

Original Research

Immunohistochemical Assessment of the P53 Protein as a Predictor of Non-Small Cell Lung Cancer Response to Immunotherapy

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Abstract

Background: Determining predictive biomarkers for immune checkpoint inhibitors (ICIs) is a current challenge in oncology. Previous studies on non-small cell lung cancer (NSCLC) have shown how *TP53* gene mutations are correlated with different responses to ICIs. Strong and diffuse immuno-expression of p53 by immunohistochemistry (IHC) is interpreted as a likely indicator of a *TP53* gene mutation. We aimed to assess the p53 protein expression via IHC in NSCLC as a predictive biomarker of the response to ICIs. **Methods:** This was a retrospective hospital-based study of patients with NSCLC treated with Nivolumab in the University Hospital of Salamanca. All diagnostic biopsies were studied via IHC (measuring p53 protein expression, peroxidase anti-peroxidase immunohistochemistry technique using Leica BOND Polymer development kits). Survival analysis was performed by subgroups of expression of p53 and other factors using the Kaplan-Meier estimator and Cox proportional-hazards model. **Results:** Seventy-three patients were included (59 men and 14 women). The median age was 68 (44–84) years. Thirty-six biopsies were adenocarcinoma, 34 were squamous, and three were undifferentiated. In 41 biopsies (56.2%), the cellular expression of p53 was <5% (Group A), and in 32 biopsies (43.8%), the expression was ≥5% (Group B). In the general analysis, no differences were observed in overall survival (OS) (A: 12 months vs B: 20 months; $p = 0.070$) or progression-free survival (PFS) (A: 4 m vs B: 7 m; $p = 0.064$). Significant differences were observed in adenocarcinomas for both OS (A: 8 m vs B: median not reached; $p = 0.002$) and PFS (A: 3 m vs 8 m; $p = 0.013$). No differences in PFS and OS were observed in squamous cell carcinoma. Significant differences were observed in OS in the PD-L1 negative group (0% expression) (A: 13 m vs B: 39 m; $p = 0.024$), but not in PFS (A: 3 m vs B: 7 m; $p = 0.70$). No differences were observed in the PD-L1 positive group. **Conclusions:** A trend toward a greater response to ICIs was observed in the PFS and OS of patients with high expression of p53 by IHC (*TP53* mutation), especially in the PD-L1 negative adenocarcinoma subgroup. These results will make it possible to make future modifications to the clinical guidelines of NSCLC according to the expression of p53.

Keywords: p53 protein; non-small cell lung cancer; immunotherapy; immune checkpoint inhibitors; biomarkers

1. Introduction

Among malignancies, lung cancer is a with the highest incidence worldwide, with 2.1 million newly diagnosed cases per year [1]. In terms of mortality, a total of 1.8 million people die from lung tumours each year (representing 18.4% of deaths from cancer) [2]. The aetiology is well known; tobacco is the main cause, responsible for 90% of lung cancers [3]. Other known risk factors include asbestos,

radon, and polycyclic aromatic hydrocarbons [4]. The most widely used histological classification for the characterisation of lung cancer divides these tumours into four variants: (1) adenocarcinoma, (2) squamous cell carcinoma, (3) large cell carcinoma, and (4) small cell or small cell carcinoma. The first three tumour types are grouped under the term non-small cell lung cancer (NSCLC), which encompasses 85% of lung tumours.



The introduction of immunotherapy treatments [immune checkpoint inhibitors (ICIs)] has revolutionized NSCLC treatment. The inhibition of T cells by tumour cells is performed through two main inhibitory pathways best described for T cells: the cytotoxic T-lymphocyte antigen 4 (CTLA-4) protein receptor and the programmed cell death protein 1 (PD-1) [5,6]. These are the pathways through which ICIs act by negatively regulating these receptors and consequently stimulating the T lymphocytes. Various factors, such as gene mutations, are known as predictors or modifiers of the response to immunotherapy or, more accurately, to ICIs in solid tumours [7].

The most pertinent somatic mutations in NSCLC are those related to the *RAS* (Rat Sarcoma Virus), *RB* (Retinoblastoma Protein), *TP53* (Tumour Protein 53), *AKT* (Protein Kinase B), *LKB1* (Liver Kinase B1), and *BRAF* (Murine Sarcoma Viral Oncogene Homolog B) genes. Alterations in the *TP53* gene occur in approximately 50% of NSCLC cases [8]. This percentage is higher in epidermoid tumours and lower in adenocarcinomas because it is associated with tobacco use [9]. The involvement of the *TP53* gene in different tumours is widely known because of its function as a suppressor gene. The loss or mutation of the *TP53* gene in tumour cells has been shown to influence immune recognition through mechanisms, such as the presentation of major histocompatibility complex 1 or the recruitment of suppressor myeloid cells or Treg lymphocytes [10]. Not only is *TP53* key in the direct regulation of the immune system, but it also plays a role in establishing the tumour microenvironment [11]. Therefore, several studies have evaluated the implications of *TP53* mutations in predicting the response to ICIs in solid tumours, especially NSCLC [12,13].

Immunohistochemical (IHC) staining for p53 is mainly correlated with the presence of missense mutations in *TP53* [14,15]. Historically, p53 IHC has been interpreted as negative or positive based on the percentage of stained tumour cell nuclei using variable cut-offs ranging from 5 to 10% [16]. However, the current classification of p53 expression is subdivided into three expression patterns: overexpression, the complete absence of expression, and cytoplasmic expression. Only 5% of tumours with mutated *TP53* show p53 protein expression by the IHC wild type. This three-pattern classification correlates with all possible *TP53* mutations, the most common being missense. For all these reasons, the protein expression of p53 by IHC (in all its forms) can be used as a highly sensitive surrogate marker for mutations in *TP53* with a failure rate of only 5% [17].

TP53 mutations have traditionally been correlated with resistance to radiation therapy and chemotherapy [18]. Studies on immunotherapy and NSCLC have presented contradictory results regarding the mutational status of *TP53*. A study by Assoun *et al.* [19] demonstrated a greater benefit of immunotherapy in patients with NSCLC and mutated *TP53*. However, a study by Zhao *et al.* [20] presented

contrary results, with lower survival of NSCLC patients treated with immunotherapy treatment in patients with mutated *TP53*. These results were more marked in patients with low immune expression measured as the tumour mutation burden (TMB), and were aligned with the findings of Carlisle *et al.* [21], who reported a trend toward a greater response in tumours with high expression of PD-L1.

In this research, our primary objective was to determine whether the protein expression of p53, as a surrogate marker of the mutational status of *TP53*, was correlated with the response to immunotherapy treatments in patients with NSCLC. As secondary objectives of the study, we investigated whether this response to ICIs, based on the mutational status of *TP53*, was influenced by various other factors, such as sex, histology, and the expression of PD-L1.

2. Materials and Methods

2.1 Data Collection and Construction of the Cohort

A retrospective hospital-based study was carried out in selected patients within the Complejo Asistencial Universitario de Salamanca (Salamanca, Spain). The inclusion criteria were a diagnosis of advanced or metastatic NSCLC and second-line immunotherapy treatment with an anti-PD1 drug (Nivolumab independent of PD-L1 expression in the tumour, according to clinical trials CheckMate 017 and CheckMate 057) [22,23]. The fundamental data collected and studied were age (years), sex, histology, sites of metastases, progression-free survival (PFS) in months, overall survival (OS) in months, the number of doses received, the best response obtained with immunotherapy, and immunotoxicity presented. The study was carried out according to the ethical protocols of the hospital and with the informed consent of the patients for the extraction of the sample. If the information found in this study is favourable for the immunotherapy treatment, it will be included in the NSCLC treatment protocols of the Complejo Asistencial Universitario de Salamanca.

According to the data available in the literature, a p53 protein expression level of 5% is considered to determine a high probability of *TP53* mutation (based on the results of a study carried out by Kim *et al.* [24]. Several studies have shown that the 5% cut-off point has the shortest sensitivity and specificity for assessing *TP53* mutations, both in solid tumours (such as adenocarcinoma of the lung or ovary) and in haematological neoplasms [15,25]. Together with the above, for evaluating the secondary endpoint, the patients were subdivided by positive or negative PD-L1 expression (this subdivision was made because it is the most widely used in clinical trials in NSCLC and second-line immunotherapy, such as the trials CheckMate 017 and CheckMate 057). To carry out this classification of patients, we relied on published clinical trials of second-line immunotherapy in which the main factor for dividing the subgroups was the positive or negative expression of PD-L1 [26,27].

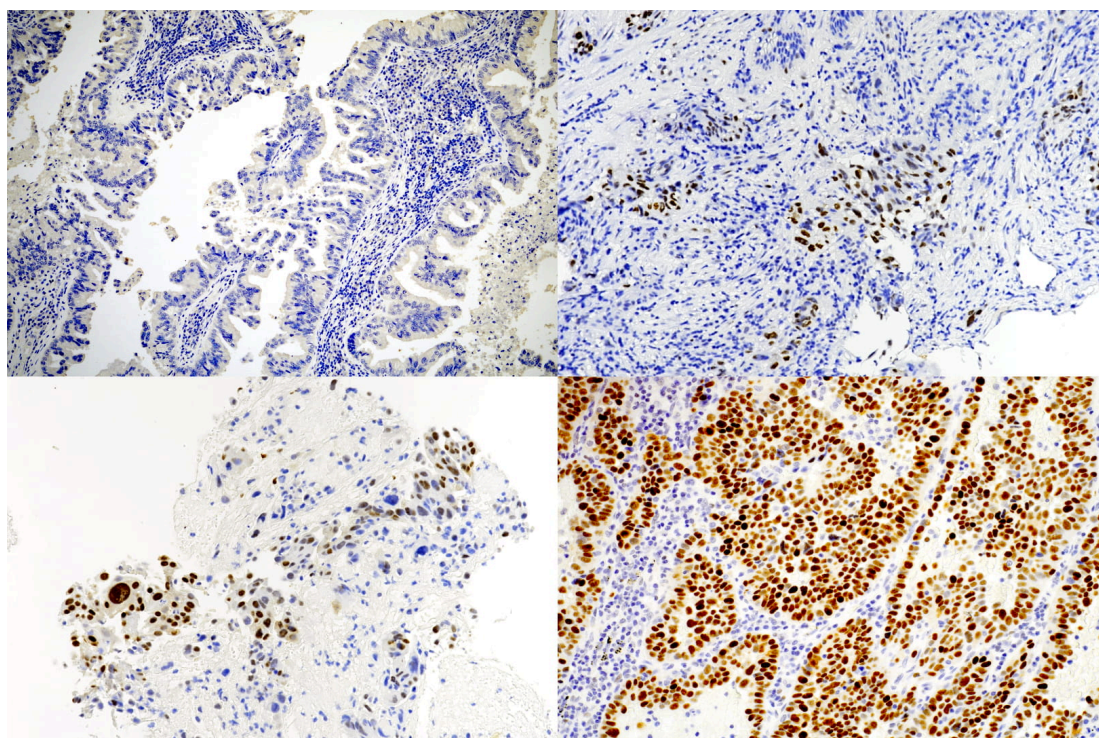


Fig. 1. Assessment of the level of protein expression of p53 by IHC. The figure shows 0% p53 expression (upper left), 30% (upper right), 50% (lower left), and 100% (lower right) staining.

2.2 Analysis of p53 Protein Expression by IHC

The expression analysis of the p53 protein was performed with the peroxidase anti-peroxidase immunohistochemistry technique using the Leica BOND Polymer development kits. Leica BOND III automatic machines were subsequently used. P53 clon D07 Leica prediluted. The level of expression of p53 was quantified as a percentage, with 0% indicating no expression and 100% indicating complete expression of the protein in the cell (Fig. 1). The values of p53 are described in quantitative terms according to the main studies in the literature on IHC in p53 [28,29]. We carried out a semi-quantitative study, analysing ten fields of 20× (Supplementary material).

The samples were evaluated by two independent people to avoid bias. Microscopic analysis was carried out using a Nikon Eclipse Ci microscopy device (Tokyo, Kanto, Japan). According to the existing literature on IHC as a surrogate marker for mutations in the *TP53* gene (in both solid and haematological malignancies), it has been considered that a p53 protein expression level of $\geq 5\%$ is a reliable cut-off point indicating a mutated *TP53* gene [24].

2.3 Statistical Analysis

For statistical analysis, first, PFS was calculated as the months from the initiation of immunotherapy treatment to clinical or radiological progression. OS was calculated as the period (in months) from initiating the patient's immunotherapy treatment until death. Survival rates were cal-

culated as medians with 95% confidence intervals (CIs). Survival as a function of p53 expression was calculated using the Kaplan-Meier method (log-rank and Breslow test) and Cox regression analysis. To avoid confounding factors, subgroup analyses were performed for sex, age, and histology. Additional proportional-hazards models that included the above potential confounders were constructed. The statistical significance for the analyses in this study was established at $p < 0.05$. The software used was SPSS, version 25 (IBM®, Armonk, NY, USA). The results were expressed using the log-rank test, except those specifically requiring the Breslow test.

3. Results

3.1 General Analysis of the Sample

A total of 73 biopsies were examined, which were taken from patients with NSCLC who were treated with anti-PD1 as second-line therapy. The median age was 68 (44–84) years; 59 patients were men (80.8%), and 14 were women (19.2%). Histology corresponded to adenocarcinoma in 36 patients, squamous cell carcinoma in 34 patients, and undifferentiated carcinoma in three patients. The most frequent sites of metastatic involvement were the lung with metastatic secondary nodules (45 patients, 61.6%) and lymph nodes (39 patients, 53.4%), followed by the bone (14 patients, 19.2%) and liver (ten patients, 13.7%). The mean PD-L1 expression was 2%. PD-L1 expression was negative (0%) in 27 patients and positive ($\geq 1\%$) in 46 patients. PD-

Table 1. General characteristics of the sample.

Sample	Overall, 73 (100%)	Adenocarcinoma, 36 (49.3%)	Squamous, 34 (46.6%)
Age (range)	68 (44–84)	68 (44–84)	68 (44–84)
Sex (M/W)	59/14 (80.8/19.2%)	25/11 (69.4/30.6%)	31/3 (91.2/8.8%)
P53 expression			
• 0%	35 (47.9%)	20 (55.5%)	13 (38.2%)
• <5%	41 (56.2%)	12 (33.3%)	14 (41.2%)
• ≥5%	32 (43.8%)	24 (66.7%)	20 (58.8%)
• ≥10%	26 (35.6%)	7 (19.4%)	19 (55.9%)
• ≥20%	22 (30.1%)	6 (16.6%)	16 (47.1%)
• ≥50%	14 (19.2%)	3 (8.3%)	8 (23.5%)
PD-L1			
• Negative	27 (37%)	18 (50%)	9 (26.5%)
• Positive	46 (63%)	18 (50%)	25 (73.5%)
Survival (months)			
• Overall	13 (95% CI 8.2–17.8)	16 (95% CI 3.5–28.5)	12 (95% CI 9–15)
• Progression-free	5 (95% CI 3.8–6.2)	4 (95% CI 2.5–5.5)	5 (95% CI 2–8)
Response			
• Progression	40 (54.8%)	22 (61.1%)	16 (47.1%)
• Stabilisation	16 (21.9%)	8 (22.2%)	8 (23.5%)
• Partial response	13 (17.8%)	3 (8.3%)	9 (26.5%)
• Complete response	4 (5.4%)	3 (8.3%)	1 (2.9%)
Toxicity			
• Not observed	47 (64.4%)	24 (66.7%)	22 (64.7%)
• Asthenia	12 (16.4%)	5 (13.9%)	7 (20.6%)
• Endocrine	4 (5.5%)	2 (5.6%)	2 (5.9%)
• Dermal	1 (1.4%)	1 (2.8%)	0 (0%)
• Gastrointestinal	3 (4.1%)	1 (2.8%)	1 (2.9%)
• Hepatic	4 (5.5%)	2 (5.6%)	2 (5.9%)
• Renal	7 (9.6%)	5 (13.9%)	2 (5.9%)
• Cardiac	1 (1.4%)	1 (2.8%)	0 (0%)
• Pulmonary	1 (1.4%)	1 (2.8%)	0 (0%)

The table shows the demographic variables of the study population of patients with advanced or metastatic NSCLC under treatment with immunotherapy at the University Hospital of Salamanca. Patients with undifferentiated carcinomas have been excluded from Table 1 due to its lack of representation (three patients).

L1 expression was $\geq 10\%$ in 21 patients and $< 10\%$ in 52 patients. Only four patients had expression levels of $\geq 50\%$. The protein expression of p53 determined by IHC was 0% in 35 patients. The p53 expression level was $\geq 5\%$ in 32 patients, and in 41 patients, the expression level was $< 5\%$. The characteristics of the sample are presented in Table 1.

The OS of the sample was 13 months (95% CI 8.2–17.8). The PFS was 5 months (95% CI 3.8–6.2). The OS of patients with adenocarcinoma histology was 16 months (95% CI 3.5–28.5), with a 4-month PFS [95% CI 2.5–5.5]. The OS of patients with squamous cell carcinoma histology was 12 months (95% CI 9–15) with a 5-month PFS (95% CI 2–8). The PFS of the PD-L1-negative subgroup was 4 months (95% CI 3–5), whereas it was 6 months (95% CI 3–9) in the PD-L1-positive subgroup ($p = 0.097$). The response results were as follows: (1) four patients (5.4%)

had a complete response, (2) 13 patients (17.8%) had a partial response, (3) stabilisation was observed in 16 patients (21.9%), and (4) progression was observed in 40 patients (54.8%). The mean number of doses administered was seven. The observed toxicity was grade ≥ 3 in eight patients (CTCAEv5.0).

3.2 Survival Analysis by Protein Expression of p53

The OS for patients above the 5% p53 cut-off point was 12 months (95% CI 7.5–16.5), and the OS was 20 months (95% CI 3.4–36.6) in patients below the cut-off (HR = 0.590 [95% CI 0.327–1.064]; $p = 0.070$) (Fig. 2). The PFS was 4 months in patients above the 5% cut-off point (95% CI 3.0–5.0), and it was 7 months (95% CI 3.3–10.7) in patients below the cut-off (HR = 0.630 [95% CI 0.370–

1.072]; $p = 0.064$). No statistical differences were observed with cut-off points of 1% (OS $p = 0.317$ and PFS $p = 0.343$) and 10% (OS $p = 0.149$ and PFS $p = 0.181$).

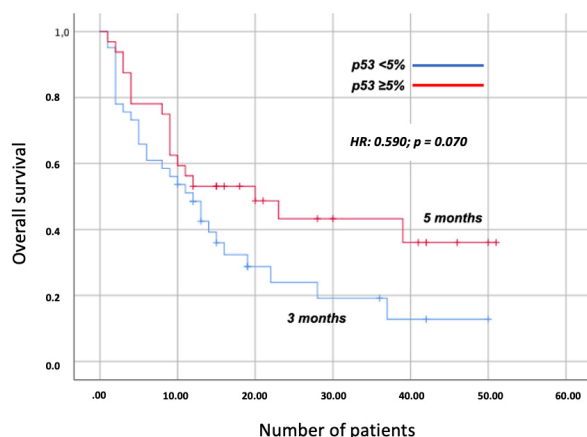


Fig. 2. Comparison of overall survival (OS) in the entire sample of patients with protein expression levels of p53 of <5% vs ≥5%. Although the results are not statistically significant, a trend toward a higher OS was observed for the entire sample in patients with expression levels of ≥5%. The OS of patients with p53 expression of ≥5% was 5 months, and 3 months in patients with p53 <5% ($p = 0.070$).

In the analysis by histology, statistically significant differences were observed for adenocarcinoma histology but not for squamous tumours. In adenocarcinomas, the OS was 8 months (95% CI 0.0–16.0) in patients with p53 expression levels of <5%, whereas the median was not reached at the time of the analysis in patients with p53 expression levels of ≥5% (HR = 0.173 [95% CI 0.049–0.616]; $p = 0.002$) (Fig. 3). The PFS was 3 months (95% CI 1.8–4.2) in patients with p53 expression levels of <5% and was 8 months [95% CI 4.6–11.4] in patients with p53 expression levels of ≥5% (HR = 0.372 [95% CI 0.155–0.894]; $p = 0.013$). In squamous cells, no differences were observed in OS ($p = 0.247$) or PFS ($p = 0.860$).

The only statistically significant differences regarding sex were observed in PFS in female patients. The PFS was 3 months (95% CI 2.2–3.8) in patients with p53 levels of <5% and was 5 months (95% CI 0.0–11.5) in patients with p53 levels of ≥5% (HR = 0.233 [95% CI 0.056–0.975]; $p = 0.020$). There were no statistically significant differences in OS ($p = 0.123$) in female patients. There were also no differences in OS ($p = 0.247$) or PFS ($p = 0.239$) in male patients. No statistically significant differences were observed in the subgroup analyses based on the age, toxicity, or site of metastatic involvement by the expression of p53.

A higher percentage of responses was observed in patients with protein expression of p53 ≥5% compared to patients with p53 <5%. All complete responses (four patients) were in tumours with p53 ≥5%. In contrast, pro-

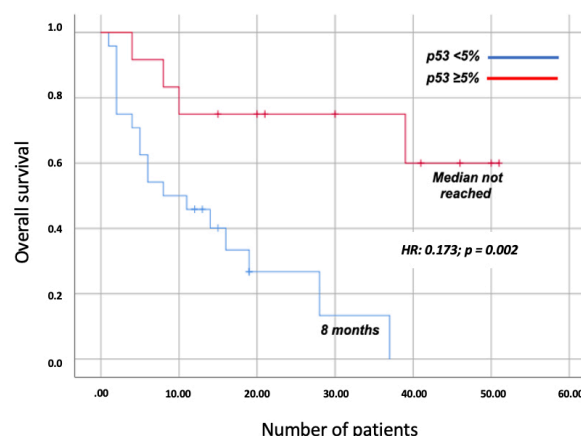


Fig. 3. Comparison of overall survival (OS) in patients with adenocarcinoma with p53 protein expression levels of <5% vs ≥5%. The results obtained are statistically significant in favour of the p53 ≥5% subgroup, with the median not reached at the time of the study for an OS of 8 months in the p53 <5% subgroup ($p = 0.002$).

gression was more frequent in patients with p53 <5% (61% of patients) vs p53 ≥5% (46.9% of patients). These findings were not statistically significant (Chi-square test, $p = 0.103$). Fig. 4 shows the responses as a function of p53 expression.



Fig. 4. Responses were obtained as a function of p53 protein expression. The absolute value is accompanied by the percentage of all patients with the same p53 expression. All complete responses were in tumours with p53 protein expression ≥5%. On the contrary, the patients with progression mostly belonged to the group with p53 expression <5% (46.9 vs 61%). *Abbreviations: CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

3.3 Survival Analysis by Expression of p53 and PD-L1

When the sample was analysed according to the positivity or negativity of PD-L1 expression, statistically signif-

icant differences were identified in the subgroup of patients with negative PD-L1. For this subgroup of patients, OS was 13 months (95% CI 11.0–15.0) in patients with p53 levels of <5% and was 39 months (95% CI 0.0–78.5) in patients with p53 levels of $\geq 5\%$ (HR = 0.298 [95% CI 0.093–0.950]; $p = 0.024$) (Fig. 5). The PFS was 3 months (95% CI 1.9–4.1) in patients with p53 levels of <5% and was 7 months (95% CI 1.5–12.5) in patients with p53 levels of $\geq 5\%$ (HR = 0.478 [95% CI 0.186–1.231]; $p = 0.070$). No differences were observed in the OS ($p = 0.449$) or PFS ($p = 0.525$) in patients with positive PD-L1. Exploratory analyses were performed with different cut-off points for PD-L1 (10%, 25%, and 50%), with no differences observed in OS or PFS between the different cohorts. Exploratory analyses have also been performed with the currently approved cut-off points for PD-L1 in NSCLC such as <1%, 1–50%, and $\geq 50\%$, without finding statistically significant results in the subgroups with PD-L1 between 1–50% (OS $p = 0.310$, PFS $p = 0.241$). Neither were differences observed by subgroups of PD-L1 as a function of age, toxicity, or other factors.

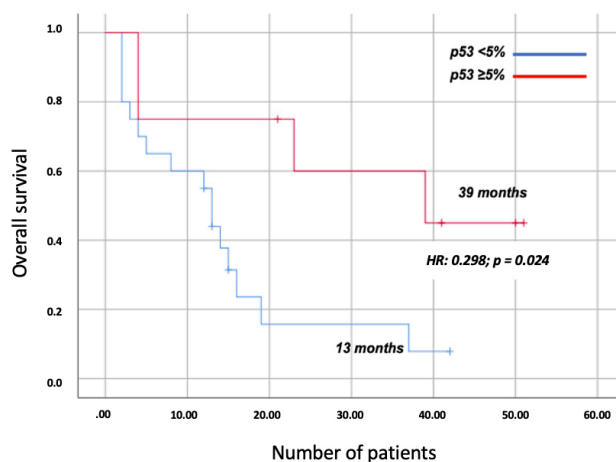


Fig. 5. The difference in overall survival (OS) of patients in the sample with negative PD-L1 expression, in which a higher OS was observed in patients with high p53 protein expression compared with those with p53 expression levels of <5%. In those with negative PD-L1, the OS of patients with p53 expression $\geq 5\%$ was 39 months, and 13 months in patients with p53 <5% ($p = 0.024$).

In the evaluation of the subgroup of patients with adenocarcinoma and negative PD-L1 expression, we observed a 14-month OS (95% CI 5–30) in patients with p53 levels of <5% compared with an unreached median OS in patients with p53 levels of $\geq 5\%$ (HR = 0.096 [95% CI 0.011–0.806]; $p = 0.010$) (Fig. 6). The PFS was 3 months (95% CI 2–4) in patients with p53 levels <5% and was 7 months (95% CI 2–11) in patients with p53 levels of $\geq 5\%$ (HR = 0.392 [95% CI 0.122–1.262]; $p = 0.078$). In the epidermoid and PD-L1 negative subgroup, no statistically significant differences

were observed in OS ($p = 0.894$) or PFS ($p = 0.624$). No statistically significant differences were observed in these analyses based on age, toxicity, or location of metastases.

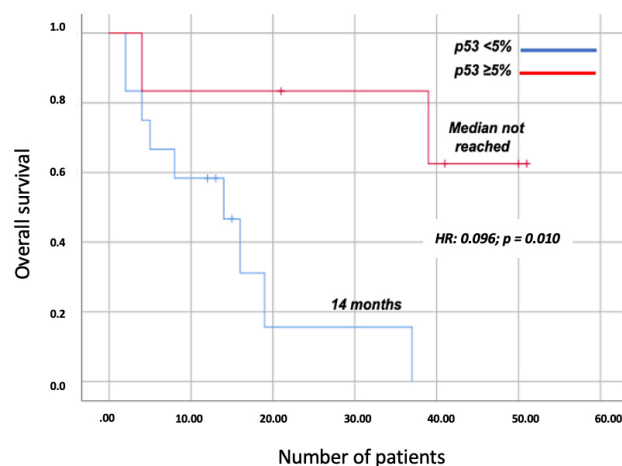


Fig. 6. The difference in overall survival (OS) between patients with advanced lung cancer, adenocarcinoma subtype, who were PD-L1 negative as a function of p53 protein expression. A greater tendency for OS was observed in patients with expression $\geq 5\%$ ($p = 0.010$).

To validate the chosen cut-off point of p53, a ROC curve has been made to show which cut-off point of p53 protein expression has the most homogeneous sensitivity and specificity. The patients were divided into two subgroups according to whether they obtained a response or not with the immunotherapy treatment, and it was compared with the percentage of p53 expression. Fig. 7 shows the ROC curve where the cut-off point of 5% is the one that presents the most homogeneous sensitivity and specificity of the entire sample.

4. Discussion

In this study, we analysed the influence of p53 mutations on the response to immunotherapy in patients with NSCLC. We performed an IHC analysis of an NSCLC sample to measure the protein expression of p53 (section 2.2). This research has a series of limitations, the first of which is due to the retrospective design of the study. Along with this, the number of patients is limited because of the difficulty of processing the samples, given that those performed by fine-needle aspiration were not accepted as valid because of technical difficulties.

Instead of genetic analysis of the tumour, we performed IHC (which can be performed at any type of centre) to determine mutations in *TP53* and assess predictive markers of response to immunotherapy [30,31]. Currently, genetic sequencing technology is not available at any centre [32]; therefore, the IHC technique was chosen as a surrogate marker of mutation in *TP53* [33]. The different studies car-

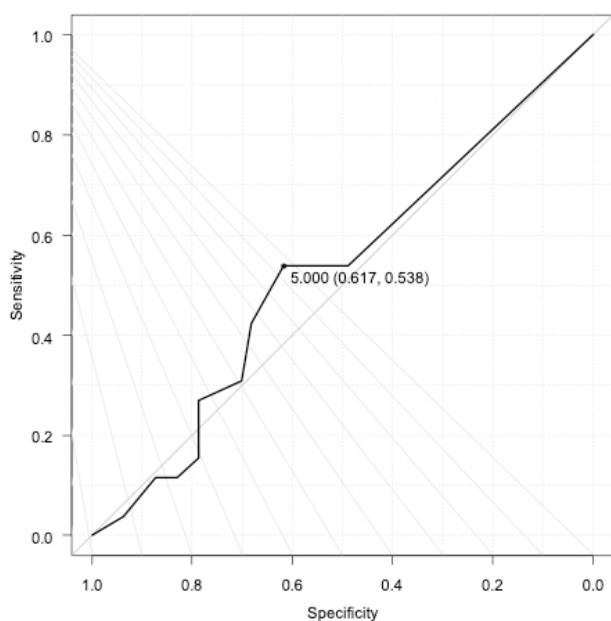


Fig. 7. ROC curve showing the cut-off point of p53 protein expression in 5% as the one that best discriminates between the possibility of mutated and non-mutated *TP53*.

ried out to date do not clearly show the influence of *TP53* on the response to immunotherapy treatments in NSCLC. Current studies are contradictory on this matter, and therefore this article aims to make a greater contribution to this field.

The choice of patients with anti-PD1 treatment as second-line therapy was a key point of our work and is crucial for understanding the analysis and the sample. Given that until 2 years ago, first-line and second-line immunotherapy treatment was classified according to PD-L1 expression levels of $\geq 50\%$ and $< 50\%$, respectively, we aspired to assemble a cohort of patients as homogeneous as possible [34]. The characteristics of the patients were similar between groups as all of them were patients with NSCLC and anti-PD1 treatment (patients without anti-PDL1 treatment were excluded) who presented with measurable disease at the beginning and during treatment with ICIs.

The main objective of the analysis was to assess whether p53 mutations (determined through IHC) were correlated with a greater response to immunotherapy treatment and, therefore, may serve as a predictive marker of response [24,25]. The global data analysis showed a trend toward enhanced survival in both OS and PFS, although the trend was not statistically significant. The absence of statistical significance was possibly due to the small sample size. These differences are consistent with those from other studies in solid tumours, such as those carried out by Assoun *et al.* [19] in NSCLC and Michel *et al.* [35] in colorectal cancer. These theoretical data are based on the increased release of antigens produced by mutations in *TP53* [36]. This creation of antigens would imply activation of the immune system

with the consequent greater response to ICIs [37].

These differences in OS and PFS can be observed in our analysis, which was dependent on the adenocarcinoma subtype and in women (two factors that are likely related in many cases). A study by Sun *et al.* [38] reported that *TP53* mutations could predict response to immunotherapy in the NSCLC adenocarcinoma subtype, although this was independent of the mutational type. According to these findings, p53 expression as a surrogate marker is very likely dependent on and exclusive to the adenocarcinoma subtype. It is possible that in adenocarcinoma subtypes, the influence of p53 is key compared to squamous cells because the TMB in the former is lower. Therefore, in squamous subtypes, high TMB would already be a biomarker of response to ICIs [39]. Together with the above, a higher percentage of responses is observed in patients with high protein expression of p53. There were only complete responses in patients with mutated *TP53*. In contrast, the percentage of progressions was 14.1% higher in patients without mutations. The results, in this case, were not statistically significant. However, it is possible that the limiting factor of the sample size accounts for this absence of statistical significance.

Another relevant finding is the fact that the high protein expression level of p53 seems more critical in tumours with negative PD-L1 expression. In these patients, OS was higher in patients with expression levels of $\geq 5\%$, and the PFS was also higher, but the difference was not statistically significant. This might be explained by the fact that the presence of *TP53* mutations may have a greater influence on immunotherapy treatment in patients with negative PD-L1 expression, who present a poorer response than patients with positive PD-L1 expression [40]. In these latter patients, the response to immunotherapy was greater because of the PD-L1 positive status, and the influence of p53 expression was lower; therefore, the results were not positive for this finding [41]. Because of this, the changes produced in the microenvironment by the *TP53* mutations have greater influence in tumours with a less activated immune system (tumours with negative PD-L1) than in tumours with a microenvironment favourable to the activation of the immune system (PD-L1 positive) [11,42].

Taken together, our results favour the determination of p53 before the start of treatment with immunotherapy, mainly in patients with the adenocarcinoma subtype. The prior determination of p53 should be evaluated in those patients whose treatment is based on immunotherapy plus chemotherapy regimens where chemotherapy could be avoided, or on the contrary in those with monotherapy ICIs, where chemotherapy addiction would be interesting.

5. Conclusions

It can be stated that survival is higher with NSCLC immunotherapy in patients with mutations in *TP53*, as assessed using a surrogate marker, such as p53 protein ex-

pression. This finding seems to be more valid in patients with the adenocarcinoma subtype. The influence of p53 on NSCLC is possibly greater on PD-L1 negative tumours due to the relatively less activation of the immune system in these tumours. Additional studies are required in the future to validate these conclusions. Due to the limitation of the sample size in the research, future studies should include a larger sample size with a greater number of patients with negative PD-L1 and adenocarcinoma subtypes.

Author contributions

Conceptualization—AO-H, EB-M, and JPM-G. Methodology—AO-H, LP-B, and LF-P. Validation—AO-H, EB-M, JPM-G, RG-S, JJC-H, MDL-C, and CP-P. Writing - original draft preparation—AO-H, JM-V, TM-G, RE-C, and RV-T. Investigation—AO-H, LF-P, and LB-H. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

This study has been approved by the ethics committee of the University Hospital of Salamanca under reference number PI 2021 10 878. It has had the informed consent of all the patients included for the analysis of the samples and has complied with the bioethical protocols current.

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Conflict of interest

The authors declare no conflict of interest.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at <https://www.imrpress.com/journal/FBL/27/3/10.31083/j.fbl2703088>.

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