PD-L1 expression by Tumor Proportion Score (TPS) and Combined Positive Score (CPS) are similar in non-small cell lung cancer (NSCLC)

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ABSTRACT

Background For non-small cell lung cancer (NSCLC) the most used method for analysing programmed cell death ligand 1 (PD-L1) expression is the Tumor Proportion Score (TPS). Nevertheless, for other tumour types, the Combined Positive Score (CPS) has been the method of choice.

Aim Evaluate and compare the predictive value of both CPS and TPS as predictors of immunotherapy response in NSCLC, and to evaluate the agreement intra-observer between both methods and inter-observer between two expert lung pathologists.

Methods 56 NSCLC patients who were treated with anti-programmed cell death 1 (PD-1)/PD-L1 therapy were included. Two pathologists evaluated all cases independently, considering the sample's adequacy for analysis, and the PD-L1 expression by TPS and CPS.

Results The Kappa coefficient for adequacy was 0.82 (95% CI 0.67 to 0.97). There was a high agreement between TPS and CPS and a high agreement between pathologists concerning the two methods. The Kappa coefficient between TPS and CPS was 0.85 for both pathologists, and between pathologists was 0.94 and 0.93 for TPS and CPS, respectively.

Conclusions Both methods proved to be equally predictive of response to anti-PD-1/PD-L1 therapy. There was both a high intra-observer agreement between the two methods and a high inter-observer agreement between pathologists. This study suggests that CPS could also be used in a routine setting for immunotherapy decision in NSCLC.

INTRODUCTION

Lung cancer is the most incident and deadliest cancer worldwide.[1] In the last few decades, targeted therapies based mainly on tyrosine kinase inhibitors have significantly improved the treatment of non-small cell lung cancer (NSCLC).[2] Despite the great improvement in the clinical management of NSCLC patients, only a subset of patients benefit from these targeted therapies.[2]

In the last few years, anti-programmed cell death 1 (PD-1)/programmed cell death ligand 1 (PD-L1) immune checkpoint inhibitors have revolutionised cancer treatment, including NSCLC.[3] In this context, a number of studies identified several predictive biomarkers of response.[4] Among these biomarkers, the PD-L1 expression, as determined by immunohistochemistry in tumour tissue, proved to be an important predictive biomarker, and several methodologies for PD-L1 immunostaining scoring have been developed.[5 6] One of the most used is the Tumor Proportion Score (TPS), which considers the percentage of tumour cells expressing PD-L1.[7–9] Limiting expression analysis to tumour cells can have some advantages, such as making the analysis easier and even allowing it to be performed on cytology cell blocks.[10] Both in the first and second-line treatment, the higher the expression of PD-L1 in tumour cells, the better the results achieved with immunotherapy, combined or not with chemotherapy.[7–9 11–14] However, for other tumour types such as gastric/gastro-oesophageal junction carcinoma, cervical carcinoma, oesophageal squamous cell carcinoma, head and neck squamous cell carcinoma, bladder and renal cancer the Combined Positive Score (CPS) which considers the expression of PD-L1 in both tumour and inflammatory cells, exhibited a better correlation with immunotherapy response.[6 15]

This study aimed to evaluate and to compare the predictive value of both CPS and TPS as predictors of response to immunotherapy in NSCLC. Additionally, were compared the concordance of both scoring systems between two distinct observers. Clinical endpoints were assessed as secondary objectives.

PATIENTS AND METHODS

Study design

This is a retrospective study that evaluated 56 NSCLC patients treated at Barretos Cancer Hospital and who have received at least one cycle of anti-PD-1/PD-L1 therapy with palliative intent and had a formalin-fixed paraffin-embedded (FFPE) tissue available for immunohistochemistry analysis of PD-L1 expression. The tissue should necessarily have been collected before the first dose of immunotherapy and for response analysis, patients should have had at least one radiological image after immunotherapy initiation to assess response to treatment.

The institutional review board approved the study protocol (CAAE 87212918.5.0000.5437) and a waiver for the written informed consent was obtained, given the retrospective nature of the study.

Table 1 Scoring methods evaluated

Method	Scoring
TPS	(No. of PD-L1 stained tumour cells/No. of tumour cells)×100
CPS	(No. of PD-L1 stained tumour and inflammatory cells*/No. of tumour cells)×100

*Lymphocytes and macrophages.
CPS, Combined Positive Score; PD-L1, programmed cell death ligand 1; TPS, Tumor Proportion Score.

Clinical data

Clinical, demographic, radiological and pathological data were collected from patient's medical records. Histological diagnosis and staging of NSCLC were based on the 2015 WHO Classification of Lung Tumors[16] and the 8th tumour, node, metastases Staging System of Lung Cancer,[17] respectively. Tumour measurement was assessed at baseline and at least at one other time point after treatment initiation. All assessments were performed by investigators using Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. Objective response rate (ORR) was defined as the proportion of patients with partial or complete radiological response to immunotherapy treatment. Overall survival (OS) and post-immunotherapy survival (PIS) were defined as the time intervals from the date of the first cycle of palliative therapy and from the first cycle of palliative immunotherapy, respectively, until death from any cause.

PD-L1 expression analysis

Immunohistochemistry staining for PD-L1 was performed on FFPE tumour tissue with Dako 22C3 pharmDx (Agilent Technologies/Dako, Carpinteria, California, USA)[18] kit, following manufacturer's instructions. PD-L1 expression was measured by TPS and CPS—table 1. Furthermore, both TPS and CPS were categorised in low (<1% for TPS and <1 for CPS), intermediate (1%–49% for TPS and 1–49 for CPS) or high (≥50% for TPS and ≥50 for TPS) expression.

Neoplastic cells had to show partial or complete membrane staining to be counted as positive, whereas immune cells were counted if any staining level was observed. A minimum number of 100 neoplastic cells were counted to consider a sample valid for its evaluation. Although, theoretically, CPS can exceed 100, the maximum score was set at 100.[6]

Two experienced lung pathologists independently evaluated all cases, considering their adequacy for analysis and the expression of PD-L1 by both TPS and CPS.

Statistical considerations

Based on the results found in gastric and oesophago-gastric junction carcinoma where the percentages of patients with PD-L1 expression ≥1% by TPS and CPS were compared (12.5% and 57.6%, respectively),[6] the sample size calculation was performed taking into account a similar difference in the proportion of positive samples, a significance level of 5% and power of 90%, what yielded a sample size of 50 tumour samples.

To analyse the agreement between the measurements (TPS vs CPS) and the reproducibility between different pathologists, it was used the Cohen's kappa coefficient (k).

The Kaplan-Meier method and the log-rank test were employed for survival analysis. A stratified Cox regression model was used to calculate HRs. Patients without an OS or PIS event were censored at the date of the last visit they were known to be alive. The OR was used to evaluate the association between response rate and PD-L1 expression by TPS and CPS.

Table 2 Clinicopathological features of NSCLC patients (n=52)

Median age=63 years old (36–81)	N (%)
Gender	
Male	33 (63.5)
Female	19 (36.5)
TNM stage	
III	6 (11.5)
IV	46 (88.5)
ECOG-PS	
0	3 (5.8)
1	44 (84.6)
2	3 (5.8)
3–4	2 (3.6)
Histology	
Adenocarcinoma	27 (51.9)
Squamous cell carcinoma	23 (44.2)
Adenosquamous	1 (1.9)
NSCLC-NOS	1 (1.9)
Smoking status	
Active	23 (45.1)
Former	20 (39.2)
Never	8 (15.7)
Unknown	1 (–)
Immunotherapy used	
Nivolumab	17 (32.6)
Pembrolizumab	18 (34.6)
Atezolizumab	4 (7.7)
Avelumab	1 (1.9)
Cemiplimab	2 (3.8)
Nivolumab+ipilimumab	9 (17.3)
Durvalumab+tremelimumab	1 (1.9)
Line of treatment	
First	30 (57.7)
Second	13 (25.0)
Third or beyond	9 (17.3)
Treatment regimen	
Anti-PD-1/PD-L1 isolated	28 (53.8)
Anti-PD-1/PD-L1+anti-CTLA4	1 (1.9)
Anti-PD-1/PD-L1+chemotherapy	14 (26.9)
Anti-PD-1/PD-L1+anti-CTLA4+chemotherapy	9 (17.3)

ECOG-PS, Eastern Cooperative Oncology Group Performance Status; NOS, not otherwise specified; NSCLC, non-small cell lung cancer; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; TNM, tumour, node, metastases.

For comparisons involving PD-L1 expression (TPS and CPS) and clinical outcomes, PD-L1 analysis performed by the pathologist 1 was used.

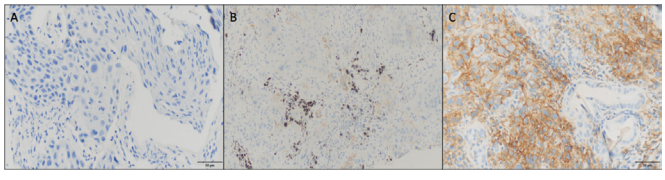

Figure 1 Examples of PD-L1 immunohistochemical staining using 22C3PhamDX kit. (A) TPS <1%; (B) TPS 1%-49%; (C) TPS ≥50% (x400 magnification). PD-L1, programmed cell death ligand 1; TPS, Tumor Proportion Score.

Table 3 PD-L1 expression analysis by TPS and by CPS according each pathologist (n=52)

TPS	P1 No. (%)	P2 No. (%)	CPS	P1 No. (%)	P2 No. (%)
PD-L1 <1%	27 (51.9)	25 (48.0)	PD-L1 <1	22 (42.3)	20 (38.4)
PD-L1 1%–49%	6 (11.5)	8 (15.3)	PD-L1 1–49	11 (21.1)	13 (25.0)
PD-L1 ≥50%	19 (36.5)	19 (36.5)	PD-L1 ≥50	19 (36.5)	19 (36.5)

CPS, Combined Positive Score; P1, pathologist 1; P2, pathologist 2; PD-L1, programmed cell death ligand 1; TPS, Tumor Proportion Score.

RESULTS

The median time between sample collection (biopsy) and FFPE tissue cutting for PD-L1 immunohistochemistry analysis was of 571 days (range from 2 to 2071 days). PD-L1 immunohistochemistry was performed in the same week (1–5 days) of FFPE tissue cutting.

Initially, the 56 cases were evaluated in terms of the adequacy of the material (at least 100 neoplastic cells). Both pathologists analysed the samples and classified them as suitable or not suitable for PD-L1 expression analysis. Most cases (n=50; 89.3%) were classified as suitable by both pathologists. One sample (1.8%) was consensually classified as unsuitable and it was not considered for further evaluation. In five (8.9%) discordant cases, they were analysed simultaneously by both pathologists, and a final consensus decision was attained. Two of these cases were considered adequate and three of them were excluded, totalising 52 samples for further analysis. The Kappa coefficient between the two pathologists for adequacy was 0.82 (95% CI 0.67 to 0.97).

The clinicopathological features of the 52 patients are summarised in table 2. Most of patients were men (63.5%) and Eastern Cooperative Oncology Group (ECOG) Performance Status 1 (84.6%). The predominant histology was adenocarcinoma (51.9%), followed by squamous cell carcinoma (44.2%) (table 2). The majority of patients presented at clinical stage IV and 15% were never smokers (table 2). Thirty patients (57.7%) were treated with anti-PD-1/PD-L1 in first-line and most of them (53.8%) received anti-PD-1/PD-L1 as monotherapy (table 2).

Regarding PD-L1 expression, the pathologist 1 (P1), using TPS, classified 27 cases (51.9%) as low, 6 cases (11.5%) as intermediate and 19 cases (36.5%) as high expression (figure 1).

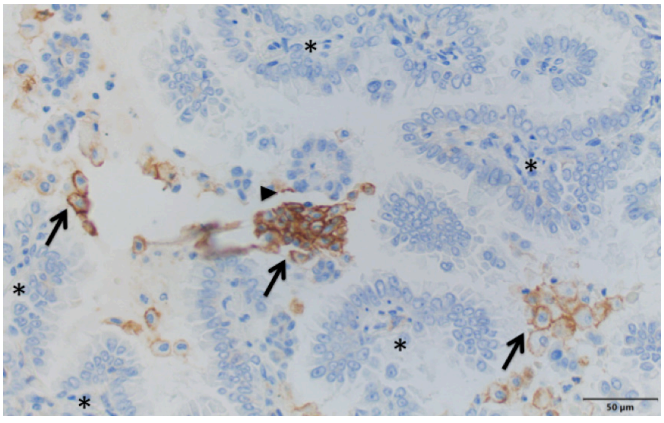

Figure 2 PD-L1 expression variability according to methodology: low PD-L1 expression by TPS (<1%) and intermediate PD-L1 expression (30) by CPS. (Arrows) Inflammatory stained cell; (Triangle) Tumoural stained cell; (Asterisks) Tumoural cell group - papillary arrangement. CPS, Combined Positive Score; TPS, Tumor Proportion Score.

Table 4 Best radiological response (Response Evaluation Criteria In Solid Tumors (RECIST) 1.1) and objective response rate among cases with TPS <1% and CPS >1, regarding at least one pathologist (n=6)

Patient ID	TPS1 (%)	CPS1	TPS2 (%)	CPS2	BRR	ORR
24	<1	02	<1	10	PD	33.3%
68	<1	30	<1	15	PD	
75	<1	05	<1	05	PR	
111	<1	32	<1	10	PR	
60	<1	01	5	10	PD	
7	<1	<1	<1	5	PD	

BRR, best radiological response; CPS1, Combined Positive Score by pathologist 1; CPS2, Combined Positive Score by pathologist 2; ORR, objective response rate; PD, Progressive Disease; PR, Partial Response; TPS1, Tumor Proportion Score by pathologist 1; TPS2, Tumor Proportion Score by pathologist 2.

Likewise, the pathologist 2 (P2), using TPS, classified 25 cases (48.0%) as low, 8 cases (15.3%) as intermediate and 19 cases (36.5%) as high expression. Considering the CPS approach, P1 classified 22 cases (42.3%) as low, 11 cases (21.1%) as intermediate and 19 cases (36.5%) as high expression, and P2 classified 20 cases (38.4%) as low, 13 cases (25.0%) as intermediate and 19 cases (36.5%) as high expression. The Kappa coefficient between TPS and CPS was 0.85 for both pathologists, and between pathologists was 0.94 and 0.93 for TPS and CPS, respectively (table 3). Only two cases (3.8%) where discordant among pathologists for TPS and CPS, and the disagreement was for cases with low and intermediate expression in both TPS and CPS methodologies. Concerning the agreement between TPS and CPS, each pathologist changed the classification of five samples, all of them from low expression to intermediate expression (figure 2). However, these cases are not exactly the same. There were four cases in which both pathologists changed the category and two cases in which only one of the pathologists changed the category, totalling six cases. The ORR observed among these patients was 33% (table 4).

For clinical endpoints all 52 cases were included and PD-L1 expression, both by TPS and CPS, was that performed by P1. Considering the response to treatment, 2 of the 52 patients were excluded since no radiological response evaluation was available. The ORR was 42.0%. The response rate was numerically higher among those who showed PD-L1 expression (intermediate or high), both by TPS (47.8% vs 37.0%) and CPS (46.4% vs 36.3%). However, the odds for response were similar regardless of the method employed (table 5). The median OS was 24.1 months (95% CI 18.3 to 30.0 months) and the median PIS was 20.2 months (95% CI 11.5 to 28.9 months). There was no statistically significant difference in survival analyses in relation to PD-L1 expression regardless of the TPS or CPS methodology employed. The median OS and PIS for TPS <1% was 21.5 months (95% CI 10.2 to 32.8 months) and 14.8 months (95% CI 6.3 to 23.3

Table 5 ORR by TPS and by CPS according to pathologist 1 (n=50)

	Total (n=50) No. (%)	Response	No response	ORR, %	OR
TPS ≥1%	23 (46)	11	12	47.8	1.56
TPS <1%	27 (54)	10	17	37.0	
CPS ≥1	28 (56)	13	15	46.4	1.51
CPS <1	22 (44)	8	14	36.3	

CPS, Combined Positive Score; ORR, overall response rate; ; TPS, Tumor Proportion Score.

Table 6 Overall survival (OS) and post-immunotherapy survival (PIS) by TPS and by CPS according to pathologist 1 (n=52)

	Median OS (months)	P value	Median PIS (months)	P value
TPS < 1%	21.5 (14.5–28.6)	0.63	14.8 (6.3–23.4)	0.29
TPS 1–49%	NR		NR	
TPS ≥50%	NR		NR	
CPS <1	21.5 (10.2–32.8)	0.59	14.8 (6.3–23.3)	0.29
CPS 1–49	32.4 (–)		NR	
CPS ≥ 50	NR		NR	

CPS, Combined Positive Score; NR, not reached; TPS, Tumor Proportion Score.

months), respectively (table 6; figures 3 and 4). For those with TPS >1%, median survival was not reached.

DISCUSSION

The effectiveness of anti-PD-1/PD-L1 therapy in NSCLC was first demonstrated in 2015 with the presentation of positive results from two phase III randomised controlled trials, CheckMate 017[11] and CheckMate 057,[12] that investigated nivolumab (anti-PD-1) as the second line of treatment for metastatic NSCLC. Despite the positive results, only about 20% of the patients had an objective response following immunotherapy. Since then, PD-L1 expression has been consolidated as the only biomarker used in clinical practice, with a consensus that PD-L1 expression on tumour cells predicts response to PD-1/PD-L1 inhibitors. However, the value of PD-L1 as the 'definitive' or agnostic biomarker is controversial and there are multiple unsolved issues such as the lack of validation for immunohistochemistry laboratory-developed tests,[19] the use of different staining platforms and antibodies, thresholds values used for PD-L1-positivity, the source and timing for sample collection and the type of cells in which PD-L1 is assessed (tumour vs immune cells).[20] To clarify this last point, we carried out this study that analysed the expression of PD-L1 by two different estimation methods, one of them including inflammatory cells in addition to tumour cells. Moreover, since PD-L1 expression is an observer-dependent measurement, NSCLC cases were independently analysed by two pathologists to reduce bias.

Among the 52 cases included, the clinicopathological features are those expected for patients with NSCLC: male predominance, adenocarcinoma histology, tobacco-related disease and stage IV. Most patients received anti-PD-1/PD-L1 treatment as the first line of treatment combined or not with anti-CTLA4 or chemotherapy, which justifies the high response rate observed and a median survival longer than 2 years. About 25% of the cases received immunotherapeutic agents not yet approved for the treatment of NSCLC such as avelumab, cemiplimab, nivolumab combined with ipilimumab and durvalumab combined with tremelimumab, since these patients were treated within randomised clinical trials.

For the analysis of PD-L1 expression, the adequacy of the material for analysis is the first issue, since at least 100 tumour cells are required and this number may not be attained due to scarcity of material, particularly in small biopsies. Regarding this stage of the process, although there was a high degree of agreement between the two pathologists, a few cases were discordant. After concurrent analysis of these five cases by the two pathologists using a double-headed microscope, there was a consensus regarding the adequacy of two out of these five samples. Although the limited number of discordant cases, it seems important to consider a double check by a second pathologist in cases considered unsuitable for analysis as it would save the patient from a new invasive procedure and avoid delays at the beginning of treatment.

Likewise, for PD-L1 expression, a high agreement between TPS and CPS and a high agreement between pathologists in relation to the two methods were reported. The main difference observed between TPS and CPS is the change from low to intermediate expression. There was no case classified as high expression only by CPS, which would potentially have stronger clinical relevance since immunotherapy alone is a robust therapeutic option in this scenario. Furthermore, there was a 100% agreement between the two pathologists in relation to samples with high expression, by both methods. There was also no difference regarding the probability of response to treatment when using TPS or CPS. Thus, external and internal reproducibility is high for both TPS and CPS and the use of CPS does not seem to demonstrate a better correlation with response to therapy based on anti-PD-1/PD-L1 agents. However, it might exist biases related to other treatments administered concomitantly with immunotherapy, which is a limitation of this study.

The expression of PD-L1 in inflammatory cells might have some role in predicting response to immunotherapy. However, only six patients were classified as TPS <1% and CPS >1 in our

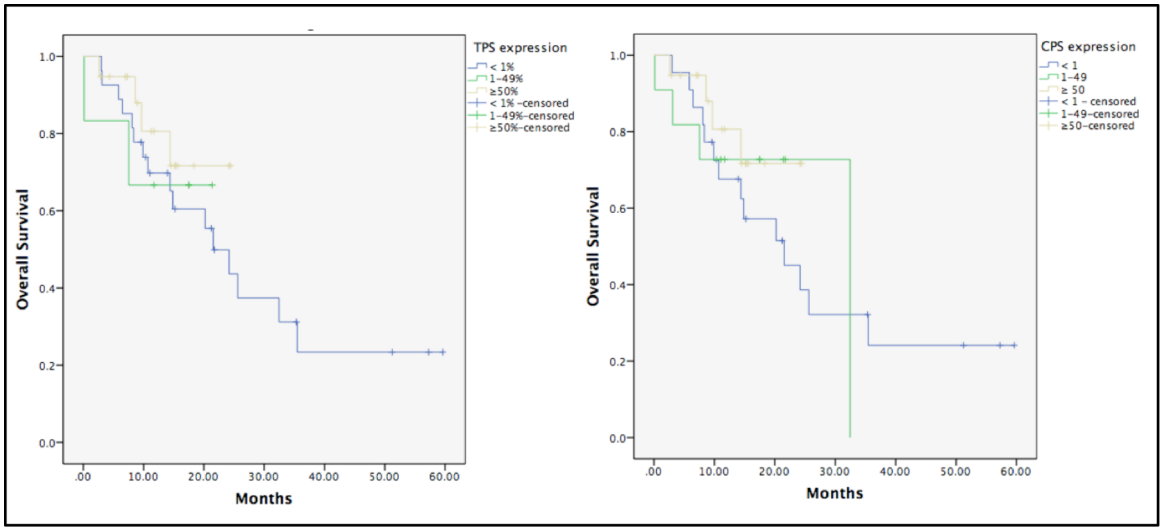

Figure 3 Overall Survival by TPS and by CPS (n=52). CPS, Combined Positive Score; TPS, Tumor Proportion Score.

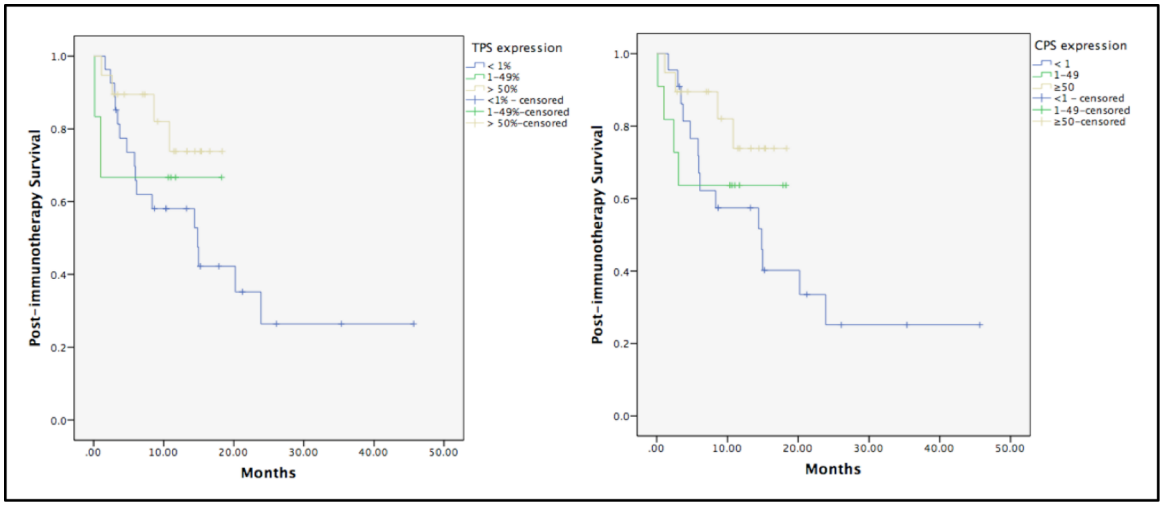

Figure 4 Post-immunotherapy Survival by TPS and by CPS (n=52). CPS, Combined Positive Score; TPS, Tumor Proportion Score.

cohort, therefore limiting any conclusion. This is a point that deserves further investigation in the future.

CONCLUSION

The determination of the adequacy of tumour samples is an essential step in the process to evaluate the expression of PD-L1 and a double-check seems to be highly recommended for cases considered unsuitable for analysis as it can save the patient from a new invasive procedure and avoid delays in treatment start.

Although the standard method to analyse PD-L1 expression in NSCLC is TPS, this study suggests that CPS can also be used and is associated with similar outcomes. However, considering that TPS's use is already consolidated in clinical practice and has a high correlation with response to anti-PD-1/PD-L1 therapy, which has been consistently demonstrated, it is more likely that TPS will remain the method of choice.

Take home messages

- Samples considered unsuitable for programmed cell death ligand 1 (PD-L1) expression analysis should be double-checked.
- For PD-L1 expression, a high agreement between Tumor Proportion Score (TPS) and Combined Positive Score (CPS) and a high agreement between pathologists in relation to the two methods were reported.
- The response rate is higher among PD-L1 positive patients, both by TPS and CPS. The odds for response were similar regardless of the method employed.

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