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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PD-L1 antigen expression was assayed in 214/448 patients from the Phase 3 MAPS trial. PD-L1 expression was higher in sarcomatoid and biphasic MPM cells than in epithelioid subtypes, negatively impacting patient outcome, though not independently. In the epithelioid subset of 179 patients, PD-L1 strong expression (>50% of tumor cells) significantly and independently impacted 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 impact survival in the epithelioid subtype, while its predictive impact for ICI efficacy must still be established in prospective randomized trials.

(96 words)

ABSTRACT **356 words**

Aims: Anti-cancer immune responses are negatively regulated by PD-1 (Programmed Death-1) T-cell membrane protein interaction with its ligand PD-L1 (Programmed Death-Ligand 1) on cancer cells. We sought to assess the prognostic role of PD-L1 expression in tumor samples from patients enrolled in the IFCT-0701 'MAPS' randomized Phase 3 trial (NCT00651456).

Methods: Tumor samples were analyzed by immunohistochemistry for percentages of PD-L1 membrane-stained tumor cells, using the E1L3N clone, and data correlated to survivals using multivariate Cox models including stratification variables.

Results: PD-L1 staining was assessed in 214/448 (47.75%) patients. Epithelioid subtype represented 83.7% (179/214). Absence of PD-L1 staining occurred in 137/214 MPM samples (64.1%), while 77/214 (35.9%) were PD-L1-positive, with 50/77 (64.9%) showing less than 50% PD-L1-expressing tumor cells. Sarcomatoid/biphasic subtypes were more commonly PD-L1-positive than the epithelioid subtype ($p<0.001$). In patients with 1% or more PD-L1-stained tumor cells, median OS was 12.3 months versus 22.2 months for other patients (HR= 1.25 [0.93-1.67], p=0.14). OS did not differ according to PD-L1 positivity in multivariate

analyses (adj. HR=1.10 [0.81-1.49], p=0.55). 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 [1.27-2.93], p=0.002). OS did not significantly differ in adjusted Cox models (adj. HR=1.20 [0.74-1.94], p=0.47). In the 179 epithelioid MPM patients, high PD-L1 staining (≥ 50% of tumor cells) negatively impacted OS, although not significantly, showing a 12.3-months median OS (95%CI: 4.3-21.6) versus 23-months (95%CI:18.5-25.2) for patients with tumor PD-L1 staining in <50% cells, p=0.071. The PFS differences were statistically significant with a longer 9.9-months median PFS in patients with low PD-L1 staining (<50% cells), compared with 6.7 months of median PFS in patients with high PD-L1 expression (≥50% cells), p-value= 0.0047.

Conclusions: 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 impacted OS and PFS, this prognosis influence remaining statistically significant for PFS after adjustment in multivariate Cox model.

Keywords: malignant pleural mesothelioma, PD-1, PD-L1, immunohistochemistry

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], the non-epithelioid subsets showing the poorest prognosis. Irrespective of the histological subtype, MPM patients have a poor survival outcome, although median overall survival (OS) of MPM patients has recently improved due to the addition of bevacizumab to the conventional chemotherapy doublet: Median OS increased from 10 months with older regimens to 15-16 months with pemetrexed-based chemotherapy, and to 18.8 months after adding bevazicumab 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 PD-1 (Programmed Death-1) and its main ligand PD-L1 (Programmed Death-Ligand 1). 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 intra-tumor 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 anti-angiogenic drugs and checkpoint inhibitors. [7]

Recently, the use of second- or third-line immune checkpoint inhibitors has been shown to potentially prolong MPM patient survival. Indeed, the Phase 2 IFCT-1501 'MAPS2' trial involving relapsing MPM patients 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 immune checkpoint inhibitors (ICI), 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 (NSCLC) 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, yet also MST1/hippo or LATS2) [9-10], by governing YAP transcriptional co-activator activity state, may likewise influence anti-tumor 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 (MDSC) [11], while cross-talks between Hippo/YAP and TGF-β or JAK-STAT pathways have been extensively reported to be involved in immune response regulation. [12] We have recently reported, in the MAPS series, that methylation and inactivation of 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 tumors cells according to different studies, with a higher frequency observed in the non-epithelioid subtype, [13-16] which 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 out of the 448 patients enrolled in the Phase 3 MAPS trial, we have investigated the largest European multicenter prospective cohort of non-resectable MPM patients who were all treated homogenously by a

pemetrexed-platinum doublet plus or minus the anti-angiogenic bevacizumab monoclonal antibody targeting the vascular endothelial growth factor (VEGF). A central pathological diagnostic assessment confirmed that MPM cells of sarcomatoid and biphasic suptypes more frequently expressed PD-L1 as compared to the epithelioid MPM subtype. PD-L1 expression negatively impacted patient outcome, yet not independently, whereas PD-L1 expression was not able to significantly predict survival following bevacizumab-based triplet therapy.

MATERIEL & METHODS

Patients and MAPS trial

From February 13, 2008, to January 5, 2014, 448 patients were randomly assigned to treatment (223 [50%] to PCB (pemetrexed plus cisplatin and bevacizumab) and 225 [50%] to PC (pemetrexed plus cisplatin)). Tumor samples from the patients were collected by the IFCT and then sent to the 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 following analysis of a representative 3µm section from each paraffin-embedded block stained with hematoxylin, eosin, and safran, along with the quantification of calretinin, WT1, EMA, CK5/6, TTF-1, and CEA expressions, all analyses conducted in a blinded manner as for both asbestos exposure and clinical context. The histopathological international classification system (WHO 2004) for mesothelioma tumors was applied.

PD-L1 immunohistochemistry and scoring

Tumor paraffin-embedded blocks were cut into 3µm slices. Slides were de-paraffinized in toluene and rehydrated using standard techniques. After antigen retrieval pretreatment with

pH 9.0 buffer, at 100°C for 30 min, slides were incubated 20 minutes at room temperature with the anti-PD-L1 clone (E1L3N, sourced from CST/Ozyme, 1:400), then revealed 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 or TPS), were evaluated by a thoracic pathologist from the MESOPATH group (CD), who was blinded in terms of clinical patient characteristics and treatments.

Statistical Analysis

The Bio-MAPS study was a pre-planned ancillary, yet exploratory study. The baseline characteristics of patients with positive or negative PD-L1 expression were compared using chi-squared tests or Fisher's exact tests for qualitative variables, and Student's t-tests or Wilcoxon-Mann-Whitney for quantitative variables, according to variable distributions.

Prognostic values for both progression-free survival (PFS) and OS, based on PD-L1 expression, were assessed using 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 employed to adjust for stratification variables (histology subtype, 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 using SPSS software SPSS for Windows Version 15.0, Chicago, IL: SPSS, Inc., 2006), and SAS software, Version 9.3 (SAS Institute Inc., Cary, NC, USA).

RESULTS

PD-L1 expression in the 214 MPM patients

PD-L1 quantification was assayed on 214 of the 448 (47.75%) 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 FFPE collected blocks actually contained no longer any cell tumor components (Figure 1A). This study population comprised 160 males (74.8%) and 54 females (25.2%). 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 **(suppl. Table 1**). Survivals did not differ significantly either, with median survivals of 18.49 95%CI (16.66-22.23) and 15.57 95%CI (14.26-17.34) months for the group with PD-L1 analysis and the group without PDL1 analysis, respectively (p=0.34).

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, *i.e.*, by evaluating the percentage of tumors cells with membrane PD-L1 expression without taking into account: *i)* staining intensity (Figure 1B); *ii)* eventual cytoplasmic staining; *iii)* stromal immune cells 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 negatives, we have systematically ensured that macrophages (positive control) were positive for PD-L1 expression (Figure 1C). Of the 77 (35.9%) MPM samples with positive PD-L1 expression, we observed that for 50 MPM (64.9%) samples, less than 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, whilst often being heterogeneous within the same tumor sample, irrespective of the histological 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. Lastly, as previously reported, we found that the

histological subtype significantly influenced PD-L1 staining positivity, sarcomatoid or biphasic cell subtypes 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 non-significant trend towards predominant negative PD-L1 staining (< 50%) in PS 0-1 patients (p=0.07) and in men (p=0.06, 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 two cutoffs for PD-L1 staining positivity (Figure 2A (OS) and 2B (PFS), upper graphs): a cutoff set at either 1% of tumor cells or at 50% of tumor cells (<50% vs. ≥50%) (Figure 2A (OS) and 2B (PFS), lower graphs). 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 TPS higher than 1%, median OS was 12.3 months versus 22.2 months for other patients (HR= 1.25 [0.93-1.67], p=0.14. However, 2-years 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 was 1.10 [0.81-1.49], being statistically not significant (p=0.55) (Figure 2A upper graph). When the analysis was performed using the 50% cutoff (<50% vs. ≥50% PD-L1 positive tumor cells), patients with a PD-L1 intensity staining higher than 50% had a median OS of 10.5 months, which was poorer than the median 19.3 months observed for patients with lower PD-L1 expression, namely less than 50% of PD-L1-expressing tumor cells (HR= 1.93 [1.27-2.93], p=0.0016). 2-years survivals were 14.8 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 adjusted HR was 1.20 [0.74-1.94], the difference being statistically not significant (p=0.47) (Figure 2A lower graph). In patients with a PD-L1 intensity staining higher than 1%, median PFS was 6.9 months versus 9.5 months for other patients (HR= 1.11 [0.83-1.48], p=0.47, Adj HR = 0.97

[0.72-1.31], p=0.84 Figure 2B upper graph). In patients with PD-L1-expressing tumor cells of more than 50%, median PFS was 6.2 months versus 9.2 months for patients with lower expression (HR= 1.99 [1.32-3.00], p=0.001, but adj HR = 1.25 [0.77-2.06], p=0.37 Figure 2B lower graph).

PD-L1 score to not significantly predict survival in the bevazicumab arm

Based on 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, as compared with 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 or more 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 neither significant for OS, despite OS tending towards statistical significance (p for interaction=0.12), nor for PFS (p for interaction=0.21). 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 four.

PD-L1 expression in the 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 (suppl. Table 2A). With a cut-off of 1% for PD-L1 positivity, there was no overall survival difference according PD-L1 expression (suppl. Figure 1), although patients with negative staining (n=126) had a slightly longer median OS (23 months, 95%CI: 18-25.76) as compared with patients with positive PD-L1 staining (19 months, 95%CI: 12-25.6), (p-value=0.8). There was no PFS difference either (data not shown) between PD-L1-positive and PD-L1-negative patients (data not shown). When the PD-L1 cut-off was set to 50%, in this sub-group of epithelioid homogeneous patients, again no differences of PD-L1 positivity was found according to age, sex, smoking, PS, or treatment arm (bevacizumab triplet or

chemo doublet) (suppl. Table 2B). With this cut-off, a clear trend, although not statistically significant yet, was found, with longer OS in patients with PD-L1 staining <50%, showing a 23 months median OS (95%CI:18.5-25.2), versus patients with PD-L1 staining ≥50%, who had a 12.3 median OS (95%CI: 4.3-21.6), p=0.071. Such not significant trend persisted after adjusting for PS, smoking status, treatment arm and PD-L1 status (<50% vs. ≥50% of PD-L1 positive tumor cells), with adj. HR=1.65, 95%CI (0.91-3.0), p-value=0.098. These trends were re-enforced by PFS analyses showing a significantly longer 9.89 months median PFS (95%CI: 3.6-10.3) in patients with PD-L1<50%, compared with 6.7 months of median PFS (95%CI: 3.6-10.35) in patients with PD-L1≥50%, p-value= 0.0047 (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 with adj. HR= 2.16, 95%CI(1.2-3.84), p=0.0087 (suppl. Table 2C)

DISCUSSION

Using the Bio-MAPS series of MPM samples, we were able to address the question of the prognostic value of the PD-L1 immune checkpoint inhibitory protein expression in a large series of patients with non-resectable 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, as compared with 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 proves to be unique, which encouraged

us to collect pathological samples in order to further investigate putative prognostic biomarkers. In this study, we were able to show 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 paper by Yamana et al. reporting that lymphocyte infiltration was correlated with an improved clinical outcome, possibly plaing a pivotal role in the antitumor immune response against MPMs. [6] Recently, using the anti-PD-L1 clone 5H1-A3 antibody, Mansfield et al. reported a 40% positivity rate in 106 patients, when both cytoplasmic and membranous staining were considered, along with a 5% cutoff. [13] 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. 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. [15] 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 non-epithelioid 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% or more tumor cells was selected as 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 very similar finding to ours and likewise correlated with non-epithelioid 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 survivals proved to be superior in the MAPS population. However the poorer prognosis was maintained when separately analyzing both histological categories, namely epithelioid and non-epithelioid subtypes, with multivariate analyses not controlling for different treatment influences. The major caveat of this study was the use of TMA for a tumor reputed for its histological heterogeneity, for which we were able to demonstrate a distinct heterogeneity level in PD-L1 staining, even within different parts of the same pathological sample when using whole slides, while exploring at least 10 fields at x 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% cut-off 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, since patients with highly PD-L1 expressing tumors had only 6.7 months median PFS as compared with 9.9 months for patients with negative or low-expressing tumors, HR=2.16, 95%CI (1.2-3.84), p=0.0087. 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, as discussed below.

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 employed a laboratory-developed test (LDT) that had previously been validated and compared with 28.8, 22C3, SP-263 PD-L1 assays on dedicated immunohistochemistry platforms, in the large French harmonization study for PD-L1 testing in NSCLC. [18] The E1L3N assay on LEICA platform showed an excellent correlation with SP263 assay used as a reference (0.78 weighted kappa coefficient), exhibiting very similar staining patterns with 28.8 and 22C3 assays, despite a well-known moderate background not-specific cytoplasmic signal[18]. Such good correlations were also found by independent groups in the U.S. Blue-Print study[19], demonstrating that with validated staining protocols, discrepancies in PD-L1 antibodies are unlikely the source of discrepancies in PD-L1 positivity results among studies, and accordingly, in prognostic differences. We cannot exclude that our study, despite its sample size, lacks sufficient power for prognosis evaluation. However, though we cannot exclude this, we feel it to be rather unlikely, since 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 very close to that of the current study, suggesting that our PD-L1 study could have been perfectly powered to detect significant survival differences. [10] Lastly, to assess the predictive value for PD-L1 staining in patients treated with immune checkpoint inhibitors, we should await the results of large on-going randomized trials assessing ICI efficacy, used either in 2nd- or 3^{d}-line monotherapy as compared with best supportive care or low-efficacy chemotherapy single-agent (vinorelbin or gemcitabine), or when employed in frontline therapy in combination with pemetrexed-platinum doublet, as compared with the chemo doublet alone. In the MAPS2 non-comparative randomized trial assessing either the anti-PD-1 nivolumab or the anti-PD1 nivolumab plus the anti-CTLA-4 ipilimumab combination, in either 2nd or 3rd line setting, we have previously reported that, in MPM patients relapsing 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 DAKO IHC 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 limited-size series (n=99), positive PD-L1 staining with 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 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.

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Legends.

Figure1. PD-L1 expression in patients with MPM from the Bio-MAPS cohort.

A) Disposition chart of patients and pathological samples.

B) Distribution of PD-L1 positive MPM samples according to the number of PD-L1positive tumor cells

C) Representative PD-L1 immunostaining. Macrophages were used as internal positive control. Among the 214 samples studied, 137 (64.1%) did not at all express PD-L1 in the tumor cell components (see “Negative PD-L1 MPM cells” panel). Among the positive PD-L1 MPM cells, sarcomatoid or biphasic cells subtypes were more commonly PD-L1 positive than the epithelioid subtype (Table1) and quantitatively expressed more PD-L1 than rhe epithelioid subtype (Table2) (s “Positive PD-L1 MPM cells” panel).

Figure2. **Kaplan-Meier curve of overall survival (A) and progression free survival (B) according to PDL1 expression (positive or negative:** upper panels, **<50% or ≥50%**: lower panels**).**

Table1. Positive PD-L1 population *versus* negative PD-L1 population comparison

Descriptive statistics			Negative PD-L1 (N=137)	Positive PD-L1 (N=77)	p-value
Sex	Male	N (%)	103 (75.2)	57 (74.0)	0.85
	Female	N (%)	34 (24.8)	20 (26.0)	
Age (years)		Mean ± SD	65.03 ± 7.85	65.90 ± 6.31	0.65
		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)	0.22
	Yes	N (%)	83 (60.6)	40 (51.9)	
PS	0-1	N (%)	134 (97.8)	72 (93.5)	0.14
	2	N (%)	3 (2.2)	5 (6.5)	
Histology	Epithelioïd	N (%)	126 (92.0)	53 (68.8)	**<0.001**
	Sarcomatoïd + Biphasic	N (%)	11 (8.0)	24 (31.2)	
Arm	A	N (%)	66 (48.2)	40 (51.9)	0.60
	B	N (%)	71 (51.8)	37 (48.1)	

PS: performance status

Table2. PD-L1 ≥50% population *versus* PD-L1 <50% population comparison

Descriptive Statistics			<50% (N=187)	>=50% (N=27)	p-value
Sex	Male	N (%)	136 (72.7)	24 (88.9)	0.07
	Female	N (%)	51 (27.3)	3 (11.1)	
Age (years)		Mean ± SD	65.18 ± 7.55	66.45 ± 5.58	0.65
		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)	0.83
	Yes	N (%)	108 (57.8)	15 (55.6)	
PS	0-1	N (%)	182 (97.3)	24 (88.9)	0.06
	2	N (%)	5 (2.7)	3 (11.1)	
Histology	Epithélioïde	N (%)	166 (88.8)	13 (48.1)	**<0.001**
	Sarcomatoïd + Biphasic	N (%)	21 (11.2)	14 (51.9)	
Arm	A	N (%)	92 (49.2)	14 (51.9)	0.80
	B	N (%)	95 (50.8)	13 (48.1)	

PS: performance status

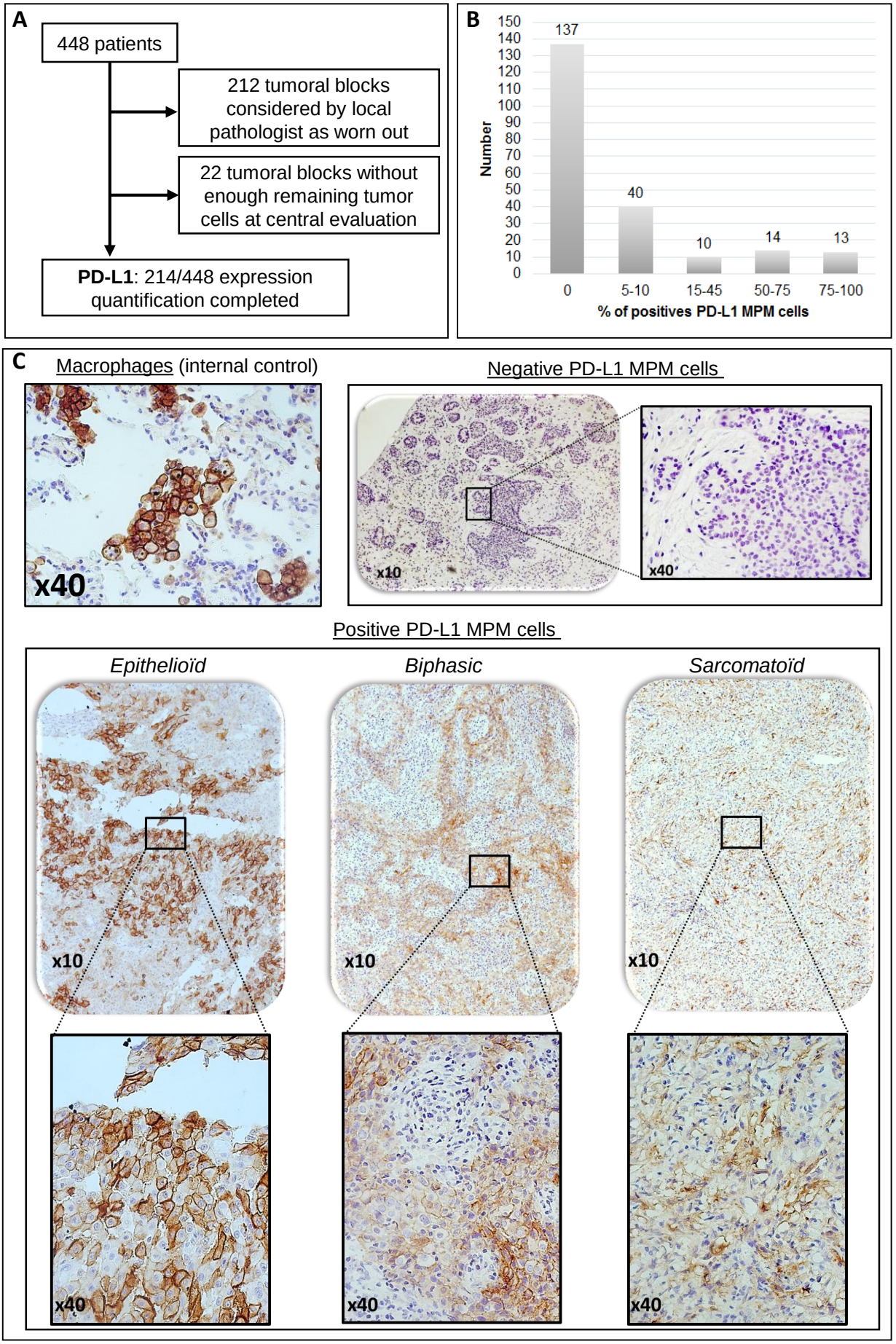
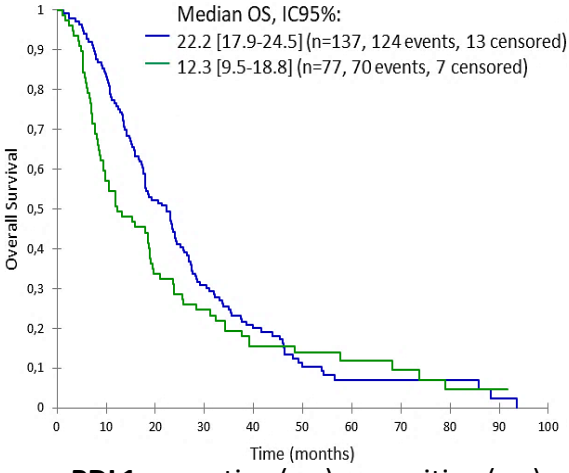

A
448 patients
212 tumoral blocks considered by local pathologist as worn out
22 tumoral blocks without enough remaining tumor cells at central evaluation
PD-L1: 214/448 expression quantification completed
B
Number
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
137
40
10
14
13
0
5-10
15-45
50-75
75-100
% of positives PD-L1 MPM cells
C
Macrophages (internal control)
x40
Negative PD-L1 MPM cells
x10
x40
Positive PD-L1 MPM cells
Epithelioïd
Biphasic
Sarcomatoïd
x10
x10
x10
x40
x40
x40

Figure1.

A

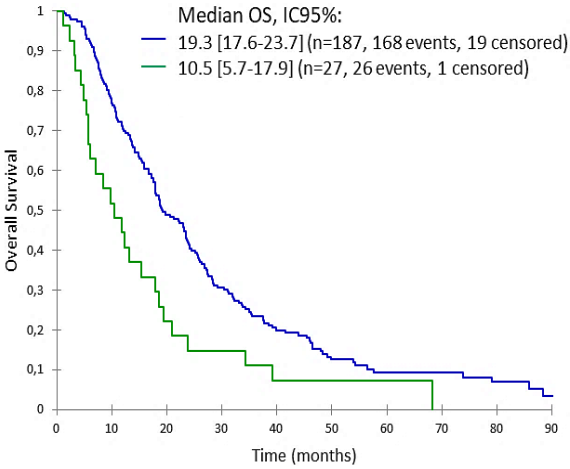
Overall Survival



PDL1 : negative (—) or positive (—)

HR= 1.25 [0.93-1.67], p=0.14

Adj HR = 1.10 [0.81-1.49], p=0.55
(Adjustment for histology, PS, smoking, treatment arm)



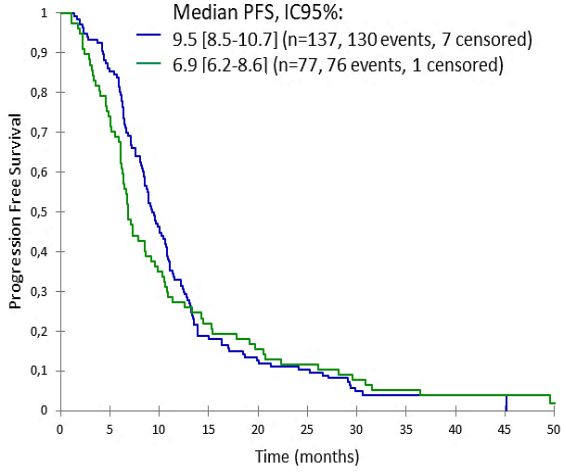
PDL1 : <50% (—) or ≥ 50%(—)

HR= 1.93 [1.27-2.93], p=0.002

Adj HR = 1.20 [0.74-1.94], p=0.47
(Adjustment for histology, PS, smoking, treatment arm)

B

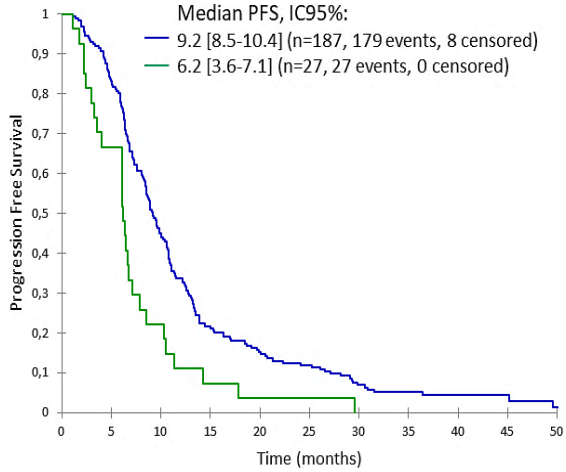
Progression Free Survival



PDL1 : negative (—) or positive (—)

HR= 1.11 [0.83-1.48], p=0.47

Adj HR = 0.97 [0.72-1.31], p=0.84
(Adjustment for histology, PS, smoking, treatment arm)



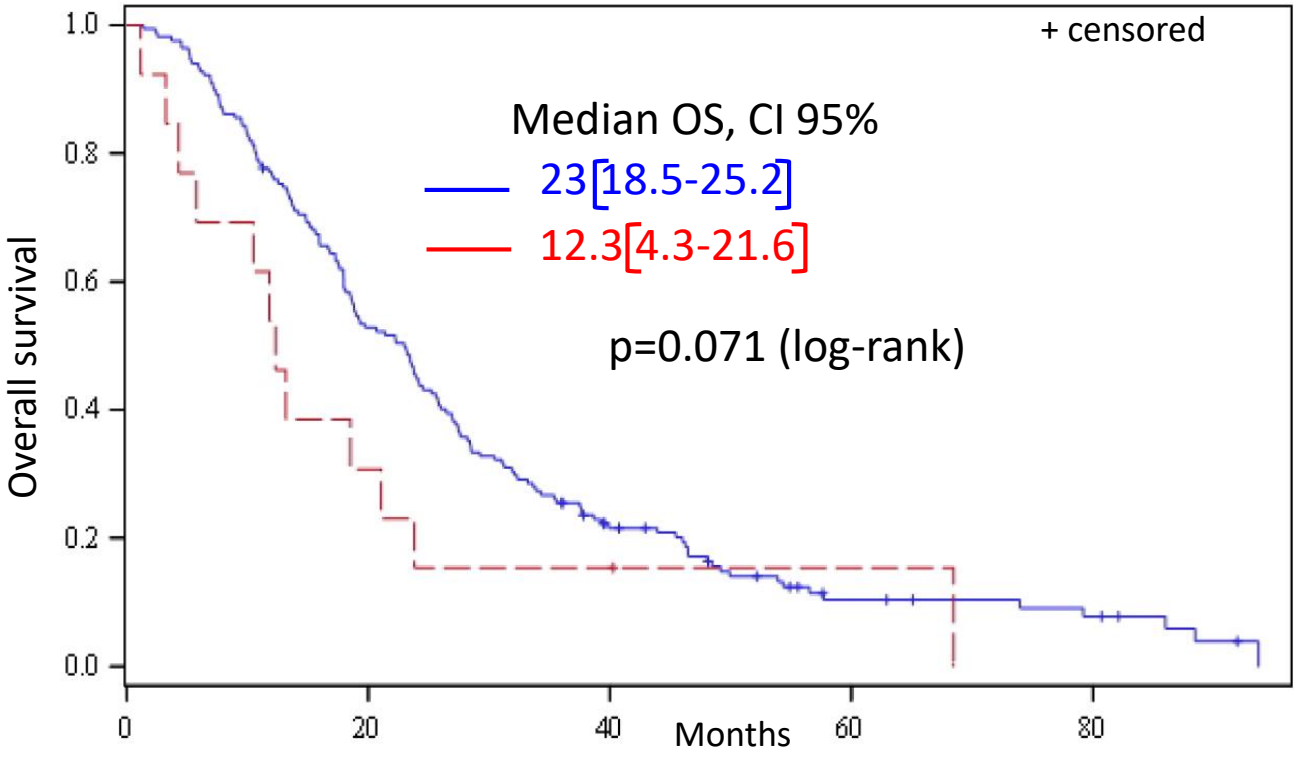
PDL1 : <50% (—) or ≥ 50%(—)

HR= 1.99 [1.32-3.00], p=0.001

Adj HR = 1.25 [0.77-2.06], p=0.37
(Adjustment for histology, PS, smoking, treatment arm)

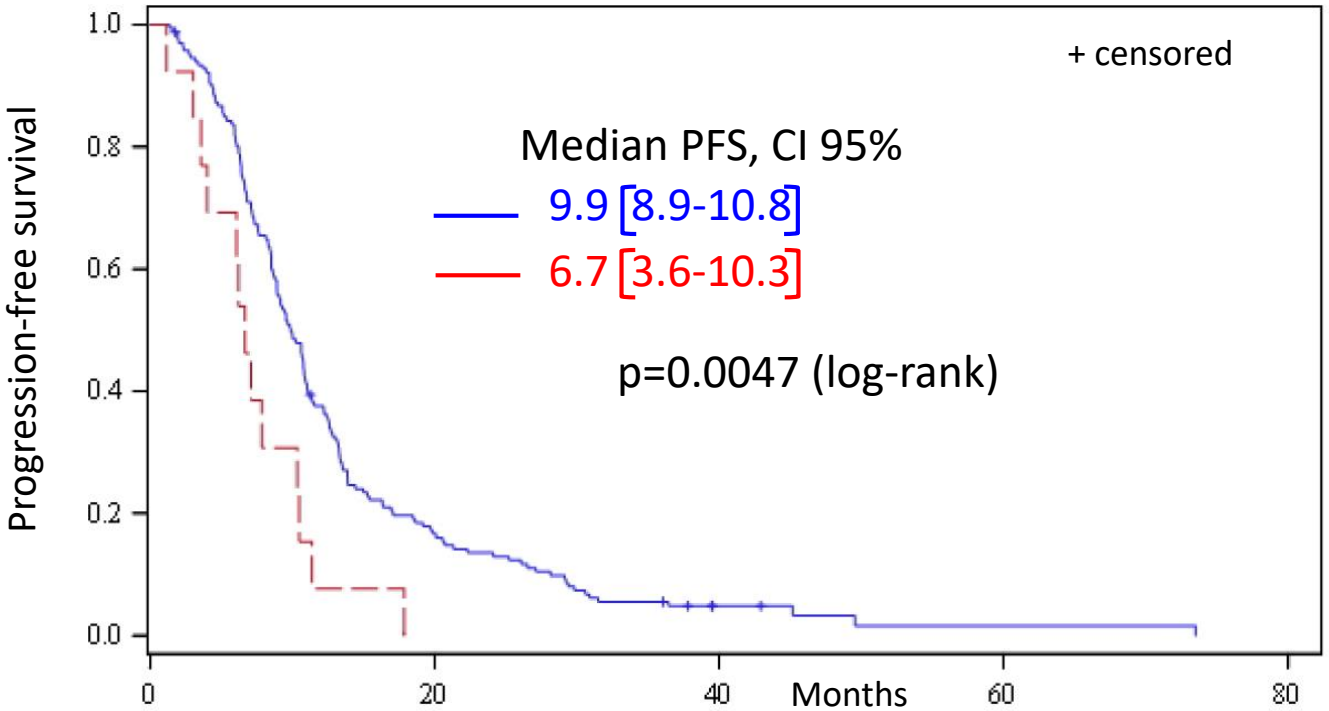
Figure 2.

Figure 3. **Survival according to PD-L1 staining in the epithelioid MPM subset**



PD-L1 - (<50%)	166	87	31	10	6
PDL-1+ (≥50%)	13	4	2	1	0

HR=1.71, 95%CI (0.95-3.1), p=0.075 (Univariate Cox model)
Adj.HR= 1.65, 95%CI (0.91-3), p= 0.098 (adjustment for PS, smoking, Tt arm)



PD-L1 - (<50%)	166	27	4	1	0
PDL-1+ (≥50%)	13	0	0	0	0

HR=2.24, 95%CI (1.26-3.97), p=0.006 (univariate Cox model)
Adj.HR= 2.16, 95%CI (1.21-3.84), p= 0.0087 (adjustment for PS, smoking, Tt arm)