Prognostic impact of PD-1 and PD-L1 expression in malignant pleural mesothelioma: an international multicenter study

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Background: Programmed cell death 1/programmed death ligand 1 (PD-1/PD-L1) immune-checkpoint blockade is a promising new therapeutic strategy in cancer. However, expression patterns and prognostic significance of PD-L1 and PD-1 are still controversial in human malignant pleural mesothelioma (MPM).

Methods: Formalin-fixed paraffin-embedded (FFPE) tumor samples from 203 MPM patients receiving standard treatment without immunotherapy were collected from 5 European centers. PD-L1 and PD-1 expression of tumor cells (TCs) and tumor-infiltrating lymphocytes (TILs) were measured by immunohistochemistry and correlated with clinical parameters and long-term outcome.

Results: High (>10%) PD-L1 TC and PD-1 TILs expressions were found in 18 (8%) and 39 (24%) patients, respectively. PD-L1 was rarely expressed by TILs [≥1%, n=13 (8%); >10%, n=1]. No significant associations were found between the PD-L1 or PD-1 expression of TCs or TILs and clinicopathological parameters such as stage or histological subtype. Notably, patients with high (>10%) TC-specific PD-L1 expression exhibited significantly worse median overall survival (OS) (6.3 *vs.* 15.1 months of those with low TC PD-L1 expression; HR: 2.51, P<0.001). In multivariate cox regression analysis adjusted for clinical parameters, high TC PD-L1 expression (>10%) proved to be an independent negative prognostic factor for OS (HR: 2.486, P=0.005). There was no significant correlation between PD-L1 or PD-1 expression of TILs and OS.

Conclusions: In this multicenter cohort study, we demonstrate that high (>10%) PD-L1 expression of TCs independently predicts worse OS in MPM. Further studies are warranted to investigate the value of PD-L1/PD-1 expression as a marker for treatment response in MPM patients receiving immunotherapy.

Keywords: Malignant pleural mesothelioma (MPM); prognosis; programmed death ligand 1 (PD-L1); programmed cell death 1 (PD-1)

Introduction

Malignant pleural mesothelioma (MPM) is a rare and aggressive malignancy arising from the pleural mesothelium. The overall survival (OS) of MPM patients ranges from 10 to 20 months depending on stage and histological subtype (1-4). Platinum-based chemotherapy (CHT) has been used in MPM treatment and still remains the backbone for current combination strategies (5). Recent advances in multidisciplinary therapeutic approaches including surgery, CHT and radiation therapy (RT) have improved the OS in highly selected patients (1,6-9). Moreover, recent phase I/II trials have shown some benefit of immunotherapy in MPM, but single agent checkpoint inhibitors were so far not demonstrated to be superior to standard CHT in larger phase III trials (10-12). Nevertheless, a recent phase III study investigating the efficacy of first-line nivolumab plus ipilimumab (*vs.* platinum doublet CHT) showed promising results with regards to OS (13). Of note, however, the progression-free survival (PFS) was similar between the treatment arms even in case of combination immunotherapy (13). Altogether, selecting MPM patients for appropriate therapeutic approaches remains a key problem, resulting in an unmet need to identify prognostic markers to predict the OS and to individualize treatment based on expected prognosis.

Besides their potential to predict the efficacy of immunotherapy, programmed death ligand 1 (PD-L1) and programmed cell death 1 (PD-1) expressions have shown conflicting results in correlating with prognosis in several tumor types (14). Specifically, high PD-L1 level was found to be a negative prognostic factor in renal and gastric cancers, while in primary colorectal and thymic carcinomas PD-L1 was associated with favourable outcome (14-16). In lung cancer, immunotherapy is a well-established first-line treatment and its use is based on the predictive role of PD-L1 expression (17). However, several studies have reported favourable whereas some have shown dismal outcome based on high PD1/PD-L1 expressions and thus the prognostic role of these tissue biomarkers to date remains unclear in lung cancer (18-21). In MPM, currently only limited data is available on the prevalence and prognostic role of PD-L1 and PD-1 expressions and the exact role of these tissue biomarkers in predicting MPM outcome remains thus controversial (15,22-25). To further explore the expression and prognostic impact of PD-L1 and PD-1 of tumor cells (TCs) and tumor-infiltrating lymphocytes (TILs), our multi-institutional study investigated the expression patterns of these molecules and their relationship with clinicopathologic parameters and long-term outcome in human MPM.

We present the following article in accordance with the REMARK reporting checklist (available at http://dx.doi.org/10.21037/tlcr-20-1114).

Methods

Study population

In this multicenter study, we included 203 patients with histologically confirmed MPM, diagnosed in the following 5 Central European medical centers between 2000 and 2016 (*Tables 1,2*): Department for Respiratory Diseases Jordanovac, University of Zagreb School of Medicine, Zagreb, Croatia (cohort #1, n=42); National Koranyi Institute of Pulmonology, Budapest, Hungary (cohort #2, n=39); Department of Thoracic Surgery, Medical University of Vienna, Austria (cohort #3, n=38); Department for Pulmonology, University Clinic Golnik, Golnik, Slovenia (cohort #4, n=46), and Department of Respiratory Medicine, University Hospital, Palacky University, Olomouc, Czech Republic (cohort #5, n=38). Only patients with adequate clinical data and sufficient amount

Table 1 Patient characteristics and PD-L1 expression of TCs in human MPM

Variables	No. of patients (%)	PD-L1 expression		P value[a]
		PD-L1 ≤10%	PD-L1 >10%	
All patients	203	185 (91.1%)	18 (8.9%)	
Age (years)[b]				
<65	104 (51.2)	95	9	0.245
≥65	99 (48.8)	90	9	
Gender[c]				
Male	145 (71.4)	133	12	0.639
Female	58 (28.6)	52	6	
Histology[c]				
Epithelioid	151 (74.4)	140	11	0.328
Non-epithelioid	39 (19.2)	34	5	
No data	13 (6.4)	11	2	
Treatment[c]				
Multimodality	29 (14.3)	28	1	0.306
Other[d]	113 (55.7)	99	14	
No data	61 (30.0)	58	3	
Stage[c]				
Early (I/II)	63 (31)	56	7	0.837
Late (III/IV)	99 (48.8)	89	10	
No data	41 (20.2)	40	1	
Medical center[c]				
#1	42 (20.7)	38	4	0.285
#2	39 (19.2)	36	3	
#3	38 (18.7)	34	4	
#4	46 (22.7)	45	1	
#5	38 (18.7)	32	6	

[a], P values refer to PD-L1$_{high}$ versus PD-L1$_{low}$ subgroups; [b], Student's t-test is used in case of continuous variable (age); [c], χ^2 test or Fisher's exact test are used between categorical variables; [d], CHT, RT, CHT/RT or BSC. PD-L1, programmed death ligand 1; TC, tumor cell; MPM, malignant pleural mesothelioma.

of formalin-fixed paraffin-embedded (FFPE) tumor tissue were included. Staging was performed according to the IMIG TNM staging system (7th edition) (26). Clinical data regarding patient age, gender, clinical stage, histological subtype, treatment and survival data for the included patients were retrospectively collected from medical records and/or records from the National Health Insurance Offices or Central Statistical Offices of each participating country.

Tissue samples

Tissue samples were acquired during diagnostic procedures or surgery. The FFPE tissue specimens were routinely processed and examined for routine diagnostic work-up and consequently stored at room temperature. To re-confirm the diagnosis of MPM of each case and to evaluate tumor content, serial sections were cut at 4 μm and stained

Table 2 Patient characteristics and PD-1 expression of TILs in human MPM

Variables	No. of patients (%)	PD-1 expression		P value[a]
		PD-1 ≤10%	PD-1 >10%	
All patients	164	125 (76.2%)	39 (23.8%)	
Age (years)[b]				
<65	92 (56.1)	69	23	0.754
≥65	72 (43.9)	56	16	
Gender[c]				
Male	118 (72.0)	92	26	0.401
Female	46 (28.0)	33	13	
Histology[c]				
Epithelioid	121 (73.3)	95	26	0.826
Non-epithelioid	30 (18.3)	23	7	
No data	13 (7.9)	7	6	
Treatment[c]				
Multimodality	27 (16.5)	19	8	0.541
Other[d]	76 (46.3)	58	18	
No data	61 (37.2)	48	13	
Stage[c]				
Early (I/II)	39 (23.8)	29	10	0.604
Late (III/IV)	84 (51.2)	66	18	
No data	41 (25.0)	30	11	
Medical center[c]				
#1	41 (25)	32	9	0.362
#2	39 (23.8)	32	7	
#3	38 (23.2)	25	13	
#4	46 (28.0)	36	10	
#5	No data	No data	No data	

[a], P values refer to PD-1$_{high}$ versus PD-1$_{low}$ subgroups; [b], Student's t-test is used in case of continuous variable (age); [c], χ^2 test or Fisher's exact test are used between categorical variables. [d], CHT, RT, CHT/RT or BSC. PD-1, programmed death 1; TILs, tumor-infiltrating lymphocytes.

first with hematoxylin and eosin. Further sections were used for the immunohistochemical analyses. In 23 (11%) cases, samples were collected at the time of surgery after administration of neoadjuvant platinum-based CHT. All other samples were retrieved from CHT-naïve patients.

Immunohistochemistry

The monoclonal PD-L1 and PD-1 antibodies (Cell Signaling, clone E1L3N, dilution 1:25 and R&D Systems, # AF 1086, dilution 1:20, respectively) for immunostaining were used as reported by the standardized protocols of the

ISO-certified laboratory of the 1st Department of Medicine, Medical University of Vienna. Briefly, after deparaffinization and rehydration of the 4 µm-thick sections, the slides were incubated in a 0.3% H_2O_2 solution for 10 minutes in order to reduce the nonspecific background staining, and heated afterwards for 10 minutes in 10 mM citrate buffer (pH 6.0) in a pressure cooker. Slides were then incubated for 5 minutes at room temperature with Ultra V Block (UltraVision LP detection system, Lab Vision Corporation, Thermo Fisher Scientific Inc., Pittsburgh, MA, USA), followed by PD-L1 and PD-1 antibody incubation for 30 minutes at room temperature. Antibody binding was detected by using The UltraVision LP detection system following the manufacturer's recommendations (Lab Vision Corporation). Color development and antibody visualizations were performed with 3-3'-diaminobenzidine and counterstained with hematoxylin. PD-L1 and PD-1 expressions were examined blinded to clinical data of the patients by an expert pathologist. Slides were examined at 400× magnification, and the staining rate (percentage of all TCs and TILs showing positive staining) was determined. PD-L1 and PD-1 staining intensity was scored as 0 (absent), 1 (weak), 2 (moderate), and 3 (strong). The degrees of PD-L1 and PD-1 expressions were classified separately according to the percentage of positive TCs or TILs as follows: (TC low) 0–10% TCs positive, (TC high) >10% TCs positive, (TIL low) 0–10% TILs positive, (TIL high) >10% TILs positive.

Treatment

All diagnostic and therapeutic approaches were conducted in accordance with the individual institutional and with the current National Comprehensive Cancer Network (NCCN) guidelines with no major differences across the 5 institutions (27). Accordingly, after the diagnosis and tissue sampling, patients were treated with either platinum-based CHT, palliative radiotherapy (RT), combined chemoradiotherapy (CHT-RT) or best supportive care (BSC). Multimodality treatment (MMT) was defined as the combination of surgery by either pleurectomy/decortication (P/D) or extrapleural pneumonectomy (EPP) with either chemo- and/or radiotherapy. None of the included patients received immunotherapy or other targeted agents.

Statistical analyses

All statistical analyses were performed using the SPSS Statistics 26.0 package (SPSS Inc., Chicago, IL, USA) and GraphPad Prism Version 8. Data distribution was verified by the Kolmogorov-Smirnov normality test. Categorical and ordinal parameters such as gender (male vs. female), clinical stage (I–II vs. III–IV), histological subtype (epithelioid vs. non-epithelioid) and dichotomized PD-L1/PD-1 expression (low vs. high), were analysed by χ^2 test or Fisher's exact test. Age as a continuous variable was analysed in the different PD-L1/PD-1 subgroups by Mann-Whitney U test and Student's t-test. The primary endpoint of our study was the OS, which was estimated from the time of diagnosis until death of any cause, or the last available follow-up visit. Follow-up was completed until March 2018. Survival curves were estimated by Kaplan-Meier plots and the differences between different groups were compared using the log-rank test. The independent prognostic value of the clinicopathological variables was studied with a multivariate Cox proportional hazard regression model, which was adjusted for PD-L1 expression, age (as a continuous variable), gender, IMIG clinical stage, histological type and therapeutic approaches. For an additional exploratory multivariate analysis, multiple imputations by chain equation (MICE) were employed to handle missing data, in order to avoid the omission of valuable information. Continuous data are always shown as median or mean and corresponding range or, in case of OS, as median and corresponding 95% confidence interval (CI). All reported P values are two-sided, and a level of 0.05 or less was considered statistically significant.

Ethics statement

The study was conducted in accordance with the guidelines of the Helsinki Declaration (as revised in 2013) of the World Medical Association and the Good Scientific Practice guidelines of the Medical University of Vienna with the approval of the national level ethics committee (Medical University of Vienna; EK#: 904/2009). Due to the retrospective nature of the study, the requirement for written informed consent was waived. Tissue and data collection were approved in all institutions. After clinical
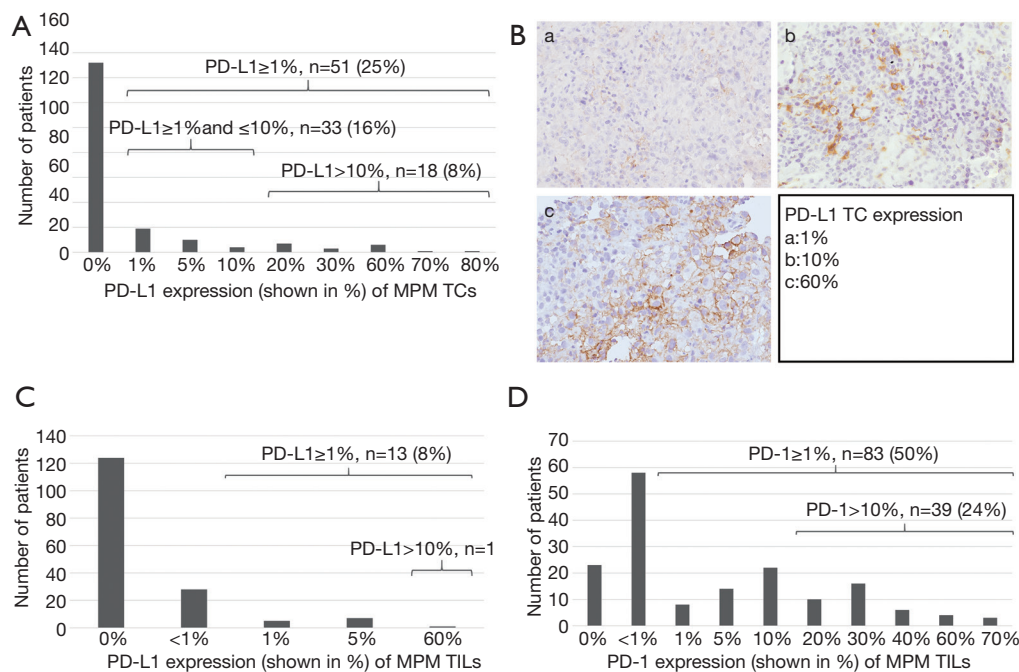
Figure 1 PD-L1 and PD-1 expression of TCs and TILs in MPM patients. (A) Of all 203 patients, 51 (25%) showed any (≥1%) PD-L1 expression in their TCs. Out of these patients, 18 (8%) were categorized as TC PD-L1 "high". (B) Representative images of PD-L1 expressing TCs in MPM. Immune staining was performed with monoclonal PD-L1 antibodies (Cell Signaling, clone E1L3N, dilution 1:25). All images were captured at a magnification of ×200. (C) No or low (<1%) PD-L1 TIL expression was detected in 152 (92%) patients, while PD-L1 TIL expression of ≥1% was found in 13 (8%) patients. (D) ≥1% PD-1 TIL expression was found in 83 (50%) patients. Of these cases, 39 (24%) patients had a PD-1 TIL expression higher than 10%. PD-L1, programmed death ligand 1; PD-1, programmed cell death 1; TC, tumor cell; TIL, tumor-infiltrating lymphocytes; MPM, malignant pleural mesothelioma.

information was collected, patient identifiers were removed, and subsequently, patients could not be identified either directly or indirectly. Tissue staining and data analysis was performed at the Medical University of Vienna (center #3).

Results

Correlation of clinicopathological variables with PD-L1/ PD-1 expression

In total, 203 MPM patients were enrolled in the study whose clinicopathological characteristics are summarized in *Tables 1,2*. The full cohort comprised 151 (74%) epithelioid and 39 (19%) non-epithelioid (i.e., biphasic or sarcomatoid) MPMs. Thirteen (6%) cases were classified as MPM not otherwise specified (NOS). Median age of all cases was 64 years (range, 27–86 years) and patients were predominantly male (71.4%). At diagnosis, 63 (31%) and 99 (49%) cases had IMIG/TNM stage I–II and stage III–IV disease, respectively. Twenty-nine (14%) patients received

multimodality treatment including surgery (MMT), while 113 (56%) patients underwent other therapeutic approaches such as CHT, RT, CHT/RT or BSC. In case of 61 patients, treatment-related data was not available.

PD-L1 expression was measured in both of the TC and TIL populations. Meanwhile, PD-1 expression was analyzed solely in TILs because we did not observe any positivity on TCs. Out of all 203 cases, 152 (75%) cases did not show any TC PD-L1 expression. Of the 51 (25%) cases who were categorized as TC/PD-L1 positive (≥1%), the tumor samples of 33 (16%) and 18 (9%) patients were categorized by TC/PD-L1 scores "low" and "high", respectively (*Figure 1A*). Representative images of PD-L1 expressions of TCs are shown in *Figure 1B*. Eligible MPM tissue for investigating PD-L1 expression of TILs was available from 165 patients. PD-L1 TIL expression was rarely seen. Positive staining (PD-L1 TIL expression ≥1%) was found in 13 (8%) patients, and only 1 case exhibited a PD-L1 TIL expression >10% (*Figure 1C*). PD-1 expression of TILs

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Table 3 Univariate survival analysis for 203 MPM patients from 5 European centers

Variables	Subgroups	Median OS (mo)	P[c]	HR	95% CI
Age	<65[a]	12.8	0.164	1.23	0.92–1.65
	≥65[a]	14.4			
Gender	Female	11.2	0.725	1.06	0.77–1.45
	Male	15.1			
Histology	Epithelioid	13.2	0.012	0.64	0.39–0.89
	Non-epithelioid	12.7			
Treatment	MMT	28.7	<0.001	0.32	0.22–0.47
	Other	11.8			
Stage	I/II	18.6	0.01	0.66	0.47–0.92
	III/IV	11.3			
PD-L1 TCs	≤10%	15.1	<0.001	0.39	0.18–0.86
	>10%	6.3			
PD-L1 TILs[a]	<1%	15.1	0.508	0.82	0.43–1.56
	≥1%	11.8			
PD-1 TILs[b]	<1%	15.0	0.703	1.04	0.77–1.47
	≥1% and ≤10%	15.6		0.87	0.59–1.33
	>10%	12.7			

[a], performed in 165 cases; [b], performed in 164 cases; [c], P value was calculated with log-rank test. OS, overall survival; mo, months; HR, hazard ratio; CI, confidence interval; MMT, multimodality treatment including surgery; TCs, tumor cells; PD-L1, programmed death ligand 1; PD-1, programmed cell death 1; TILs, tumor infiltrating lymphocytes.

could be measured in 164 patients. TIL PD-1 positivity (i.e., ≥1%) was found in 83 (50%) patients. A higher than 10% TIL PD-1 expression was observed in 39 (24%) patients (*Figure 1D*).

Next, we studied the correlation between clinicopathological parameters and PD-L1 and PD-1 expression of TCs and TILs. No significant correlation was found between PD-L1 or PD-1 TC or TIL expressions and clinical variables such as age, gender, histological subtype or tumor stage when patients were dichotomized into PD-L1 and PD-1 negative (no staining) *vs.* positive (≥1% staining) categories. Of note, using cut-off values of 10% (*Tables 1,2*) or 50% (data not shown) for PD-L1 or PD-1 expressions did not yield significant associations either. It is also important to mention that we did not find significant associations between TC or TIL PD-L1/PD-1 expressions and histological subtypes or therapeutic modality (*Tables 1,2*).

Prognostic parameters and OS

The median follow-up time for all 203 patients was 12.8 months. Median OS of the full cohort was 13.2 months (95% CI, 10.6–15.8). First, we performed a univariate survival analysis in order to identify clinical prognostic factors for OS (*Table 3*). We found that patients with epithelioid histological subtype exhibited significantly improved OS compared to those with non-epithelioid MPM (median OSs were 13.2 *vs.* 12.7 months, respectively; HR 0.64, P=0.012, *Figure 2A*). Patients with stage I/II MPM (*vs.* stage III/IV, respectively, HR 0.66, P=0.01, *Table 3* and *Figure 2B*) and patients receiving MMT (*vs.* other therapies, HR 0.32, P<0.001, *Table 3* and *Figure 2C*) were also associated with significantly improved OS. There were no significant associations between OS and gender (*Figure 2D*) or age (dichotomized at a cut-off of 65 years, data not shown).
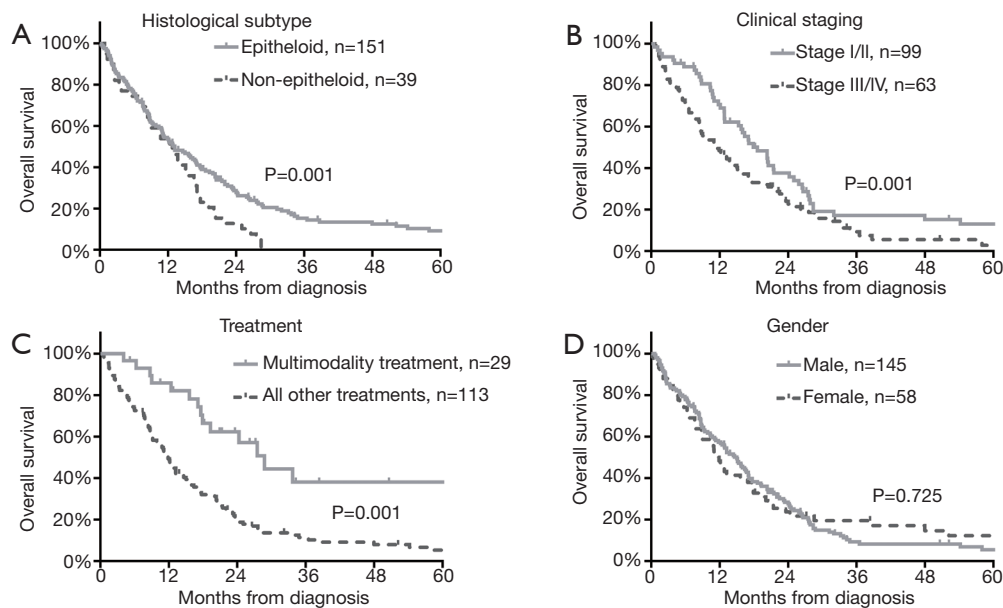
Figure 2 Kaplan-Meier estimates for OS in patients with MPM according to clinicopathological parameters. (A) Patients with epithelioid subtype exhibited significantly superior OS compared to those with other non-epithelioid histotypes (i.e., sarcomatoid or biphasic) (median OSs were 13.2 *vs.* 12.7 months, respectively; HR 0.64, P=0.012). (B) Early stage MPM (I and II) at diagnosis conferred significantly longer OS (*vs.* stages III/IV; median OSs were 18.6 *vs.* 11.3 months, respectively; HR 0.66, P=0.014). (C) Patients treated with MMT including surgery had significantly improved OS (*vs.* those receiving other treatments; median OSs were 28.7 *vs.* 11.8 months, respectively; HR 0.32, P<0.001). (D) No significant differences in OS have been observed between male and female patients (median OSs were 15.1 *vs.* 11.2 months, respectively; HR 106, P=0.725). OS, overall survival; MPM, malignant pleural mesothelioma; HR, hazard ratio.

Next, we examined the prognostic value of PD-L1 and PD-1 expression of TCs and TILs (*Table 3*). Our initial statistical analyses indicated that patients whose TCs did not express PD-L1 (median OS 14 months) had comparable OS to those with PD-L1 TC expressions between 1% and 10% (median OS 16 months, P=0.194, *Figure 3A*). We grouped patients accordingly into low (≤10%) and high (>10%) PD-L1 TC categories and found that low PD-L1 expression was significantly associated with improved OS (HR 0.39, P<0.001, *Table 3* and *Figure 3B*). PD-L1 was rarely expressed by TILS and there was no difference in the OS of patients whose tumor samples were categorized by a PD-L1 TIL score <1% (n=152) *vs.* ≥1% (median OSs were 15.1 *vs.* 11.8 months, HR 0.82, P=0.508, *Table 3* and *Figure 3C*). Similarly, we could not show prognostic information from the PD-1 expression of TILs when patients were grouped into PD-1 TIL <1% *vs.* ≥1% and ≤10% *vs.* >10% categories (*Table 3* and *Figure 3D*).

In order to assess if the prognostic value of PD-L1 TC expression was independent from significant clinical

prognostic factors, we performed a multivariate Cox regression analysis with available data from 126 (62%) patients (*Table 4*). The model was adjusted for clinical factors such as age, gender, histological subtype, tumor stage at diagnosis and treatment. We found that PD-L1 TC expression at a 10% cut-off remained a significant prognostic factor for OS (low *vs.* high expression; HR 0.405, P=0.005; *Table 4*). Histological subtype (epithelioid *vs.* non-epithelioid; HR 0.504, P=0.009), tumor stage (I–II *vs.* III-IV; HR 0.545, P=0.007) and treatment (MMT *vs.* other therapies, HR 0.351, P<0.001) also independently influenced OS. As 126 (62%) patients only had completely available data for the multivariate model, we performed an exploratory multivariate Cox regression analysis, using a dataset after multiple imputation by MICE approach, including all 203 cases, in order to avoid the omission of data. In this exploratory analysis, PD-L1 TC expression remained as a significant prognostic factor for OS (HR 0.443, P=0.004), independent from age, gender, histologic subtype, stage and treatment (data not shown).
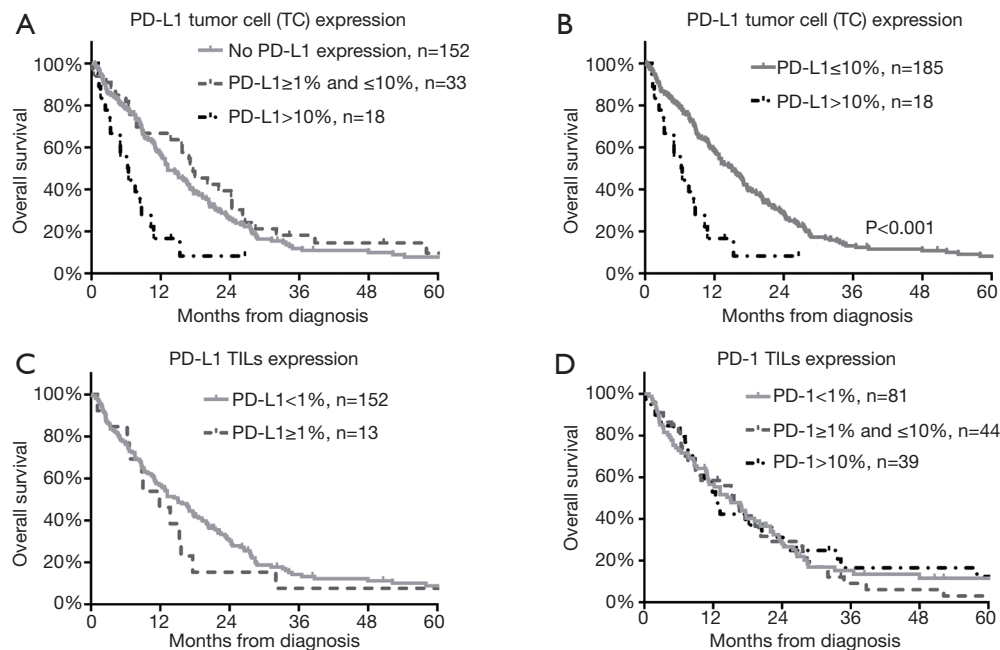
Figure 3 Kaplan-Meier estimates for OS according to PD-L1 and PD-1 expression of TCs and TILs in human MPM. (A) OS of patients with no *vs.* ≥1% and ≤10% PD-L1 TC expression was similar, whereas OS in patients with high (>10%) PD-L1 TC expression was significantly worse. (B) Patients with PD-L1 TC expression ≤10% had significantly longer OS than those in the PD-L1 TC high (>10%) group (median OSs were 15.1 *vs.* 6.2 months, respectively, P<0.001, log rank test). (C) Patients with a positive PD-L1 TILs staining (≥1%) had a similar OS compared to patients without PD-L1 TILs expression (median OS 15.1 *vs.* 11.8 months, HR 0.82, P=0.508). (D) PD-1 expression on TILs did not have any impact on OS, as OS was similar among three groups of different expression levels (<1% *vs.* ≥1% and ≤10% *vs.* >10%; P=0.703). OS, overall survival; PD-L1, programmed death ligand 1; PD-1, programmed cell death 1; TC, tumor cell; TIL, tumor-infiltrating lymphocytes; MPM, malignant pleural mesothelioma; HR, hazard ratio.

Discussion

The poor survival outcome in MPM and the lack of effective therapies require the development of novel therapeutic strategies. Hence, there is an urgent need for identifying specific prognostic and predictive markers that enable clinicians to effectively allocate patients to appropriate treatment. Previous studies suggest that high PD-L1 expression might be associated with impaired survival outcomes in MPM, yet the prognostic value and clinicopathological significance of both PD-L1 and PD-1 are still controversial (1,15,24). Therefore, the aim of this study was to evaluate the expression of PD-L1 and its receptor PD-1 in MPM and to correlate their expression patterns with clinicopathological parameters and long-term outcome by analyzing a large patient cohort in a multicenter setting.

The majority of MPM cases are caused by prior exposure to asbestos leading to the increase of local infiltrating immune cells and malignant transformation of mesothelial cells (22,28,29). High numbers of TILs have been associated with a better prognosis, while high numbers of TAMs and low lymphocyte to monocyte ratio (LMR) in peripheral blood or tissue have a negative impact on prognosis (2,22,30-32). The PD-L1/PD-1 pathway plays a pivotal role in normal immune system regulation, but also in tumor immune escape control since the interaction of TC PD-L1 with T-cell PD-1 reduces the effector functions of T cells (33). Accordingly, immunogenic tumors can easily bypass the anti-tumor responses of the organism by overexpressing PD-L1, and thus escaping the immune surveillance (33). On the other hand, by blocking the PD-L1/PD-1 pathway with therapeutic antibodies a durable anti-tumor activity and favorable response rates can be achieved in multiple tumor types, including skin melanoma, lung cancer, and partly in MPM as well (14,34).

In our international multicenter study, we found that 25% of cases were categorized as positive (≥1%) for TC

Table 4 Multivariate Cox regression model for OS adjusted for clinicopathological variables (n=126)

Variables	Number
Age (continuous)	
HR	1.008
95% CI	(0.987–1.028)
P	0.472
Gender (female *vs.* male)	
HR	0.855
95% CI	(0.546–1.340)
P	0.495
Histology (epithelioid *vs.* non-epithelioid)	
HR	0.504
95% CI	(0.301–0.843)
P	0.009
IMIG clinical stage (I+II *vs.* III+IV)	
HR	0.545
95% CI	(0.352–0.844)
P	0.007
Treatment (MMT *vs.* other)	
HR	0.351
95% CI	(0.194–0.633)
P	<0.001
PD-L1 expression of TCs (PD-L1 >10% *vs.* ≤10%)	
HR	0.405
95% CI	(0.216–0.759)
P	0.005

OS, overall survival; PD-L1, programmed death ligand 1; HR, hazard ratio; CI, confidence interval; IMIG, International Mesothelioma Interest Group; MMT, multimodality treatment.

PD-L1 expression. These results are in agreement with two recent MPM studies reporting that 18% to 24% of the patients had PD-L1 expressing tumors (24,35). Additionally, we found that only a small number of patients (n=18; 8%) had a PD-L1 TC expression higher than 10%. Previous studies have shown that PD-L1 can be expressed by multiple components of the tumor microenvironment. Therefore, we investigated PD-L1 expression by TILs too (24). However, PD-L1 was rarely expressed by TILs in our cohort. These results are only partly in line with the findings of Herbst and colleagues, who studied 732 different tumor types and observed PD-L1 positivity on both TCs and immune cells (36). A possible explanation for the relatively low number of cases with PD-L1 expressing TILs might be that TIL PD-L1 positivity is usually seen in sarcomatoid MPM, whereas the majority of patients included in our study had epithelioid type MPM (37).

So far, two major studies investigated the detailed expression pattern of PD-1 in MPM (37,38). In our study, PD-1 expression of TILs could be measured in 164 patients, whereas we did not observe any PD-1 positive TCs. Our results are in line with the findings of Marcq and colleagues who demonstrated that PD-1 is expressed to a great extent on immune cells in MPM (37). Additionally, they also showed that PD-1 positive TCs are rarely seen in these patients (only 4 of 54 patients had PD-1 positive TCs in their study) (37). PD-1 is primarily expressed by activated lymphocytes and upon triggering by its ligands (PD-L1 and PD-L2) it can repress Th1 cytotoxic immune responses (14,39). Interestingly, half of our patients have been categorized as positive for TIL PD-1 expression, and 24% of them had high (>10%) PD-1 expression. Interestingly, the significance of PD-1-expressing tumor infiltrating CD8$^+$ T cells in predicting anti-PD-1 therapeutic response in MPM is still unclear (40). Of note, in case of other solid tumors, such as skin melanoma it is suspected that the presence of activated PD-1$^+$ CD8$^+$ T cells might be associated with therapeutic efficacy (40,41). As mentioned before, previous studies have reported higher PD-L1 expression in non-epithelioid (especially sarcomatoid) MPM compared to other histological subtypes (37,38). We did not find a significant association between PD-L1 or PD-1 expression and histological subtype. Our results are, therefore, in contrast to previous studies (23,24,38). A possible explanation for this discordance might be related to different cut-off values. In our study, "PD-L1/PD-1 high" patients were defined as those with PD-L1/PD-1 expression >10%. Meanwhile, others used alternative threshold values or grouped the patients solely based on positivity irrespective of the expression percentage. Additionally, the relatively low ratio of patients with non-epithelioid MPM in our study might also explain these divergent results.

To date, no threshold PD-L1 expression level predicting treatment response or survival probability in MPM has been defined (15). In contrast to previous studies applying cut-off levels of 1% or 5% (15,24,42,43), in the present study we investigated the correlation between PD-L1 expression and OS using cut-off levels of 1% and 10%. PD-

L1 and PD-1 expression have been shown to correlate with survival in several tumor types including hepatocellular, breast, esophageal and thymic carcinomas (14,16,44-46). As for MPM, the small number of available studies has yielded conflicting results partly due to different threshold values (15,24,35,42,43). In our study, we found that high (>10%) TC PD-L1 expression was associated with impaired median OS, with a clinically relevant difference of 8.8 months between low and high subgroups. In addition, by performing multivariate analysis, high (>10%) PD-L1 expression was significantly associated with OS, regardless of histology, stage or treatment. Of note, similar survival probabilities between PD-L1 negative patients (<1%) and those with PD-L1 expression between 1–10% might suggest the need for higher cut-off values compared to previous studies. PD-L1 protein expression was previously shown to correlate with tumor aggressiveness and may be a critical factor to promote tumor growth and metastases (1,24,46-48). Accordingly, the worse OS related to higher PD-L1 TC expression levels may be partly explained by PD-L1 acting as a surrogate marker for an unfavorable tumor behavior.

As for the prognostic impact of PD-1 expression by TILs, previous studies suggest that PD-1 expression by immune cells correlates with increased OS in patients with triple-negative breast cancer, gastric cancer or skin melanoma (40,49,50). Meanwhile, in case of other solid tumors including nasopharyngeal carcinoma no such association was found (51). To the best of our knowledge, ours is so far the largest study to investigate the prognostic relevance of PD-1 expression by TILs in MPM patients. Although Marcq *et al.* also examined the prognostic importance of PD-1 on immune cells, their study included only 54 patients (37). In the present study, we could not detect any statistically or clinically relevant difference in the OS of subcohorts with different PD-1 TILs expressions. Accordingly, our results suggest that PD-1 TIL expression may not serve as a suitable prognostic biomarker in MPM.

The present study is partly limited by its retrospective nature and also by the lack of a validation set. Accordingly, results have to be interpreted with caution. Additionally, the use of PD-L1 expression as a prognostic biomarker can be confounded by multiple unresolved issues, including variability in antibody characteristics, tissue processing and expression threshold values. In this study, we used the commercially available E1L3N antibody for PD-L1 staining. Importantly, however, not all antibody clones show a similar staining pattern and positivity (52). Therefore,

our results should preferentially be considered when using the E1L3N antibody clone. Finally, our results should be interpreted with the caveat that both PD-L1 and PD-1 expressions are variable over time, and although the majority of included patients were CHT-naïve at biopsy, the application of CHT prior to tissue sampling can possibly confound expression patterns (37,53,54). Nevertheless, this study examined a relatively large number of patients in a multicenter setting and we used multiple cut-off values in order to get a clearer insight into the expression pattern and prognostic impact of both PD-L1 and PD-1.

To conclude, the results of this study show that PD-L1 is uniformly expressed by both TCs and TILs in MPM. Furthermore, this is the largest study that comprehensively evaluates the prognostic value of PD-1 by TILs in a multicenter cohort of MPM patients. Our study also demonstrates that high (>10%) TC PD-L1 expression is associated with a clinically relevant survival disadvantage and, furthermore, that it is an independent prognostic factor in MPM. Altogether, by shedding light on the expression patterns and prognostic relevance of PD-L1 and PD-1 our results might provide support for further MPM trials investigating PD-L1/PD-1 inhibition, especially in patients with high PD-L1 expression and consequently poor survival.

Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at http://dx.doi.org/10.21037/tlcr-20-1114

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the guidelines of the Helsinki Declaration (as revised in 2013) of the World Medical Association and the Good Scientific Practice guidelines of the Medical University of Vienna with the approval of the national level ethics committee (Medical University of Vienna; EK#: 904/2009). Due to the retrospective nature of the study, the requirement for written informed consent was waived. Tissue and data collection were approved in all institutions. After clinical information was collected, patient identifiers were removed, and subsequently, patients could not be identified either directly or indirectly. Tissue staining and data analysis was performed at the Medical University of Vienna (center #3).

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