b>
<b>Gender</b>	
Male	229 (77.1)
Female	68 (22.9)
<b>Age</b>	
Mean (SD)	67.1 (9.9)
≤75 years	237 (79.8)
>75 years	60 (20.2)
<b>Cigarette smoking history</b>	
Never smoker (≤100 cigarettes/lifetime)	30 (10.1)
Former smoker (≥1 year)	149 (50.2)
Smoker	115 (38.7)
Unknown	3 (1)
Years smoking, mean (SD)	42.8 (10.8)
<b>ECOG (n = 295)</b>	
0	82 (27.8)
1	137 (46.4)
≥2	76 (25.8)
<b>Status of stage IV (n = 296)</b>	
0	82 (27.8)
1	137 (46.4)
≥2	76 (25.8)
<b>Histological subtype</b>	
Squamous	53 (17.8)
Non squamous	244 (82.2)
<b>Site of metastases<sup>†</sup></b>	
Lung	126 (42.4)
Bone	116 (39.1)
Adrenal glands	61 (20.5)
Brain (active)	52 (17.5)
Liver	45 (15.2)
Pleura	36 (12.1)
Lymph nodes	32 (10.8)
Brain (not active)	13 (4.4)
Muscular	12 (4)
Other <sup>‡</sup>	32 (10.8)
<b>Autoimmune disease at time of diagnosis</b>	
Crohn's disease	2 (0.7)
Ulcerative colitis	1 (0.3)
Rheumatoid arthritis	2 (0.7)
<b>Viral infections</b>	
HIV	1 (0.3)
Hepatitis B	3 (1)
Hepatitis C	3 (1)

<sup>†</sup>A single patient might report more than one localization of metastases.  
<sup>‡</sup>Other localization of metastases (n = 32): peritoneum n = 7; soft tissue n = 5; spleen n = 3; kidney n = 3; pancreas n = 2; pericardium n = 2; celiac trunk n = 1; kidney/small intestine n = 1; mediastinum n = 1; pancreas/intestine n = 1; peritoneum and soft tissue n = 1; pancreas n = 1; skin and peritoneum n = 1; small intestine n = 1; soft tissue and hypothalamic pituitary n = 1; thyroid n = 1.  
ECOG: Eastern Co-operative Oncology Group; SD: Standard deviation.

Table 2. PD-L1 expression: characterization and description.	
Characteristic	n (%)
PD-L1 expression	287 (100.0)
<1%	115 (40.1)
1–49%	77 (26.8)
≥50%	92 (32.1)
Not evaluable	3 (1.0)
Site of biopsy	274 (100.0) <sup>#</sup>
Primary	178 (65.0)
Metastatic	96 (35.0)
Method of diagnosis	188 (100.0) <sup>#</sup>
Surgical resection specimen	17 (9.0)
Lymph node biopsy	5 (2.7)
Core/bronchial biopsy	136 (72.3)
Cytology	28 (14.9)
Other <sup>†</sup>	2 (1.1)
Cytology method <sup>‡</sup>	28 (100.0) <sup>#</sup>
Bronchial wash	4 (14.3)
Pleural effusion	3 (10.7)
Fine needle aspiration	21 (75.0)
Cytology sample format	28 (100.0) <sup>#</sup>
Cell block	23 (82.1)
Cytological smear	5 (17.9)
Fixation agent	287 (100.0)
Alcohol	1 (0.3)
Formalin	285 (99.3)
Other <sup>§</sup>	1 (0.3)
Control	287 (100.0)
Tonsil	186 (64.8)
Placenta	19 (6.6)
Other <sup>¶</sup>	82 (28.6)
PD-L1 antibody used	287 (100)
PharmDx kit	245 (85.3)
22C3 pharmDx kit	157 (64)
28–8 pharmDx kit	51(20.8)
SP263 pharmDx kit	38 (15.5)
SP142 pharmDx kit	1 (0.40)
Free antibody	42 (16.6)
22C3 laboratory-developed test option	1 (2.4)
28–8 laboratory-developed test option	1 (2.4)
SP263 laboratory-developed test option	39 (92.8)
SP142 laboratory-developed test option	1 (2.4)
Unknown	1 (0.3)

<sup>†</sup>List of other methods of biopsy (n = 2): biopsy of pleural metastasis n = 1; biopsy of soft tissue metastasis n = 1.  
<sup>‡</sup>Percentages based on n = 28 patients with cytology as diagnostic material for PD-L1.  
<sup>§</sup>List of other fixation agents (n = 1): unknown n = 1.  
<sup>¶</sup>List of other control (n = 82): cell lines n = 40; lung n = 40; bone n = 1; unknown n = 1.  
<sup>#</sup>n values change depending on available data.

Statistically significant differences were observed in treatment selection regarding the histological subtype (p = 0.0387): more patients with nonsquamous NSCLC received platinum doublet in combination with immunotherapy compared with squamous NSCLC patients (17.7 vs 2%, respectively; p = 0.0387), whereas NSCLC

**Table 3. Treatment regimen by clinicopathological characteristics.**

Overall n = 265	Platinum doublet	CT monotherapy	IO monotherapy	Platinum doublet+IO	IO+IO	p-value <sup>†</sup>
	142 (53.6%)	5 (1.9%)	71 (26.8%)	39 (14.7%)	6 (2.3%)	
<b>Age groups, n (%)</b>						
≤75 years	118 (54.1)	3 (1.4)	53 (24.3)	37 (17.0)	5 (2.3)	0.0666
>75 years	24 (51.1)	2 (4.3)	18 (38.3)	2 (4.3)	1 (2.1)	
p-value <sup>‡</sup>	0.7025	–	0.0495	–	–	
<b>Gender, n (%)</b>						
Male	114 (55.9)	4 (2.0)	55 (27.0)	26 (12.7)	4 (2.0)	0.4589
Female	28 (45.9)	1 (1.6)	16 (26.2)	13 (21.3)	2 (3.3)	
p-value <sup>‡</sup>	0.1704	–	0.910	–	–	
<b>Smoking history, n (%)</b>						
Current/former smoker	129 (54.7)	4 (1.7)	63 (26.7)	33 (14.0)	5 (2.1)	0.4854
Never smoked	10 (38.5)	1 (3.8)	8 (30.8)	6 (23.1)	1 (3.8)	
p-value <sup>‡</sup>	0.1162	–	0.6570	–	–	
<b>ECOG, n (%)</b>						
0–1	109 (52.2)	2 (1.0)	54 (25.8)	36 (17.2)	6 (2.9)	<b>0.0277</b>
≥2	33 (61.1)	3 (5.6)	15 (27.8)	3 (5.6)	0 (0.0)	
p-value <sup>‡</sup>	0.2392	–	0.7726	–	–	
<b>Histology, n (%)</b>						
Squamous	34 (68.0)	1 (2.0)	12 (24.0)	1 (2.0)	2 (4.0)	<b>0.0387</b>
Nonsquamous	108 (50.2)	4 (1.9)	59 (27.4)	38 (17.7)	4 (1.9)	
p-value <sup>‡</sup>	0.0233	–	0.6206	–	–	
<b>PD-L1 tumor proportion score, n (%)</b>						
<1%	73 (73.0)	4 (4.0)	1 (1.0)	21 (21.0)	0 (0.0)	<b>&lt;0.0001</b>
1–49%	52 (74.3)	1 (1.4)	4 (5.7)	10 (14.3)	2 (2.9)	
≥50%	11 (12.9)	0 (0.0)	65 (76.5)	6 (7.1)	3 (3.5)	
Not evaluable	2 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)	
<b>Brain metastasis, n (%)</b>						
Yes	32 (54.2)	1 (1.7)	13 (22.0)	11 (18.6)	1 (1.7)	0.8141
No	110 (53.4)	4 (1.9)	58 (28.2)	28 (13.6)	5 (2.4)	
p-value <sup>‡</sup>	0.9092	–	0.3493	0.3340	–	
<b>Liver metastasis, n (%)</b>						
Yes	21 (56.8)	1 (2.7)	8 (21.6)	6 (16.2)	1 (2.7)	0.9442
No	121 (53.1)	4 (1.8)	63 (27.6)	33 (14.5)	5 (2.2)	
p-value <sup>‡</sup>	0.6767	–	0.4437	–	–	
<b>Comorbidities, n (%)</b>						
Yes	92 (58.2)	3 (1.9)	41 (25.9)	22 (13.9)	0 (0.0)	0.0299
No	50 (46.7)	2 (1.9)	30 (28.0)	17 (15.9)	6 (5.6)	
p-value <sup>‡</sup>	0.0655	–	0.7062	–	–	

<sup>†</sup>χ-square test in categorical variables.

<sup>‡</sup>z-ratio, two independent proportion.

Statistical significance is indicated in bold by p &lt; 0.05.

 From a clinical practice perspective, when selecting the treatment, the scheme options that are considered include: CT monotherapy, double CT, IO monotherapy, CT + IO or IO + IO. The classification using IO ± combination, CT ± antiangiogenic or IO monotherapy has no practical meaning. See [Supplementary Table A](#) for additional information. CT: Chemotherapy; EOG: Eastern Co-operative Oncology Group; IO: Immuno-oncology.

patients with squamous carcinomas were treated more frequently with platinum doublet than patients with non-squamous NSCLC (68 vs 50.2%, respectively; p = 0.0233) (Table 3).

The percentage of patients treated with platinum doublet plus immunotherapy was significantly higher when ECOG was 0–1 versus ECOG ≥2 (17.2 vs 5.6%, respectively). Treatment distribution was also statistically different depending on the presence of comorbidities (p = 0.0155). Thus patients with comorbidities were more frequently treated with platinum doublet compared with patients without comorbidities (58.2 vs 46.7%, respectively) (Table 3).

Concerning treatment selection based on PD-L1 expression, patients with PD-L1  $\geq 50\%$  were more frequently treated with immunotherapy monotherapy than the other PD-L1 expression groups (76.5% in the group with PD-L1  $> 50\%$ ; 5.7% in the group with PD-L1 1–49%; and 1% in the group with PD-L1  $< 1\%$ ), while they were less frequently treated with platinum doublet (12.9% in the group with PD-L1  $> 50\%$ ; 74.3% in the group with PD-L1 1–49%; and 73% in the group with PD-L1  $< 1\%$ ;  $p < 0.0001$ ) (Table 3).

A trend was observed regarding age groups: the percentage of patients treated with immunotherapy monotherapy was higher among patients aged  $> 75$  years (38.3 vs 24.3%;  $p = 0.0666$ ) (Table 3). No statistically significant differences were observed in treatment selection regarding gender, smoking history or presence of brain, bone or liver metastasis. Likewise, no differences were found based on history or presence of autoimmune disease or presence of viral disease (data not shown).

### PD-L1 expression regarding diagnostic & clinicopathological characteristics

There were no statistically significant differences in the diagnostic variables (institution that performed the test, site of biopsy, tumor sample and antibody test used) regarding the specific PD-L1 expression (Table 4).

Statistically significant differences were observed in the expression of PD-L1 based on histological subtypes ( $p = 0.0433$ ). Specifically, the percentage of squamous carcinoma subtype was higher compared with nonsquamous carcinoma in patients with PD-L1 1–49% (41.2 vs 24%, respectively;  $p = 0.0126$ ). In addition, there were no statistically significant differences in demographic (gender, age, smoking history) or clinicopathological characteristics (ECOG performance, status of stage IV NSCLC, history or presence of autoimmune disease, viral infections) related to the PD-L1 status (Supplementary Table 2).

### Turnaround timelines in different institutions

There were statistically significant differences in the time between PD-L1 testing request and testing results, depending on the laboratory that performed the PD-L1 test: median of 5.00 days (25th/75th percentiles: 0.0/12.0) for in-house testing versus 15.50 days (25th/75th percentiles: 5.5/19.0) for testing performed in centralized laboratories ( $p = 0.0079$ ).

### Correlation of PD-L1 expression with patient demographic & clinicopathological characteristics: multivariate analysis

An initial multivariate analysis, using a logistic regression, was conducted to study the factors related to PD-L1 status as well as those related to the most frequent systemic treatments used. Those variables with  $p$ -value  $< 0.10$  in the univariate analysis were entered in the multivariate analysis. With regard to factors related to PD-L1 status, only two categories – age and histological subtype – met a  $p$ -value  $< 0.10$  and therefore were entered in the first multivariate analysis. A squamous histological subtype was the single factor related to PD-L1 1–49% positivity results, with odds ratio (OR) = 2.320 ( $p = 0.0235$ ) compared with PD-L1  $< 1\%$  (OR: 2.089;  $p = 0.0569$ ) compared with PD-L1  $\geq 50\%$ .

A second multivariate analysis was performed in order to study the factors related to the most frequent systemic treatment (platinum doublet chemotherapy, immunotherapy monotherapy or platinum doublet–immunotherapy) used. Following the criterion of a  $p$ -value  $< 0.1$ , age (as a continuous variable), ECOG score (as a dichotomy:  $\geq 2$  versus 0–1), histological subtype and PD-L1 status (except for ‘not evaluable’; 1–49% vs  $< 1\%$ ;  $\geq 50\%$  vs  $< 1\%$ ) were entered in the logistic regression. Only age, ECOG score and PD-L1 expression were factors related to the type of systemic treatment administered. Patients treated with platinum doublet chemotherapy or immunotherapy monotherapy were older than patients in platinum doublet–immunotherapy regimens in model 1: Platinum-doublet vs platinum-doublet IO (OR: 1.040;  $p = 0.0545$ ) and model 2: monotherapy vs platinum-doublet IO (OR: 1.087;  $p = 0.0053$ ). Patients treated with platinum doublet chemotherapy or immunotherapy monotherapy reported higher percentages of ECOG  $\geq 2$  than patients treated with platinum doublet–immunotherapy in model 1 (OR: 3.597;  $p = 0.0487$ ) and model 2 (OR: 9.784;  $p = 0.0124$ ). The factor that was strongly associated with treatment was, as expected, the expression of PD-L1. Patients with PD-L1 expression  $\geq 50\%$ , as compared with those with no PD-L1 expression ( $< 1\%$ ) were more likely to receive treatment with immunotherapy in monotherapy (OR: 0.002;  $p < 0.0001$ ) (Table 5).



Table 4. PD-L1 expression by diagnostic characteristics.

Diagnostic variables	n <sup>†</sup>	PD-L1 expression, n (%)				p-value <sup>‡</sup>
		PD-L1 <1%	PD-L1 1–49%	PD-L1 ≥50%	PD-L1 not evaluable	
Institution performing PD-L1 testing	287	115 (40.1)	77 (26.8)	92 (32.1)	3 (1.0)	0.5237
In-house	273	107 (39.2)	75 (27.5)	88 (32.2)	3 (1.1)	
Centralized laboratory	14	8 (57.1)	2 (14.3)	4 (28.6)	0 (0.0)	
p-value <sup>§</sup>	–	0.1812	–	–	–	
Site of biopsy <sup>¶</sup>	274	106 (38.7)	76 (27.7)	90 (32.8)	2 (0.7)	0.4173
Primary	178	71 (39.9)	44 (24.7)	61 (34.3)	2 (1.1)	
Metastatic	96	35 (36.5)	32 (33.3)	29 (30.2)	0 (0.0)	
p-value <sup>§</sup>	–	0.5782	0.1288	0.4946	–	
Cytology						
Sample format <sup>#</sup>	28	17 (60.7)	2 (7.1)	8 (28.6)	1 (3.6)	0.5018
Cell block	23	15 (65.2)	2 (8.7)	5 (21.7)	1 (4.3)	
Cytological smear	5	2 (40.0)	0 (0.0)	3 (60.0)	0 (0.0)	
p-value <sup>§</sup>	–	–	–	–	–	
PD-L1 antibody used – grouped, excluding SP142	285	114 (40.0)	77 (27.0)	91 (31.9)	3 (1.1)	0.7199
22C3	157	60 (38.2)	45 (28.7)	50 (31.8)	2 (1.3)	
28–8	51	26 (51.0)	11 (21.6)	14 (27.5)	0 (0.0)	
SP263	77	28 (36.4)	21 (27.3)	27 (35.1)	1 (1.3)	
PD-L1 antibody used <sup>††</sup>	287	115 (40.1)	77 (26.8)	92 (32.1)	3 (1.0)	
22C3 pharmDx kit	157	60 (38.2)	45 (28.7)	50 (31.8)	2 (1.3)	0.8187
22C3 laboratory-developed test option (free antibody)	1	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0.2787
28–8 pharmDx kit	51	26 (51.0)	11 (21.6)	14 (27.5)	0 (0.0)	0.3722
28–8 laboratory-developed test option (free antibody)	1	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0.2787
SP263 pharmDx kit	38	14 (36.8)	10 (26.3)	13 (34.2)	1 (2.6)	0.6019
SP263 laboratory-developed test option (free antibody)	39	14 (35.9)	11 (28.2)	14 (35.9)	0 (0.0)	0.8721
SP142 pharmDx kit	0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
SP142 laboratory-developed test option (free antibody)	1	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1.0000 <sup>(f)</sup>
Other <sup>†††</sup>	1	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0.5993 <sup>(f)</sup>

<sup>†</sup>Based on total sample of patients with each of the clinical management variables.

<sup>‡</sup> $\chi^2$ -squared test or Fisher's exact test.

<sup>§</sup>z-ratio, two independent proportions.

<sup>¶</sup>Percentages based on patients with site of biopsy as diagnostic material for PD-L1.

<sup>#</sup>Percentages based on patients with cytology as diagnostic material for PD-L1.

<sup>††</sup>A single patient might report more than one PD-L1 antibody (only two patients reported >1 PD-L1 antibody: n = 1 patient with 28–8 pharmDx kit + 28–8 laboratory-developed test option; and n = 1 patient with 22C3 pharmDx kit + 22C3 laboratory-developed test option).

<sup>†††</sup>Unknown, n = 1.

## Discussion

The aims of the present study were to retrospectively analyze the standard clinical management of newly diagnosed stage IV NSCLC patients with neither tumor-activating *EGFR* or *BRAF* mutations nor *ALK* or *ROS1* rearrangements as well as to characterize and describe how PD-L1 expression testing was performed in Spain.

The study is an observational study that describes the status of PD-L1 expression testing and treatment selection for patients with stage IV NSCLC in Spain during 2019. Historically, despite the fact that since 2016 treatment selection with certain checkpoint inhibitors as second-line therapy was based on PD-L1 expression, the determination of this biomarker was not implemented in clinical practice in Spain until the approval of pembrolizumab as first-line treatment for patients with PD-L1 expression >50% in August 2017. Although its adoption was gradual, this study shows that in 2019 the testing was already fully implanted routinely and that its results had a great influence on treatment selection.

**Table 5. Multivariate analysis of factors related to systemic treatment.**

Factors	OR	95% CI (OR)	p-value <sup>†</sup>	R <sup>2</sup> Nagelkerke
<b>Model 1: platinum-doublet vs platinum doublet+IO</b>				
Age	1.040	0.999–1.082	0.0545	0.6221
ECOG				
≥2 vs 0–1	3.597	1.008–12.838	0.0487	
PD-L1				
1–49% vs <1%	1.713	0.727–4.036	0.0902	
≥50% vs <1%	0.629	0.202–1.964	0.1925	
1–49% vs ≥50%	2.722	0.800–9.258	0.0902	
<b>Model 2: IO monotherapy vs platinum doublet+IO</b>				
Age	1.087	1.025–1.153	0.0053	
ECOG				
≥2 vs 0–1	9.784	1.639–58.409	0.0124	
PD-L1				0.6221
1–49% vs <1%	10.739	1.011–114.115	0.5169	
≥50% vs <1%	340.865	33.786–>999.999	<0.0001	
1–49% vs ≥50%	0.032	0.007–0.151	0.5169	
<b>Model 3: platinum doublet vs IO monotherapy</b>				
Age	0.956	0.907–1.008	0.0985	0.6221
ECOG				
≥2 vs 0–1	0.368	0.093–1.457	0.1543	
PD-L1				
1–49% vs <1%	0.159	0.017–1.502	0.0811	
≥50% vs <1%	0.002	<0.001–0.016	< 0.0001	
1–49% vs ≥50%	86.398	23.811–313.495	0.0811	

<sup>†</sup> p: Wald  $\chi^2$ -squared test; n = 241.  
 Model 1 and model 2: platinum doublet+IO used as reference category; model 3: IO monotherapy used as reference category.  
 The factors with p < 0.10 in the univariate analysis have been entered in the regression model, and only factors with p < 0.05 in the multivariate analysis have been reported in this table.  
 ECOG: Eastern Co-operative Oncology Group; IO: Immuno-oncology; OR: Odds ratio.

The baseline clinical and demographic characteristics of the patients included in this study are very similar to those from previous epidemiological studies in NSCLC. A higher incidence of NSCLC in males compared with females was observed (77.1 vs 22.9%), despite the increase in lung cancer rates among females that was recently reported [29]. The mean age of the participants was 67.1 years, with 79.8% younger than 75 years. Regarding tobacco use, the smoking habit in 90% of the patients supports its etiopathogenic role in the disease: half of the patients were former smokers (50.2%), with a mean of 37.3 years’ smoking, and 38.7% were current smokers, with a mean of 42.8 years’ smoking. Only 10% of the patients were nonsmokers. This low percentage can be explained because patients with driver mutations, who are mostly nonsmokers, were excluded from the study. With respect to the percentage of squamous histology, it is noteworthy that the percentage reported in this study (17.8%) is lower than the percentage recently reported by the Spanish Thoracic Tumors Registry (27.7%) [30]. It is also important to highlight two factors: the high percentage of patients with comorbidities (>50%) and the percentage of patients with poor ECOG score (ECOG ≥2: 25%), which reflects the limitations that physicians face in treatment selection in real life.

Regarding treatment, the results of the present study show that a very high percentage (almost 90%) of the patients with stage IV NSCLC in Spain who are referred to medical oncology units receive active systemic treatment. This approach included not only chemotherapy alone but also immunotherapy, either alone or in combination. In our study, elderly patients, those with comorbidities and those with poor ECOG scores also received systemic treatment: 71% of ECOG ≥2 patients received systemic treatment. Significant differences were observed in treatment selection based on ECOG status: patients with ECOG ≥2 received less chemotherapy + immunotherapy than those with ECOG 0–1, whereas most of the ECOG ≥2 patients were treated with double chemotherapy. Monotherapy schedules were only chosen in 1.9% of the cases. This could be explained by the thorough knowledge and

experience of the participating centers with their specialized oncology units. Furthermore, the time elapsed between diagnosis and the initiation of treatment (mean time: ~1 month) was correlated with the complexity of the lung cancer patient journey, from the diagnosis (suspicion) in ambulatory setting until the patient was referred to a tertiary hospital.

The univariate analysis of the data showed that treatment decisions were mainly guided by the presence of comorbidities, ECOG score, tumor histology and PD-L1 expression. However, the multivariate analysis did not confirm comorbidities as a significant factor. This disparity may be due to the fact that patients with comorbidities received platinum doublet chemotherapy at a higher frequency than those without comorbidities. In addition, patients without comorbidities were more likely to be enrolled in clinical trials, a treatment option that was not included in the multivariate analysis. Additionally, although age was not identified as a statistically significant factor for treatment selection in the univariate analysis, patients treated with platinum doublet plus immunotherapy tended to be younger. This may reflect an initial concern regarding the toxicity of the combination in potentially fragile subjects. In fact, in the multivariate analysis, age (as a continuous variable, not as a dichotomy) and ECOG score were statistically significantly related to treatment selection. It is of note that the number of patients with a history or presence of an autoimmune or viral disease was too small to draw any conclusion.

According to the data, treatment decisions were guided by PD-L1 expression, in accordance with the current clinical guidelines [4,5]. However, it must be noted that the therapy selection was limited by treatment availability in Spain at that time (2019). Heterogeneous access to new immunotherapy regimens in combination with platinum doublet chemotherapy was reported among the participant hospitals. In most of them, the delay between approval by the EMA and access to immunotherapy treatments ranged from 9 to 26 months, due to the refund negotiation process [24].

With regard to treatment selection, the only immunotherapy that was approved in all hospitals was pembrolizumab in monotherapy for patients with PD-L1 expression  $\geq 50\%$ , which explains the high percentage of patients (76.5%) treated with this therapeutic option in our study, as well as why this was the most significant factor related to treatment decision, both in the univariate and the multivariate analysis. This fact also justified why the histological subtype did not reach statistical significance in the multivariate analysis, because pembrolizumab in monotherapy was approved for both histologies. After the approval of the combinations of platinum doublet-immunotherapy  $\pm$  antiangiogenic agent in nonsquamous NSCLC by the EMA, these treatments were only available in some of the institutions during the study period. This fact justifies the higher percentage of nonsquamous NSCLC patients with PD-L1 expression  $< 50\%$  who were treated with platinum doublets instead of a combination with immunotherapy. Treatment of squamous NSCLC with immunotherapy in combination was only reported anecdotally and mostly restricted to the context of clinical trials.

PD-L1 expression was determined in all the patients in whom biomarker studies were performed, reflecting the clinical implementation of its determination. PD-L1 percentages were consistent with those previously reported in clinical trials [17,18,31–35] and did not appear to be influenced by different clinicopathological characteristics except for histology, a new finding not reported previously and which should be confirmed in further real-world studies.

The implementation of in-house, validated PD-L1 tests in most of the hospitals played a key role in reducing the turnaround times, delivering PD-L1 expression status prior to treatment decision. The results of the present study confirm the use of mainly 22C3, followed by SP263 and 28–8 antibody-based platforms, all with a similar analytical performance for assessment of PD-L1 expression, and the significantly lower use of SP-142, due to its lesser sensitivity [35]. In our study, PD-L1 determination was performed in tissue samples and cytological samples in 85 and 14.9% of the cases, respectively. Although the PD-L1 testing assays have been validated only for tissue samples, more data are now available reporting their usefulness when using cytological samples, with the results obtained being comparable to those using tissue samples in centers with high experience [31].

The limitations of the study are those derived from its retrospective design, such as missing data. In addition, the small sample size in some of the subgroups may have limited the statistical significance and may not allow the extrapolation of the results to the entire Spanish population.

## Conclusion

The study is an observational study that describes the status of PD-L1 expression testing and treatment selection for patients with stage IV NSCLC in Spain during 2019. Since 2016 the treatment selection with certain checkpoint inhibitors as second-line therapy has been based on PD-L1 expression, and the adoption of this biomarker was

gradual. This study shows that in 2019 it was already fully implanted routinely and that it had a great influence over treatment selection.

The amount of sample is one of the main limiting factors for treatment selection in lung cancer, and in NSCLC patients the identification of driver mutations is prioritized. The study shows that at that time this was not a handicap for the determination of PD-L1 in this population (82% of the population evaluated). In addition, pembrolizumab in combination with chemotherapy was approved in Europe in September 2018, showing benefit regardless of PD-L1 expression level. However, in Spain, this scheme only began to be available for patients with stage IV lung adenocarcinoma PD-L1 <50% from September 2019, although several centers had access before that date and for all patients with nonsquamous NSCLC.

During the period in which this observational study was carried out, these approvals did not influence the PD-L1 determination, confirming that PD-L1 testing had been routinely established in our environment, regardless of the delay in access to drugs. In addition, both the technique and the type of sample used were analyzed in the study. The results show that the amount of material, the technique used for PD-L1 determination and the time required for treatment selection did not represent limitations for PD-L1 determination.

It can be concluded that PD-L1 testing is implemented in clinical practice and seems to guide treatment decisions in centers, according to the regulations in Spain.

### Future perspective

Immunotherapy has become part of standard of care for patients with locally advanced or metastatic NSCLC. In the next few years we will probably see how the different combinations of immunotherapy are consolidated in first line of treatment.

PD-L1 testing has already been implemented in daily practice and, although new immunotherapy biomarkers are being explored, PD-L1 will continue to be important in treatment decisions, especially depending on the approvals of the different national agencies.

#### Summary points

- PD-L1 testing is implemented in clinical practice and seems to guide treatment decisions in centers, according to the regulations in Spain.
- Most patients with stage IV non-small cell lung cancer in Spain who are referred to the medical oncology units, are given active systemic treatment. Systemic treatment availability changed during the study period.
- Treatment decisions seem to be guided by age, Eastern Co-operative Oncology Group performance status and PD-L1 expression.
- For almost all stage IV patients, the PD-L1 expression status is available prior to the treatment decision.
- PD-L1 testing is performed mainly in-house and using clinically validated methods.
- PD-L1 percentages in this study are consistent with those previously reported in clinical trials and do not appear to be influenced by different clinicopathological characteristics except for histology.

#### Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: [www.futuremedicine.com/doi/suppl/10.2217/lmt-2021-0008](http://www.futuremedicine.com/doi/suppl/10.2217/lmt-2021-0008)

#### Author contributions

All authors provided substantial contributions to the conception and design of the work, acquisition, analysis and interpretation of data; drafting the manuscript and revising it critically for important intellectual content, have provided final approval of the version to be published and have agreed to be accountable for all aspects of the study in ensuring that questions related to its accuracy and integrity in part or as whole, are appropriately investigated and resolved.

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### Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human experimental investigations. According to current Spanish regulation for non-interventional observational studies in Spain, written informed consent had to be granted for those studies that require an interview with the subject or in those in which it is not possible to adopt a procedure of safe dissociation. In the present study, only already existing data were collected from existing clinical reports and were securely and adequately dissociated, and no personal data were therefore included. In addition, subjects were given a unique screening number via eCRF that was used to identify the subject in the study as safe dissociation method. The confidentiality of personal data was guaranteed at all times in accordance with the provisions of Organic Law 3/2018, of December 5th, on the protection of personal data. In any case, an Ethic Committee evaluated the protocol and consequently approves or rejects and informed consent waiver. In this study and Informed Consent was required only in two sites (Hospital de Santa Creu i Sant Pau and Hospital Universitario Virgen de las Nieves) and the other sites accepted the exemption due to the type of study. The authors state that they have obtained verbal and written informed consent from the patient/patients for the inclusion of their medical and treatment history within this case report.

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