Original Study

Shorter Survival in Malignant Pleural Mesothelioma Patients With High PD-L1 Expression Associated With Sarcomatoid or Biphasic Histology Subtype: A Series of 214 Cases From the Bio-MAPS Cohort

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Abstract

In an analysis of programmed death ligand 1 (PD-L1) antigen expression from the phase 3 MAPS trial, PD-L1 expression was higher in sarcomatoid and biphasic malignant pleural mesothelioma (MPM) cells than in epithelioid subtypes, negatively affecting patient outcome, though not independently. In the epithelioid subset, PD-L1 strong expression significantly and independently affected progression-free survival. PD-L1 staining failed to show a prognostic role in the whole population of MPM patients, but PD-L1 high expression could affect survival in the epithelioid subtype.

Background: Anticancer immune responses are negatively regulated by programmed cell death 1 (PD-1) T-cell membrane protein interaction with its ligand, programmed death ligand 1 (PD-L1), on cancer cells. We sought to assess the prognostic role of PD-L1 expression in tumor samples from patients enrolled onto the IFCT-0701 MAPS randomized phase 3 trial (NCT00651456). **Patients and Methods:** Tumor samples were analyzed by immunohistochemistry for percentages of PD-L1 membrane-stained tumor cells using the E1L3N clone, and data were correlated to survival by multivariate Cox models including stratification variables. **Results:** PD-L1 staining was assessed in 214 (47.75%) of 448 patients. Epithelioid subtype represented 83.7% (179/214). Absence of PD-L1 staining occurred in

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137 (64.1%) of 214 malignant pleural mesothelioma (MPM) samples, while 77 (35.9%) of 214 were PD-L1 positive, with 50 (64.9%) of 77 showing < 50% PD-L1−expressing tumor cells. Sarcomatoid/biphasic subtypes were more commonly PD-L1 positive than epithelioid subtype ($P < .001$). In patients with 1% or more PD-L1−stained tumor cells, median overall survival (OS) was 12.3 months versus 22.2 months for other patients (hazard ratio [HR] = 1.25; 95% confidence interval [CI], 0.93-1.67; $P = .14$). OS did not differ according to PD-L1 positivity in multivariate analyses (adjusted HR = 1.10; 95% CI, 0.81-1.49; $P = .55$). With a 50% cutoff, PD-L1−positive patients displayed a 10.5 months median OS versus 19.3 months for patients with lower PD-L1 expression (HR = 1.93; 95% CI, 1.27-2.93; $P = .002$). OS did not significantly differ in adjusted Cox models (adjusted HR = 1.20; 95% CI, 0.74-1.94; $P = .47$). In the 179 epithelioid MPM patients, high PD-L1 staining (≥ 50% of tumor cells) negatively affected OS, although not significantly, showing a 12.3-month median OS (95% CI, 4.3-21.6) versus 23-month (95% CI, 18.5-25.2) for patients with tumor PD-L1 staining in < 50% cells ($P = .071$). The progression-free survival (PFS) differences were statistically significant, with a longer 9.9-month median PFS in patients with low PD-L1 staining (< 50% cells) compared to 6.7 months of median PFS in patients with high PD-L1 expression (≥ 50% cells) ($P = .0047$). **Conclusion:** Although high PD-L1 tumor cell expression was associated with poorer OS in MPM patients from the MAPS trial, its prognostic influence was lost in multivariate analyses in the whole cohort, while PD-L1 expression was strongly associated with the sarcomatoid/biphasic subtypes. In the epithelioid MPM subset of patients, high PD-L1 tumor expression (≥ 50%) negatively affected OS and PFS, with this prognostic influence remaining statistically significant for PFS after adjustment in multivariate Cox model.

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Introduction

Malignant pleural mesothelioma (MPM) is an aggressive tumor, histologically divided into epithelioid, sarcomatoid, and biphasic subtypes according to the World Health Organization classification of pleural tumors,[1] with the nonepithelioid subsets showing the poorest prognosis. Irrespective of the histologic subtype, MPM patients have a poor survival outcome, although median overall survival (OS) of MPM patients has recently improved as a result of the addition of bevacizumab to the conventional chemotherapy doublet. Median OS increased from 10 months with older regimens to 15 to 16 months with pemetrexed-based chemotherapy, and to 18.8 months after adding bevacizumab to the cisplatin/pemetrexed doublet.[2]

The tumor microenvironment plays a major role in the progression of several cancers.[3] Host immune responses against cancer cells were shown to be tightly and negatively regulated by the complex programmed cell death 1 (PD-1) and its main ligand, programmed death ligand 1 (PD-L1). The up-to-date view implies that cancer cells expressing PD-L1 either inhibit $CD4^+$ and $CD8^+$ T-cell activation or lead to T-cell apoptosis, thereby enabling tumor growth.[4] Of note is that PD-L1 antigen is similarly expressed by normal immune cells or endothelial cells.[5] However, through chronic inflammation due to asbestosis fiber deposits in the pleural space or into the lung, the immune system has been suspected to play a major role in MPM pathogenesis, which is yet imperfectly understood. Improved outcome was reported to correlate with higher intratumor infiltration of cytotoxic T $CD8^+$ cells.[6] Moreover, the modulation of angiogenic vasculature, leading to vessel normalization, has been shown to ease the influx of T cells, thereby favoring immunotherapy efficacy in resistant tumors, which further supports the combination of antiangiogenic drugs and checkpoint inhibitors.[7]

Recently, the use of second- or third-line immune checkpoint inhibitors (ICIs) has been shown to potentially prolong MPM patient survival. Indeed, the phase 2 IFCT-1501 MAPS2 trial involving patients with relapsing MPM initially treated by a pemetrexed/platinum doublet reported an OS of 12 and 16 months for the anti−PD-1 nivolumab monoclonal antibody or the nivolumab plus the anti−CTLA-4 ipilimumab monoclonal antibody combination, respectively.[8]

While the clinical efficacy of ICIs, which eventually resulted in registration of these drugs, has been claimed to correlate with high tumor mutational burden, as observed in melanoma or non−small-cell lung cancer patients, mesothelioma was consistently demonstrated to harbor a low mutation frequency per megabase of genomic DNA.[9] For this reason, these tumors were considered unlikely to exhibit specific sensitivity to ICI targeting PD-1/PD-L1. Nevertheless, it is still unclear which genes could possibly drive efficacy in MPM patients. The *p16* and *BAP-1* inactivating mutations, along with respective loss of expression, could possibly drive such an effect, as they both regulate cell cycle arrest and DNA repair or chromatin remodeling. Hippo gene pathway alterations (*RASSF1A* and *NF2*, but also *MST1/hippo* or *LATS2*),[9,10] by governing YAP transcriptional coactivator activity state, may likewise influence antitumor immune responses. Actually, YAP has been demonstrated to control transcription of multiple immune genes like the cytokine *CXLC5*, able to attract *CXRC2*-expressing myeloid-derived suppressor cells,[11] while cross-talks between Hippo/YAP and transforming growth factor β or JAK-STAT pathways have been extensively reported to be involved in immune response regulation.[12] We recently reported in a MAPS series that methylation and inactivation of the *MST1* gene (*hippo* in *Drosophila melanogaster*), encoding the upstream kinase leading to YAP inactivation, were associated with a worse prognosis in MPM patients.[10]

It must, however, be mentioned that the link between the host immune response and cancer mesothelioma cells is still poorly understood.

In MPM samples, PD-L1 has been reported to be expressed by 18% to 28% of tumor cells according to different studies, with a higher frequency observed in the nonepithelioid subtype,[13-16] which is correlated with a shorter OS in studies using PD-L1 SP142[16] or E1L3N clones.[15,17] However, these studies involved a single-center tumor sample collection, with limited patient numbers, heterogeneous tumor stages, or heterogeneous treatments applied.

In the current study, by assessing PD-L1 expression in 214 of 448 patients enrolled onto the phase 3 MAPS trial, we investigated the largest European multicenter prospective cohort of patients with nonresectable MPM who were all treated homogenously by a pemetrexed/platinum doublet with or without the antiangiogenic bevacizumab monoclonal antibody targeting the vascular endothelial growth factor. A central pathologic diagnostic assessment confirmed that MPM cells of sarcomatoid and biphasic subtypes more frequently expressed PD-L1 compared to the epithelioid MPM subtype. PD-L1 expression negatively affected patient outcome, yet not independently, whereas PD-L1 expression was not able to significantly predict survival after bevacizumab-based triplet therapy.

Patients and Methods

Patients and MAPS Trial

From February 13, 2008, to January 5, 2014, 448 patients were randomly assigned to treatment, with 223 (50%) assigned to pemetrexed plus cisplatin and bevacizumab and 225 (50%) to pemetrexed plus cisplatin. Tumor samples from the patients were collected by the Intergroupe Francophone de Cancérologie Thoracique (IFCT) and then sent to Caen University Hospital for biomarker characterization.

A specific informed consent was obtained for biological studies (Bio-MAPS) and approved by the trial's appointed ethics committee (CPP Ref 2007-20 Nord-Ouest III, France).

The central certification of MPM diagnosis was performed by the French National panel MESOPATH after analysis of a representative 3 µm section from each paraffin-embedded block stained with hematoxylin, eosin, and saffron, along with the quantification of calretinin, WT1, EMA, CK5/6, TTF-1, and CEA expressions, with all analyses conducted in a blinded manner as for both asbestos exposure and clinical context. The 2004 World Health Organization histopathologic international classification system for mesothelioma tumors was applied.

PD-L1 Immunohistochemistry and Scoring

Paraffin-embedded tumor blocks were cut into 3 µm slices. Slides were deparaffinized in toluene and rehydrated using standard techniques. After antigen retrieval pretreatment with pH 9.0 buffer at 100°C for 30 minutes, slides were incubated 20 minutes at room temperature with the anti−PD-L1 clone (E1L3N, sourced from CST/Ozyme, 1:400), then revealed the using Bond polymer refine detection kit on Leica Bond III autostainer, as previously described.[18] Positive internal controls were systematically evaluated (macrophage), whereas for negative controls, the primary antibody was omitted.

All slides were examined without knowledge of individual patient data. Percentages of PD-L1−stained cells (tumor proportion score) were evaluated by a thoracic pathologist from the MESOPATH group (C.D.), who was unaware of patients' clinical characteristics and treatments.

Statistical Analysis

The Bio-MAPS study was a preplanned ancillary yet exploratory study. The baseline characteristics of patients with positive or negative PD-L1 expression were compared by the chi-square test or the Fisher exact tests for qualitative variables, and the Student *t* test or Wilcoxon-Mann-Whitney test for quantitative variables, according to variable distributions.

Prognostic values for both progression-free survival (PFS) and OS, based on PD-L1 expression, were assessed by Cox models. Interaction tests were applied to evaluate predictive values. Median follow-up was estimated using the reverse Kaplan-Meier method. Multivariate Cox models were used to adjust for stratification variables (histology subtype, performance status [PS], and smoking) and treatment arm (bevacizumab-based triplet or pemetrexed/cisplatin doublet).[2] Interaction tests adjusted for stratification variables were applied to assess the PD-L1 predictive value. The data were analyzed by SPSS 15.0 for Windows (IBM, Armonk, NY) and SAS version 9.3 (SAS Institute, Cary, NC) software.

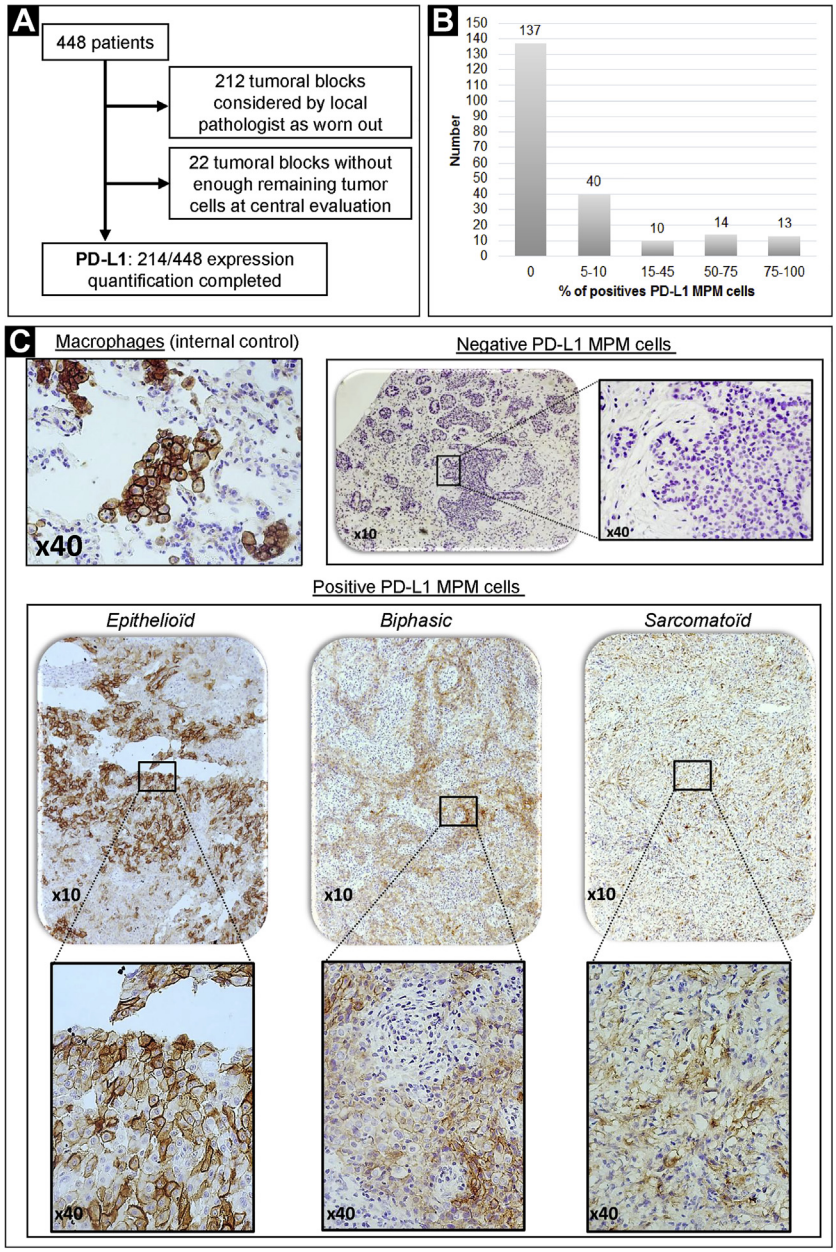
Results

PD-L1 Expression in 214 MPM Patients

PD-L1 quantification was assayed on 214 (47.75%) of 448 MPM patients accrued to the MAPS phase 3 randomized trial, given that 212 blocks, used for previous studies,[2,10] were considered exhausted by the referring pathologists, and that 22 additional formalin-fixed, paraffin-embedded collected blocks no longer actually contained any cell tumor components (Figure 1A). This study population comprised 160 male (74.8%) and 54 female (25.2%) subjects. The median age was 66.85 years (range 34.7-75.9), with occupational exposure to asbestos documented in the majority of patients by face-to-face questioning about prior professional activities. In this population, epithelioid subtype was observed in 83.7% (179/214) and sarcomatoid or biphasic subtypes in 16.3% (35/214). This patient subset did not significantly differ from the whole MAPS trial population in terms of baseline characteristics and treatment arm allocation (Supplemental Table 1 in the online version). Survival did not differ significantly either, with median survivals of 18.49 months (95% confidence interval [CI], 16.66-22.23) and 15.57 months (95% CI, 14.26-17.34) for the group with PD-L1 analysis and the group without PD-L1 analysis, respectively ($P = .34$).

PD-L1 quantification was determined in the same manner as for other cancers, with anti−PD-L1 antibodies largely used as described in the literature—that is, by evaluating the percentage of tumors cells with membrane PD-L1 expression without taking into account staining intensity (Figure 1B), eventual cytoplasmic staining, or stromal immune-cell staining. The mean score was 10.79% ± 24.11%. Of the 214 samples studied, 137 (64.1%) did not at all express PD-L1 in tumor cell components. In these cases, to discard false-negative results, we have systematically ensured that macrophages (positive control) were positive for PD-L1 expression

Figure 1 PD-L1 Expression in Patients With MPM From Bio-MAPS Cohort. (A) Disposition Chart of Patients and Pathologic Samples. (B) Distribution of PD-L1–Positive MPM Samples According to Number of PD-L1–Positive Tumor Cells. (C) Representative PD-L1 Immunostaining. Macrophages Were Used as Internal Positive Control. Among 214 Samples Studied, 137 (64.1%) Expressed No PD-L1 in Tumor Cell Components. Among Positive PD-L1 MPM Cells, Sarcomatoid or Biphasic Cells Subtypes Were More Commonly PD-L1 Positive than Epithelioid Subtype and Quantitatively Expressed More PD-L1 than Epithelioid Subtype



Abbreviations: MPM = malignant pleural mesothelioma; PD-L1 = programmed death ligand 1.

(Figure 1C). Of the 77 MPM samples (35.9%) with positive PD-L1 expression, we observed that for 50 MPM samples (64.9%), < 50% of tumor cells expressed PD-L1, whereas in the other 27 MPM samples (35.1%), 50% or more of tumor cells were PD-L1 positive. In addition, the PD-L1 staining intensity varied slightly between tumor samples while often being heterogeneous within the same tumor sample, irrespective of the histologic subtype (epithelioid, biphasic, and/or sarcomatoid) (Figure 1C). Tumor cells expressed PD-L1 either in localized areas, at the tissue surface (lining the pleural cavity), or within the thickness of the tumor mass. Finally, as previously reported, we found that the histologic subtype significantly influenced PD-L1 staining positivity, with sarcomatoid or biphasic cell subtypes

Table 1 Patient Characteristics by Presence or Absence of PD-L1

Characteristic	Variable	Value	Negative PD-L1 (N = 137)	Positive PD-L1 (N = 77)	*P*
Sex	Male	N (%)	103 (75.2)	57 (74.0)	.85
	Female	N (%)	34 (24.8)	20 (26.0)	
Age (y)		Mean ± SD	65.03 ± 7.85	65.90 ± 6.31	.65
		Median	66.58	67.12	
		Range	34.7-75.9	48.3-75.6	
		Q1; Q3	62.33; 70.18	62.69; 70.28	
Smoking	No	N (%)	54 (39.4)	37 (48.1)	.22
	Yes	N (%)	83 (60.6)	40 (51.9)	
PS	0-1	N (%)	134 (97.8)	72 (93.5)	.14
	2	N (%)	3 (2.2)	5 (6.5)	
Histology	Epithelioid	N (%)	126 (92.0)	53 (68.8)	< .001[a]
	Sarcomatoid + biphasic	N (%)	11 (8.0)	24 (31.2)	
Study arm	Chemotherapy	N (%)	66 (48.2)	40 (51.9)	.60
	Chemo+bevacizumab	N (%)	71 (51.8)	37 (48.1)	

Abbreviations: PD-L1 = programmed death ligand 1; PS = performance status; Q1 = first quartile; Q3 = third quartile; Q1-Q3 = interquartile range; SD = standard deviation.
[a]Statistically significant ($P < .05$), chi-square test.

being more commonly PD-L1 positive (Table 1) and quantitatively expressing more PD-L1 than the epithelioid subtype (Table 2). When MPM patients were stratified according to PD-L1 score ≥ 50% or < 50%, there was a nonsignificant trend toward predominant negative PD-L1 staining (< 50%) in PS 0-1 patients ($P = .07$) and in men ($P = .06$, Table 2).

PD-L1 Score and MPM Survival Outcome

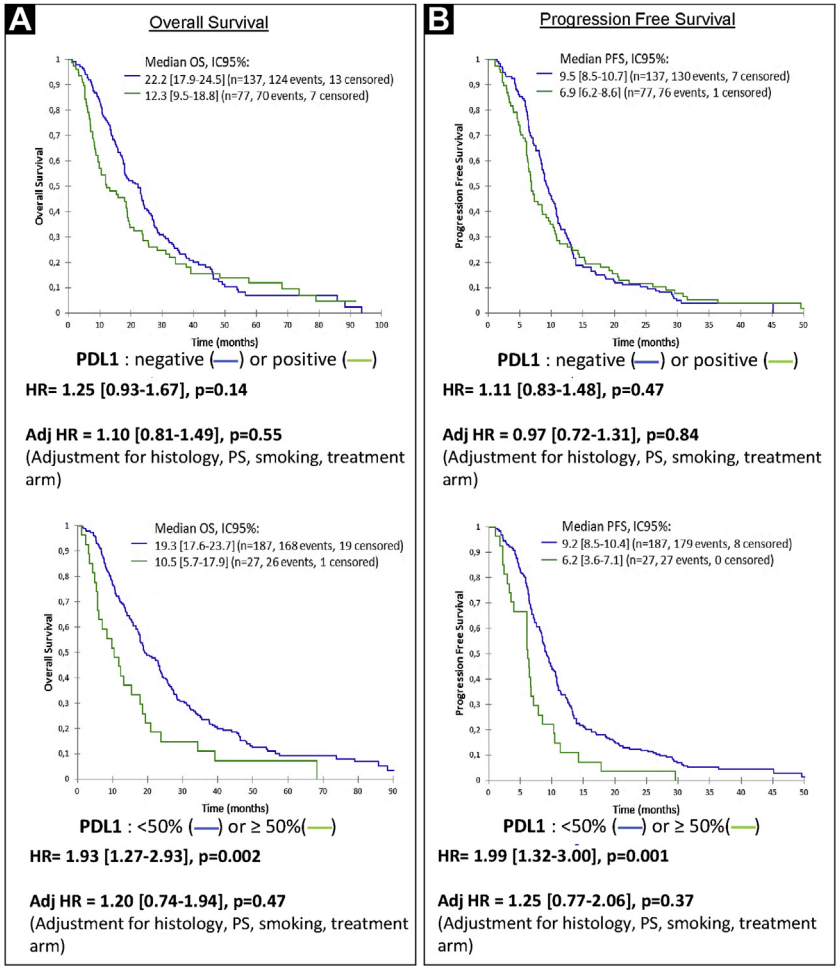
Influence of PD-L1–positive tumor cell percentages on OS and PFS in MPM patients is illustrated in Figure 2 by comparing 2 cutoffs for PD-L1 staining positivity (Figure 2, top): a cutoff set at either 1% of tumor cells or at 50% of tumor cells (< 50% vs. ≥ 50%) (Figure 2, bottom). The MPM patients' stratification appeared more informative when considering PD-L1 scoring with < 50% positive tumor cells versus ≥ 50% positive tumor cells. In patients with a PD-L1 tumor proportion score higher than 1%, median OS was 12.3 months versus 22.2 months for other patients (hazard ratio [HR] = 1.25; 95% CI, 0.93-1.67; $P = .14$). However, 2-year survivals were 28.6% (95% CI, 19-39) and 43.5% (95% CI, 35-51.6), respectively. In multivariate analyses, after adjusting for histology subtype, PS, smoking, and treatment arm, the adjusted HR (aHR) was 1.10 (95% CI, 0.81-1.49), which was not statistically significant ($P = .55$) (Figure 2A, top). When the analysis was performed using the 50% cutoff (< 50% vs. ≥ 50% PD-L1–positive tumor cells), patients with a PD-L1 intensity staining > 50% had a median OS of 10.5 months, which was worse than

Table 2 Patient Characteristics by PD-L1 ≥ 50% and < 50%

Characteristic	Variable	Value	PD-L1		*P*
			< 50% (N = 187)	> = 50% (N = 27)	
Sex	Male	N (%)	136 (72.7)	24 (88.9)	.07
	Female	N (%)	51 (27.3)	3 (11.1)	
Age (y)		Mean ± SD	65.18 ± 7.55	66.45 ± 5.58	.65
		Median	66.61	67.32	
		Range	[34.7-75.9]	[54.1-74.3]	
		Q1; Q3	62.30; 70.20	62.77; 70.73	
Smoking	No	N (%)	79 (42.2)	12 (44.4)	.83
	Yes	N (%)	108 (57.8)	15 (55.6)	
PS	0-1	N (%)	182 (97.3)	24 (88.9)	.06
	2	N (%)	5 (2.7)	3 (11.1)	
Histology	Epithelioid	N (%)	166 (88.8)	13 (48.1)	< .001[a]
	Sarcomatoid + biphasic	N (%)	21 (11.2)	14 (51.9)	
Study arm	Chemotherapy	N (%)	92 (49.2)	14 (51.9)	.80
	Chemo+bevacizumab	N (%)	95 (50.8)	13 (48.1)	

Abbreviations: PD-L1 = programmed death ligand 1; PS = performance status; Q1 = first quartile; Q3 = third quartile; Q1-Q3 = interquartile range; SD = standard deviation.
[a]Statistically significant ($P < .05$), chi-square test.

Figure 2 Kaplan-Meier Survival Curves. (A) OS and (B) PFS According to PD-L1 Expression (Positive or Negative: Upper Panels, < 50%; Lower Panels, ≥ 50%)



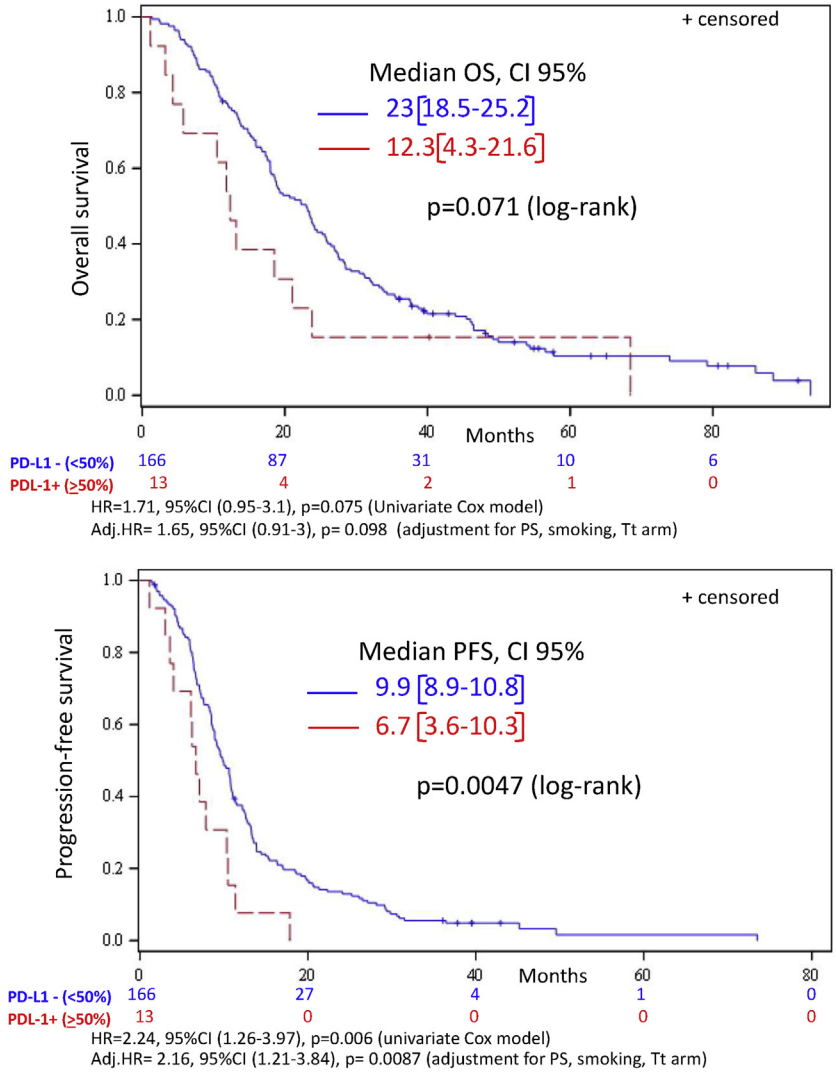
Abbreviations: OS = overall survival; PD-L1 = programmed death ligand 1; PFS = progression-free survival.

the median 19.3 months observed for patients with lower PD-L1 expression, namely < 50% of PD-L1–expressing tumor cells (HR = 1.93; 95% CI, 1.27-2.93; $P = .0016$). Two-year survivals were 14.8 months (95% CI, 4.7-30.5) and 41.5 months (95% CI, 34.4-48.4), respectively. Again, in multivariate analyses, after adjusting for histology subtype, PS, smoking, and treatment arm, the aHR was 1.20 (95% CI, 0.74-1.94), with a difference that was not statistically significant ($P = .47$) (Figure 2A, bottom). In patients with a PD-L1 intensity staining > 1%, median PFS was 6.9 months versus 9.5 months for other patients (HR = 1.11; 95% CI, 0.83-1.48; $P = .47$; aHR = 0.97; 95% CI, 0.72-1.31, $P = .84$) (Figure 2B, top). In patients with PD-L1–expressing tumor cells of > 50%, median PFS was 6.2 months versus 9.2 months for patients with lower expression (HR = 1.99; 95% CI, 1.32-3.00; $P = .001$; but aHR = 1.25; 95% CI, 0.77-2.06; $P = .37$) (Figure 2B, bottom).

Survival Prediction in Bevacizumab Arm

On the basis of the rationale for a functional interaction between immune checkpoint signaling and tumor vasculature regulation, we have investigated whether PD-L1 expression could predict the prognosis of patients treated with bevacizumab compared to patients receiving pemetrexed/platinum chemotherapy doublet only. The interaction term was therefore analyzed in order to examine whether PD-L1 expression in ≥ 50% of tumor cells could predict survival in patients undergoing the bevacizumab–pemetrexed/cisplatin triplet therapy. However, after adjusting for the stratification variables of the MAPS trial, the adjusted interaction test was not significant for OS, despite OS tending toward statistical significance (P for interaction = .12), or for PFS (P for interaction = .21). This lack of significance may, however, be accounted for by a lack of statistical power for such an analysis, with the test's statistical power divided by a factor of 4.

Figure 3 Survival According to PD-L1 Staining in Epithelioid MPM Subset



Abbreviations: MPM = malignant pleural mesothelioma; PD-L1 = programmed death ligand.

PD-L1 Expression in 179 Epithelioid MPM Patients

PD-L1 expression was available in 179 epithelioid patients of this series. There were no differences of PD-L1 expression according to age, sex, smoking, PS, or randomization arm in this subset (Supplemental Table 2 in the online version). With a cutoff of 1% for PD-L1 positivity, there was no OS difference according PD-L1 expression (Supplemental Figure 1 in the online version), although patients with negative staining (n = 126) had a slightly longer median OS (23 months; 95% CI, 18-25.76) compared to patients with positive PD-L1 staining (19 months; 95% CI, 12-25.6) (P = .8). Nor was there any difference in PFS (data not shown) between PD-L1−positive and PD-L1−negative patients (data not shown). When the PD-L1 cutoff was set to 50%, in this subgroup of epithelioid homogeneous patients, again no differences of PD-L1 positivity were found according to age, sex, smoking, PS, or treatment arm (bevacizumab triplet or chemotherapy doublet) (Supplemental Table 3 in the online version). With this cutoff, a clear trend, although not statistically significant, was found, with longer OS in patients with PD-L1 staining < 50%, showing a 23-month median OS (95% CI, 18.5-25.2), versus patients with PD-L1 staining ≥ 50%, who had a 12.3-month median OS (95% CI, 4.3-21.6; P = .071). This nonsignificant trend persisted after adjusting for PS, smoking status, treatment arm, and PD-L1 status (< 50% vs. ≥ 50% of PD-L1 positive tumor cells) (aHR = 1.65; 95% CI 0.91-3.0; P = .098). These trends were reinforced by PFS analyses showing a significantly longer 9.89-month median PFS (95% CI, 3.6-10.3) in patients with PD-L1 < 50% compared to 6.7 months of median PFS (95% CI, 3.6-10.35) in patients with PD-L1 ≥ 50% (P = .0047) (Figure 3). Such difference translated into long-term

PFS differences with 1-year PFS of 37.55% (95% CI, 30.2-45.0) and 7.69% (95% CI, 0.5-29.2) and 2-year PFS of 13.5% (95% CI, 8.8-19.3) versus 0, respectively. Multivariate analysis showed that strong PD-L1 expression (≥ 50%) was actually an independent prognosis factor (aHR = 2.16; 95% CI, 1.2-3.84; $P = .0087$) (Supplemental Table 4 in the online version).

Discussion

Using the Bio-MAPS series of MPM samples, we were able to address the question of the prognostic value of PD-L1 ICI protein expression in a large series of patients with nonresectable MPM, homogeneously treated with platinum plus pemetrexed-based combinations, either with or without bevacizumab. The academic IFCT-GFPC 0701 MAPS phase 3 trial laid the foundation of modern MPM treatment, indicated for PS 0-1 patients of maximally 75 years age, without cardiovascular comorbidities, demonstrating a significant PFS and OS advantage for bevacizumab-containing triplet therapy, compared to the historical pemetrexed/platinum doublet. This triplet therapy resulted in an extension of median OS to 18.8 months without altering quality of life, at the cost of manageable toxicities. Such a patient series has proved to be unique, which encouraged us to collect pathologic samples in order to further investigate putative prognostic biomarkers.

We found that PD-L1 tumor cell expression in MPM samples was proven to be low at diagnosis, found in 35.9% of patients, with a low mean PD-L1 score of about 11% of PD-L1–positively stained tumor cells, though inflammatory stroma has previously been reported to be the MPM hallmark. These findings are in line with previous reported series, such as the seminal article by Yamada et al[6] reporting that lymphocyte infiltration was correlated with an improved clinical outcome, possibly explaining a pivotal role in the antitumor immune response against MPMs. Recently, using the anti–PD-L1 clone 5H1-A3 antibody, Mansfield et al[13] reported a 40% positivity rate in 106 patients when both cytoplasmic and membranous staining were considered, along with a 5% cutoff. When restricting the analysis to exclusive membranous staining, such as in the MAPS series, which appears more relevant and specific, only 24% of their specimens scored positive. Likewise, Cedrés et al[15] revealed 20% positivity in their 77 specimens pertaining to a retrospective series of 119 specimens, using the very same E1L3N monoclonal antibody from Cell Signaling Technology as the one used in our series, with a 1% positivity cutoff. However, once more, both cytoplasmic and membranous tumor cell staining was considered in a series comprising a large majority of epithelioid MPM subtypes. More recently, an Australian group[17] applied tissue microarrays and E1L3N clone on 311 specimens (with 30% of nonepithelioid subtypes), which proved to be the largest series of MPM patients analyzed in the literature to date. It should, however, be noted that in this Australian series, the MPM included were of heterogeneous stages (I to IV), as were the treatments applied, although not reported in detail. PD-L1 membranous expression in ≥ 5% tumor cells was selected as the positivity cutoff, irrespective of staining intensity. In this series, 42% of patients were considered as having PD-L1–expressing tumors, but only 9.6% had high PD-L1 positivity, whereas 12.6% of the whole series exhibited moderate to high intensity in at least 50% tumor cells. These findings are similar to ours and are likewise correlated with nonepithelioid histology, as previously reported.[16] In addition, in this latter series, demographic characteristics, treatments, and patient outcomes were retrospectively retrieved form medical records, with the known biases inherent to such a methodology, in contrast to the current Bio-MAPS series, with prospectively collected data. Moreover, PD-L1 tumor expression was reported to correlate with a significantly poorer prognosis in patients with highly positive PD-L1 staining (HR = 2.37). This trend was similar to the one observed in our series, whereas survival proved to be superior in the MAPS population. However, the poorer prognosis was maintained when separately analyzing both histologic categories, namely epithelioid and nonepithelioid subtypes, with multivariate analyses not controlling for different treatment influences. The major caveat of this study was the use of tissue microarrays for a tumor reputed for its histologic heterogeneity, for which we were able to demonstrate a distinct heterogeneity level in PD-L1 staining, even within different parts of the same pathologic sample when using whole slides, while exploring at least 10 fields at 40× magnification for PD-L1 assessment.

A major finding arising from our series is that when controlling for histology and other biological factors known to be the major prognostic variables in homogeneously treated MPM patients, PD-L1 staining was no longer significantly associated with survival. Moreover, in spite of its strong rationale, PD-L1 staining did not predict the efficacy of bevacizumab, namely the vasculature-normalizing agent used in the trial. However, when the 50% cutoff for PD-L1 positivity was applied in our series, in the more homogenous subset of 179 patients with epithelioid MPM, we found a significant and independent prognosis influence of PD-L1 strong tumor staining for PFS because patients with highly PD-L1–expressing tumors had only 6.7 months' median PFS compared to 9.9 months for patients with negative or low-expressing tumors (HR = 2.16; 95% CI, 1.2-3.84; $P = .0087$). Although the same trend was clearly observed for OS, the differences did not reach statistical significance, possibly because of a lack of power in this unplanned subgroup analysis dealing with only 179 epithelioid MPM samples.

One of the main limitations of our study relies on the use of the single E1L3N monoclonal anti–PD-L1 clone, given that discrepancies in the PD-L1 staining efficiency could actually account for differences in the positivity rate observed, especially for lower levels of PD-L1 expression. Nonetheless, we have used a laboratory-developed test that had previously been validated and compared to 28.8, 22C3, and SP-263 PD-L1 assays on dedicated immunohistochemistry platforms in a large French harmonization study for PD-L1 testing in non–small-cell lung cancer.[18] The E1L3N assay on the Leica platform showed an excellent correlation with the SP263 assay used as a reference (0.78 weighted κ coefficient), exhibiting very similar staining patterns with 28.8 and 22C3 assays despite a well-known moderate-background nonspecific cytoplasmic signal.[18] Such good correlations were also found by independent groups in the United States such as the Blueprint study,[19] demonstrating that with validated staining protocols, discrepancies in PD-L1 antibodies are unlikely to be the source of discrepancies in PD-L1 positivity results among studies, and accordingly in

prognostic differences. We cannot exclude the notion that our study, despite its sample size, lacks sufficient power for evaluation of prognosis. However, although we cannot exclude this, we think it is unlikely because we have recently reported, while applying the same adjusted analyses, the high independent prognostic value of MST1 gene methylation in 223 patients with available specimens out of the very same 448 MAPS series. This sample size was close to that of the current study, suggesting that our PD-L1 study could have been perfectly powered to detect significant survival differences.[10] Last, to assess the predictive value for PD-L1 staining in patients treated with ICIs, we should await the results of large ongoing randomized trials assessing ICI efficacy used either in second- or third-line monotherapy compared to best supportive care or low-efficacy single-agent chemotherapy (vinorelbine or gemcitabine), or when used in frontline therapy in combination with pemetrexed/platinum doublet, compared to the chemotherapy doublet alone. In the MAPS2 noncomparative randomized trial assessing either the anti−PD-1 nivolumab or the anti−PD-1 nivolumab plus the anti−CTLA-4 ipilimumab combination in either the second- or third-line setting, we have previously reported that in MPM patients who experience relapse after frontline pemetrexed-based doublet, PD-L1 staining was associated with improved objective response and disease control rates, using either 1% or 25% cutoffs for PD-L1−positive tumor cell percentages, with either SP263 or 28.8 antibodies on the Dako immunohistochemistry platform.[8] In this former series, the rate of highly PD-L1−expressing tumors (with a cutoff set at 25%) was low (around 7%), while only 41% of tumors expressed PD-L1 in at least 1% of tumor cells. These reported observations corroborate the current MAPS data collected using the E1L3N clone. In this small-size series (n = 99), positive PD-L1 staining with the 28.8 clone was associated with longer survival, though not significantly (HR = 0.53; 95% CI, 0.23-1.19), yet only in patients treated with single anti−PD-1 nivolumab, whereas no impact of PD-L1 staining was found in patients receiving ICI combination.

Conclusion

Our data from this large MAPS trial are in line with most recently reported data, showing that PD-L1 staining may not have a major prognostic role in MPM (although we cannot exclude such an influence in the epithelioid subset), while its predictive impact for ICI efficacy must still be established in well-designed prospective randomized trials. Our data do not support routine use of PD-L1 staining in MPM patients, irrespective of the treatment they receive, until prospective data with immune checkpoint blocking agents become available.

Clinical Practice Points

- The Bio-MAPS cohort is the largest prospective cohort of MPM patients with disease not amenable to surgical resection in whom PD-L1 antigen expression was assessed (214/448, 48%, of the phase 3 trial MAPS population).
- Overall, PD-L1 expression is found in only 35% of MPM specimens. PD-L1 expression is more frequent in biphasic and sarcomatoid histologic subtypes than in epithelioid mesothelioma specimens.
- Patients with MPM expressing PD-L1 in $\geq$ 50% tumor cells had a 10.5-month median OS versus 19.3 months for patients with lower expression. This worse prognostic value was lost in the adjusted analysis when taking into account stratification variables (histologic subtype, PS, and smoking) and treatment arm.
- Despite a strong rationale linking antitumor immune response and angiogenesis, the predictive analysis failed to show any influence of PD-L1 staining according to antiangiogenic treatment with bevacizumab-based triplet therapy compared to pemetrexed/platinum doublet treatment.
- The MAPS phase 3 trial of MPM first-line treatment with bevacizumab the and MAPS2 randomized phase 2 trial of MPM second-line treatment with anti−PD-1−based therapies both support a 2-step strategy for MPM treatment.
- First-line bevacizumab-based triplet therapy is active on both sarcomatoid and epithelioid subtypes, irrespective of PD-L1 expression. Anti−PD-1 monoclonal antibodies provided at disease progression could be more active in patients with higher PD-L1 staining, who also are the patients with the worst prognosis, predominantly with sarcomatoid or biphasic histology.

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Disclosure

The authors have stated that they have no conflict of interest.

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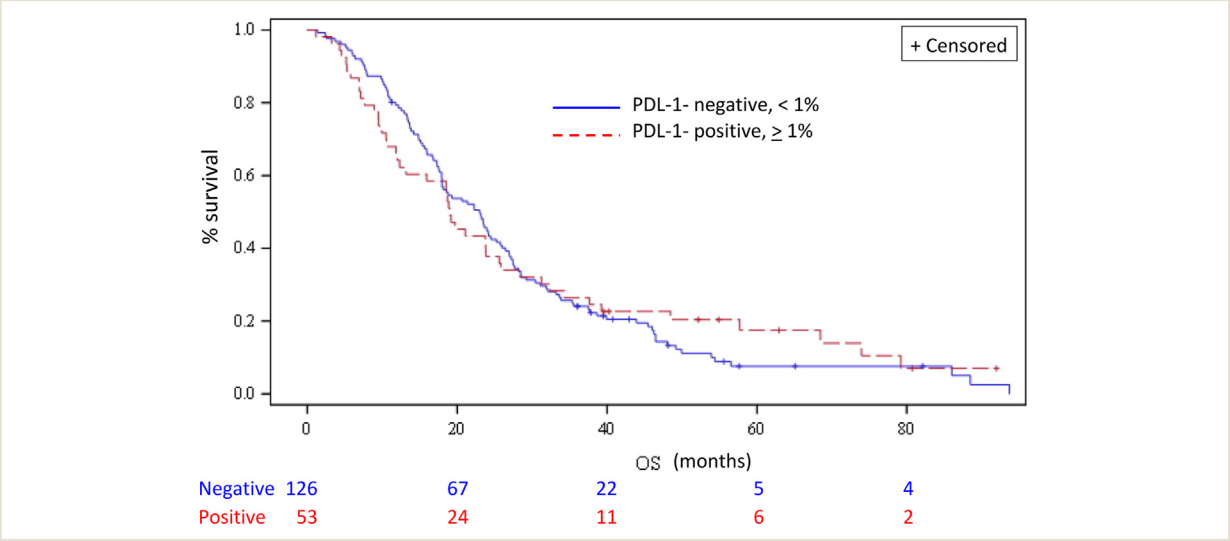
Supplemental Data

Supplemental Figure 1 OS of Epithelioid MPM Patients According to PD-L1 Tumor Expression (at 1% Cutoff)



Abbreviations: MPM = malignant pleural mesothelioma; OS = overall survival; PD-L1 = programmed death ligand 1.

Supplemental Table 1 Characteristics of 214 MPM Patients by Presence or Absence of PD-L1

Characteristic	Variable	Value	PD-L1 (N = 214)	No PD-L1 (N = 224)	*P*
Sex	Female	N (%)	54 (25.2)	54 (24.1)	.78
	Male	N (%)	160 (74.8)	170 (75.9)	
Age (y)		Mean ± SD	65.34 ± 7.33	64.56 ± 7.08	.11
		Median	66.67	64.92	
		Range	34.7-75.9	38.5-75.9	
		Q1; Q3	62.33; 70.24	60.65; 70.32	
Smoking	No	N (%)	91 (42.5)	98 (43.8)	.8
	Yes	N (%)	123 (57.5)	126 (56.3)	
PS	0-1	N (%)	206 (96.3)	217 (96.9)	.72
	2	N (%)	8 (3.7)	7 (3.1)	
Histology	Epithelioid	N (%)	179 (83.6)	177 (79)	.21
	Sarcomatoid + biphasic	N (%)	35 (16.4)	47 (21)	
Study arm	A	N (%)	106 (49.5)	116 (51.8)	.64
	B	N (%)	108 (50.5)	108 (48.2)	

Abbreviations: PD-L1 = programmed death ligand 1; PS = performance status; Q1 = first quartile; Q3 = third quartile; Q1-Q3 = interquartile range; SD = standard deviation.

Supplemental Table 2 Baseline Characteristics of Epithelioid MPM Patients With PD-L1–Positive or–Negative Tumors at 1% Cutoff

Characteristic	Variable	Value	Negative (N = 126)	Positive (N = 53)	*P*
Sex	Male	N (%)	96 (76.2)	37 (69.8)	.37
	Female	N (%)	30 (23.8)	16 (30.2)	
Age		Mean ± SD	65.36 ± 7.82	65.14 ± 6.22	.46
		Median	66.90	66.25	
		Range	34.7-75.9	48.3-74.9	
		Q1; Q3	62.77; 70.25	61.94; 69.19	
Smoking	Yes	N (%)	76 (60.3)	30 (56.6)	.64
	No	N (%)	50 (39.7)	23 (43.4)	
PS	0-1	N (%)	123 (97.6)	50 (94.3)	.36
	2	N (%)	3 (2.4)	3 (5.7)	
Study arm	A	N (%)	64 (50.8)	25 (47.2)	.66
	B	N (%)	62 (49.2)	28 (52.8)	

Abbreviations: MPM = malignant pleural mesothelioma; PD-L1 = programmed death ligand 1; PS = performance status; Q1 = first quartile; Q3 = third quartile; Q1-Q3 = interquartile range; SD = standard deviation.

Supplemental Table 3 Baseline Characteristics of Epithelioid MPM Patients With PD-L1–Positive or–Negative Tumors at 50% Cutoff

Characteristic	Variable	Value	PD-L1		*P*
			< 50% (N = 166)	≥ 50% (N = 13)	
Sex	Male	N (%)	121 (72.9)	12 (92.3)	.12
	Female	N (%)	45 (27.1)	1 (7.7)	
Age		Mean ± SD	65.2 ± 7.57	66.53 ± 3.73	.91
		Median	66.65	67.32	
		Range	34.7-75.9	59.9-72.3	
		Q1; Q3	62.33; 70.18	63.99; 69.34	
Smoking	Yes	N (%)	96 (57.8)	10 (76.9)	.18
	No	N (%)	70 (42.2)	3 (23.1)	
PS	0-1	N (%)	161 (97)	12 (92.3)	.37
	2	N (%)	5 (3)	1 (7.7)	
Study arm	A	N (%)	83 (50)	6 (46.2)	.79
	B	N (%)	83 (50)	7 (53.8)	

Abbreviations: MPM = malignant pleural mesothelioma; PD-L1 = programmed death ligand 1; PS = performance status; Q1 = first quartile; Q3 = third quartile; Q1-Q3 = interquartile range; SD = standard deviation.

Supplemental Table 4 Univariate and Multivariate Cox Analysis in 179 Epithelioid MPM Patients

Characteristic	N	Univariate Analysis			Multivariate Analysis		
		HR	95% CI	*P*	HR	95% CI	*P*
PS							
0-1	173	1			1		
2	6	4.65	2.01-10.76	.0003	4.52	1.95-10.5	.0005
Smoking							
Yes	106	1			1		
No	73	1.18	0.87-1.6	.2992	1.18	0.87-1.61	.2835
Study Arm							
A	89	1			1		
B	90	0.63	0.47-0.86	.0037	0.64	0.47-0.88	.0051
PD-L1							
<50%	166	1			1		
≥50%	13	2.24	1.26-3.97	.0060	2.16	1.21-3.84	.0087

Abbreviations: CI = confidence interval; HR = hazard ratio; PD-L1 = programmed death ligand 1; PS = performance status.