Changes in mucosa-associated lymphoid tissue 1 predicts therapeutic response and survival in patients with advanced melanoma receiving programmed cell death-1 inhibitor monotherapy

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Abstract. Advanced melanoma is an aggressive and dangerous form of skin cancer, and programmed cell death-1 (PD-1) inhibitors are recommended treatment options for patients with advanced melanoma. Mucosa-associated lymphoid tissue 1 (MALT1) impairs $CD8^+$ T-cell activation to induce immune escape, leading to a reduction in the antitumor effect of PD-1 inhibitors. The present study aimed to assess the prognostic implication of MALT1 in patients with advanced melanoma receiving PD-1 inhibitor monotherapy. Blood MALT1 levels were assessed using reverse transcription-quantitative PCR in 20 healthy controls (HCs) after enrollment and in 49 patients with advanced melanoma before (T_0), as well as 2 months (T_1) and 4 months after (T_2) PD-1 inhibitor monotherapy. The maximum level of MALT1 in HCs (3.100) was used as the cut-off in patients with advanced melanoma. MALT1 levels at T_0 were significantly increased in patients with advanced melanoma compared with in HCs (P<0.001). In patients with advanced melanoma, MALT1 was significantly decreased from T_0 to T_2 (P<0.001). Objective response rate (ORR) and disease control rate (DCR) were 28.6 and 59.2%, respectively. MALT1 levels at T_1 were significantly negatively associated with overall therapeutic response (P=0.001), ORR (P=0.009) and DCR (P=0.004). MALT1 levels at T_2 were significantly inversely associated with overall therapeutic response (P=0.021) and ORR (P=0.036). Moreover, MALT1 levels >3.100 at T_0 (P=0.027) and T_1 (P=0.045) were significantly associated with shorter progression-free survival (PFS), and MALT1 levels >3.100 at T_1 were significantly associated with a poor overall survival (OS; P=0.022). Multivariate Cox regression analysis demonstrated that MALT1 levels at T_0 (>3.100 vs. ≤3.100) were significantly associated with a poor PFS [hazard ratio (HR)=2.248; P=0.037], and MALT1 levels at T_1 (>3.100 vs. ≤3.100) were significantly associated with a poor OS (HR=4.332; P=0.007). In conclusion, MALT1 levels are reduced following PD-1 treatment, and a high MALT1 level is associated with a poor therapeutic response and shorter survival in patients with advanced melanoma receiving PD-1 inhibitor monotherapy.

Introduction

Melanoma is an aggressive type of skin cancer, with an estimated 324,635 new cases and 57,043 cancer-related deaths worldwide in 2020 (1,2). Generally, risk factors for melanoma include the number of nevi, genetic susceptibility, sun exposure and family history of the disease (3-6). The treatment strategy for melanoma depends on the stage of cancer (7-9). For early-stage melanoma, surgery is the standard therapy (7,10,11); however, when the tumor spreads, other therapies, such as immune checkpoint inhibitors, are recommended for patients with advanced melanoma (8,12-15).

Programmed cell death-1 (PD-1) inhibitors are a type of immunotherapy, which have achieved unprecedented progress in treating advanced melanoma (16). However, the therapeutic response rate is only ~40-50% and the response is unsatisfactory in ~60% of patients with advanced melanoma who receive PD-1 inhibitors, which is a crucial cause of poor prognosis in these patients (16-19). Therefore, investigating potential markers that predict therapeutic response to PD-1 inhibitors is necessary to improve the management of patients with advanced melanoma.

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Mucosa-associated lymphoid tissue 1 (MALT1) is intimately involved in the regulation of immune escape, which can further affect the response to PD-1 inhibitors and accelerate cancer progression (20-22). According to a previous study, MALT1 regulates the activation of $CD8^+$ T cells to facilitate immune escape and can further affect the antitumor effect of PD-1 inhibitors in mice models (21). At the same time, MALT1 is essential for maintaining the homeostasis and immunosuppressive function of regulatory T (Treg) cells, which may reduce the efficacy of PD-1 inhibitors (23). Furthermore, another study reported that MALT1 inhibition enhances antitumor immune responses, resulting in the attenuation of melanoma progression (22). Consequently, we hypothesize that MALT1 may possess a prognostic value for patients with advanced melanoma receiving PD-1 inhibitor monotherapy. Relevant studies are scarce; therefore, the present research aimed to assess the ability of MALT1 to predict therapeutic response and survival in patients with advanced melanoma receiving PD-1 inhibitor monotherapy.

Patients and methods

Patients and healthy subjects. The present prospective, multicenter cohort study was performed at the Affiliated Hospital of Hebei Engineering University (Handan, China), Handan Central Hospital (Handan, China) and Beijing Tsinghua Changgung Hospital (Beijing, China). A total of 49 patients with advanced melanoma receiving PD-1 inhibitor monotherapy were recruited from the aforementioned centers between July 2019 and April 2023. The inclusion criteria were as follows: i) Diagnosis of advanced melanoma with a tumor-node-metastasis (TNM) stage of III or IV; ii) aged ≥18 years; iii) inability to have a surgical resection; iv) Eastern Cooperative Oncology Group Performance Status (ECOG PS) ≤1 (24); v) ≥1 measurable lesion according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria v.1.1 (25); vi) ability to provide peripheral blood; and vii) willingness to cooperate with follow-up. The exclusion criteria were as follows: i) Other primary solid tumors or malignant hematologic diseases; ii) previous systematic anticancer treatment; iii) presence of an autoimmune disease; iv) previous organ transplantation; and v) serious liver or kidney failure. A total of 20 healthy subjects were enrolled as healthy controls (HCs), who were matched to the patients with advanced melanoma by age and sex. The inclusion criteria of the HCs were as follows: i) normal results of the physical examination; ii) aged ≥18 years; and iii) ability to provide peripheral blood. The exclusion criteria for HCs were the same as for the patients with advanced melanoma. The present study was approved by the Ethics Committee of the Affiliated Hospital of Hebei Engineering University (Handan, China; approval no. 2018K037; February 17, 2018). It should be clarified that three centers were involved, and we only obtained one ethics approval from one ethic committee, rather than three ethic committees. The reason was that: according to Guidelines for the Construction of Ethical Review Boards for Clinical Research Involving Human Subjects (https://www.cha.org.cn/site/content/393b419e529469ef3f4c0ddaddb347ca.html), a single review model could be implemented in multicenter studies. Specifically, single review referred to that in multicenter studies, participating centers only needed to obtain a single ethics approval from one ethics committee, rather than obtaining ethics approvals from every ethics committee. The patients with advanced melanoma and HCs provided written informed consent at the time of enrollment.

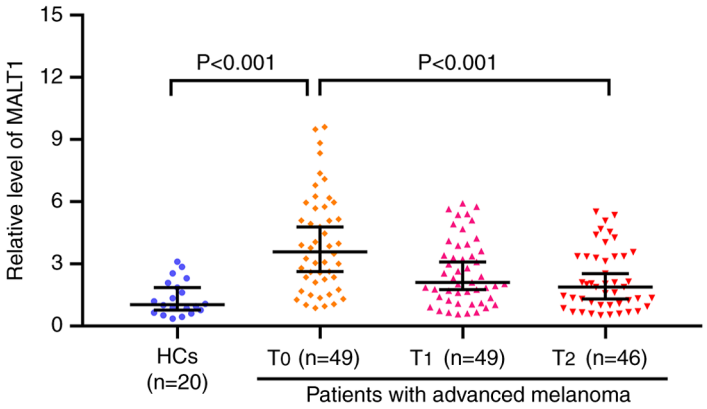
Data collection and treatment. The clinical characteristics of the patients with advanced melanoma were collected after enrollment. The PD-1 inhibitor monotherapy that each patient received was based on a combination of the patient's situation, their willingness and the physician's suggestions. The specific treatment of PD-1 inhibitors was as follows: Nivolumab (3 mg/kg, once every 2 weeks), camrelizumab (200 mg, once every 2 weeks) and pembrolizumab (200 mg, once every 2 weeks). The PD-1 inhibitor was administered until the patient's disease progressed or they became intolerant, or it was administered for two years.

Peripheral blood collection and detection. Peripheral blood was collected from the patients with advanced melanoma before treatment (T_0), after 2 months of treatment (T_1) and after 4 months of treatment (T_2). Peripheral blood was only collected once from HCs after enrollment. The peripheral blood was processed (centrifuged at 1,000 x g for 10 min at 4˚C) to obtain peripheral blood mononuclear cells (PBMCs), and reverse transcription (RT)-quantitative (q)PCR was used to detect the level of MALT1 in the PBMCs. Total RNA of PBMC was extracted using TRIzol™ reagent (Invitrogen™; Thermo Fisher Scientific, Inc.). PrimeScript™ RT reagent Kit (Perfect Real Time; cat. no. RR037A; Takara Biotechnology Co., Ltd.) was then used to obtain cDNA from the total RNA (1 cycle of 37˚C for 15 min and 85˚C for 5 sec). MALT1 levels were measured by qPCR (fluorophore: TB Green; Takara Biotechnology Co., Ltd.; 1 cycle of 95˚C for 30 sec, 40 cycles of 95˚C for 5 sec and 60˚C for 10-15 sec) and quantified using the $2^{-\Delta\Delta Cq}$ method with GAPDH used as an internal reference (26). The primer sequences for MALT1 were as follows: Forward, 5'-TCTTGGCTGGACAGTTTGTGA-3'; reverse, 5'-GCTCTCTGGGATGTCGCAA-3' (27). The primer sequences for GAPDH were as follows: Forward, 5'-GGAAGCTTGTCATCAATGGAAATC-3'; reverse, 5'-TGATGACCCTTTTGGCTCCC-3' (28).

Follow-up and evaluations. Disease progression was assessed every 2 cycles (about 1 month) during the first 4 months after treatment. Subsequently, disease progression was evaluated every 2 months. The therapeutic response rates of the patients with advanced melanoma were calculated based on the assessment data of the third month using RECIST criteria v.1.1 (25). The objective response rate (ORR) and disease control rate (DCR) were also calculated.

The patients with advanced melanoma had a normal follow-up. In detail, patients were followed up every 3-6 months for the first 2 years, every 3-12 months at 3-5 years and annually after 5 years. The median follow-up period was 9.1 months. Progress-free survival (PFS) and overall survival (OS) were calculated based on the follow-up data.

Statistical analysis. Data were analyzed using SPSS 22.0 (IBM Corp.) software. Comparisons between MALT1 levels at T_0 between the HCs and patients with advanced melanoma



MALT1	HCs	Patients with advanced melanoma		
		T_0	T_1	T_2
Quartile 1	0.673	2.175	1.295	1.000
Median	1.035	3.580	2.110	1.880
Quartile 3	2.025	5.720	3.750	3.363

Figure 1. MALT1 levels in HCs and in patients with advanced melanoma receiving programmed cell death-1 inhibitor monotherapy at T_0, T_1, and T_2. MALT1, mucosa-associated lymphoid tissue 1; HC, healthy control; T_0, before treatment; T_1, 2 months after treatment; T_2, 4 months after treatment.

were analyzed using the Mann-Whitney U test. Comparisons between MALT1 levels at T_0, T_1 and T_2 in patients with advanced melanoma was analyzed using the Friedman test. Bonferroni's correction was applied to adjust the comparison (the current P-values had already been multiplied by 2 for adjustment). The correlation between MALT1 levels and the therapeutic response of the patients was analyzed using the Spearman's rank correlation coefficient test. The association between MALT1 levels at different times (T_0, T_1 and T_2) and the prognosis of the patients with advanced melanoma was assessed using Kaplan-Meier curves and the log-rank test. The maximum level of MALT1 in HCs (3.100, relative value) was used as the cut-off value of MALT1 in the patients with advanced melanoma. The association between factors (MALT1 and all clinical characteristics) and PFS or OS was analyzed using univariate and backward stepwise multivariate Cox regression analyses. P<0.05 was considered to indicate a statistically significant difference.

Results

MALT1 levels in patients with advanced melanoma receiving PD-1 inhibitor monotherapy compared with HCs. MALT1 levels at T_0 were significantly increased in patients with advanced melanoma receiving PD-1 inhibitor monotherapy compared with that in HCs, with the median [interquartile range (IQR)], 3.580 (2.175-5.720) and 1.035 (0.673-2.025), respectively (P<0.001). In patients with advanced melanoma receiving PD-1 inhibitor monotherapy, MALT1 levels were the highest at T_0 [median (IQR), 3.580 (2.175-5.720)], followed by T_1 [median (IQR), 2.110 (1.295-3.750)] and the lowest at T_2 [median (IQR), 1.880 (1.000-3.363); P<0.001; Fig. 1].

Comparison of MALT1 levels in patients with advanced melanoma receiving PD-1 inhibitor monotherapy with different clinical features. MALT1 levels were significantly increased in patients with advanced melanoma receiving PD-1 inhibitor monotherapy with an ECOG PS score of 1 (vs. 0; P=0.018), total tumor size >5 cm (vs. ≤5 cm; P=0.015) and TNM stage IV (vs. TNM stage III; P=0.030). However, MALT1 levels were not significantly different in patients with advanced melanoma receiving PD-1 inhibitor monotherapy for other clinical features, including age (≤60 vs. >60 years; P=0.645), sex (female vs. male; P=0.337), lactate dehydrogenase (LDH) level (normal vs. abnormal; P=0.263) and programmed cell death-ligand 1 (PD-L1) level (negative vs. positive; P=0.607; Table I). The clinical characteristics of the HCs are presented in Table SI.

Association between MALT1 levels at T_0, T_1 and T_2, and therapeutic response in patients with advanced melanoma receiving PD-1 inhibitor monotherapy. In patients with advanced melanoma receiving PD-1 inhibitor monotherapy, the complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) was demonstrated to be 3 (6.1%), 11 (22.4%), 15 (30.7%) and 20 (40.8%) patients, respectively. Notably, 14 (28.6%) and 29 (59.2%) patients achieved ORR and DCR, respectively (Table II).

MALT1 levels at T_0 were not different among patients with CR, PR, SD or PD (P=0.053). Meanwhile, MALT1 levels at T_0 were not significantly associated with ORR (yes vs. no; P=0.163) or DCR (yes vs. no; P=0.070) in patients with advanced melanoma receiving PD-1 inhibitor monotherapy. MALT1 levels at T_1 were the highest in patients with PD, followed by patients with SD and PR, and the lowest in patients with CR (P=0.001). Additionally, MALT1 levels at T_1 were significantly associated with non-ORR (vs. yes, P=0.009) and non-DCR (vs. yes, P=0.004) in patients with advanced melanoma receiving PD-1 inhibitor monotherapy. MALT1 levels at T_2 were the highest in patients with PD, followed by patients with CR and SD, and the lowest in patients with PR (P=0.021). MALT1 levels at T_2 were significantly associated with non-ORR (vs. yes, P=0.036); however, it was not significantly associated with DCR in patients with advanced melanoma receiving PD-1 inhibitor monotherapy (yes vs. no; P=0.059; Table II).

Association between MALT1 levels at T_0, T_1 and T_2, and PFS and OS in patients with advanced melanoma receiving PD-1 inhibitor monotherapy. The median [95% confidence interval (CI)] PFS was 7.8 (1.0-14.6) months in patients with advanced melanoma receiving PD-1 inhibitor monotherapy (Fig. 2A). The maximum value of MALT1 in HCs (3.100) was used as the cut-off value in patients with advanced melanoma, and this

Table I. Association between MALT1 levels and clinical characteristics in patients with advanced melanoma (n=49).

Clinical characteristics	Patients with advanced melanoma, n (%)	MALT1, median (IQR)	P-value
Age, years			0.645
≤60	26 (53.1)	3.660 (1.995-6.015)	
>60	23 (46.9)	3.580 (2.350-5.080)	
Sex			0.337
Female	24 (49.0)	4.020 (2.278-6.125)	
Male	25 (51.0)	3.260 (1.930-5.040)	
ECOG PS			0.018
0	29 (59.2)	3.000 1.600-4.700)	
1	20 (40.8)	4.935 (3.043-6.188)	
Total tumor size, cm			0.015
≤5	23 (46.9)	2.580 (1.490-4.470)	
>5	26 (53.1)	4.420 (2.758-6.393)	
TNM stage			0.030
III	8 (16.3)	2.425 (1.320-3.645)	
IV	41 (83.7)	3.910 (2.355-5.965)	
LDH level			0.263
Normal	29 (59.2)	3.260 (1.585-5.385)	
Abnormal	20 (40.8)	3.675 (2.650-6.583)	
PD-L1 level			0.607
Negative	7 (14.3)	3.490 (1.520-5.760)	
Positive	42 (85.7)	3.815 (2.325-5.750)	

MALT1, mucosa-associated lymphoid tissue 1; IQR, interquartile range; ECOG PS, Eastern Cooperative Oncology Group Performance Status; TNM, tumor-node-metastasis; LDH, lactate dehydrogenase; PD-L1, programmed cell death-ligand 1.

value was applied for subsequent analyses. MALT1 levels at T_0 (P=0.027; Fig. 2B) and T_1 (P=0.045; Fig. 2C) >3.100 were significantly associated with a shorter PFS in patients with advanced melanoma receiving PD-1 inhibitor monotherapy, in comparison with MALT1 levels at T_0 and T_1 ≤3.100. However, MALT1 levels at T_2 were not significantly associated with PFS in patients with advanced melanoma receiving PD-1 inhibitor monotherapy (P=0.136; Fig. 2D).

The median (95% CI) OS was 17.3 (13.6-21.0) months in patients with advanced melanoma receiving PD-1 inhibitor monotherapy (Fig. 3A). MALT1 levels at T_0 were not significantly associated with OS in patients with advanced melanoma receiving PD-1 inhibitor monotherapy (P=0.064; Fig. 3B). MALT1 levels at T_1 >3.100 were significantly associated with a poor OS in patients with advanced melanoma receiving PD-1 inhibitor monotherapy, in comparison with those with MALT1 levels at T_1 ≤3.100 (P=0.022; Fig. 3C); however, MALT1 levels at T_2 were not significantly associated with OS (P=0.080; Fig. 3D).

Independent factors for predicting PFS and OS in patients with advanced melanoma receiving PD-1 inhibitor monotherapy. According to univariate Cox regression analysis, MALT1 levels at T_0 (>3.100 vs. ≤3.100; P=0.034), TNM stage (IV vs. III; P=0.020) and LDH level (abnormal vs. normal; P=0.020) were significantly associated with a shorter PFS in patients with advanced melanoma receiving PD-1 inhibitor monotherapy. In contrast, PD-L1 level (positive vs. negative) was significantly associated with a prolonged PFS (P=0.048; Fig. 4A). After adjustment, MALT1 levels at T_0 [>3.100 vs. ≤3.100; hazard ratio (HR)=2.248; P=0.037] and LDH level (abnormal vs. normal; HR=2.303; P=0.022) were significantly independently associated with a shorter PFS in patients with advanced melanoma receiving PD-1 inhibitor monotherapy (Fig. 4B).

Univariate Cox regression analysis revealed that MALT1 levels at T_1 (>3.100 vs. ≤3.100; P=0.028), ECOG PS score (1 vs. 0; P=0.014) and TNM stage (IV vs. III; P=0.042) were significantly associated with a poor OS (Fig. 5A). After adjustment, only MALT1 levels at T_1 (>3.100 vs. ≤3.100; HR=4.332; P=0.007) were significantly associated with a shorter OS. Furthermore, PD-L1 level (positive vs. negative; HR=0.244, P=0.018) was significantly associated with a prolonged OS in patients with advanced melanoma receiving PD-1 inhibitor monotherapy (Fig. 5B).

Discussion

MALT1 regulates the behaviors of malignant tumor cells and immune escape, thereby playing a role in the pathology of various cancers. Studies have reported the dysregulation of MALT1 in several cancers, such as metastatic colorectal cancer

Table II. Relationship between MALT1 levels at different time points and the therapeutic response in patients with advanced melanoma (n=49).

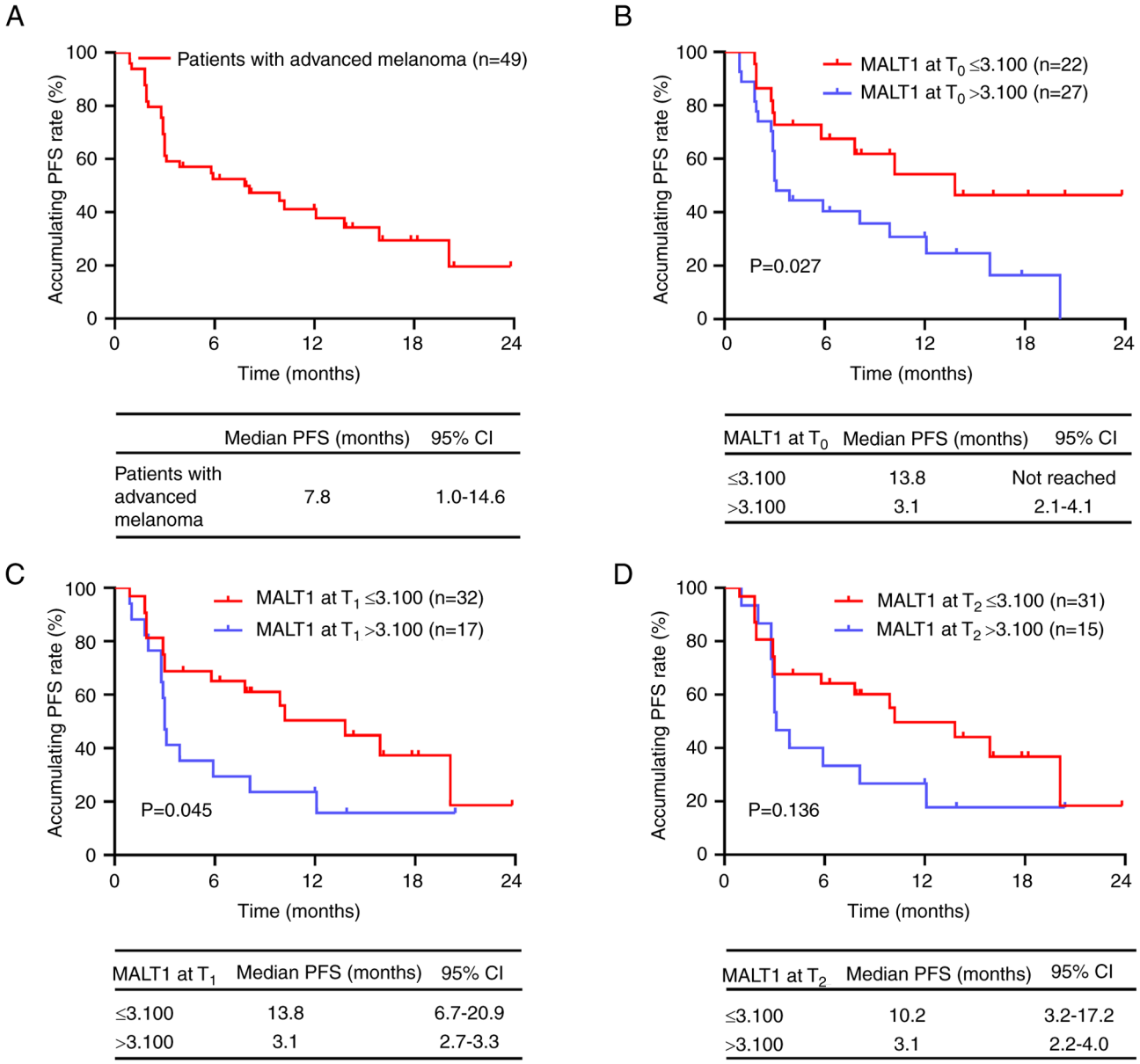
Items	Patients with advanced melanoma[a], n (%)	MALT1, median (IQR)					
		T_0 (n=49)	P-value	T_1 (n=49)	P-value	T_2 (n=46)	P-value
Response			0.053[a]		0.001[a]		0.021[a]
CR	3 (6.1)	2.250 (0.870-4.930)		1.410 (0.640-2.110)		1.580 (0.590-1.880)	
PR	11 (22.4)	3.000 (2.100-5.080)		1.610 (0.900-2.380)		1.300 (0.680-2.530)	
SD	15 (30.7)	3.315 (1.340-5.705)		1.730 (1.103-4.403)		1.405 (0.965-4.780)	
PD	20 (40.8)	3.985 (2.745-6.333)		3.155 (2.410-4.853)		2.130 (1.750-3.420)	
ORR			0.163[b]		0.009[b]		0.036[b]
Yes	14 (28.6)	2.800 (1.830-4.968)		1.510 (0.840-2.178)		1.315 (0.678-2.163)	
No	35 (71.4)	3.840 (1.910-6.125)		2.890 (1.670-4.568)		2.080 (1.180-3.930)	
DCR			0.070[b]		0.004[b]		0.059[b]
Yes	29 (59.2)	3.025 (1.433-5.088)		1.680 (1.025-3.153)		1.340 (0.748-3.153)	
No	20 (40.8)	3.985 (2.745-6.333)		3.155 (2.410-4.853)		2.130 (1.750-3.420)	

[a]Spearman's rank correlation coefficient; [b]Mann-Whitney U test. MALT1, mucosa-associated lymphoid tissue 1; T_0, before treatment; T_1, 2 months after treatment; T_2, 4 months after treatment; IQR, interquartile range; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, objective response rate; DCR, disease control rate.

(mCRC), hepatocellular cancer and prostate cancer (29-32). In the present study, it was observed that MALT1 levels was elevated in patients with advanced melanoma compared with in HCs. Potential explanations for this are as follows: i) MALT1 may strengthen melanoma cell proliferation, motility and survival by activating the Jun N-terminal Kinase/c-Jun and nuclear factor-κB pathways (33) and; ii) MALT1 may impair the activation of $CD8^+$ T cells, leading to immune escape and further induction of melanoma (21). In addition, the present demonstrated that MALT1 levels were reduced from T_0 to T_2 in patients with advanced melanoma receiving PD-1 inhibitor monotherapy. A possible reason may be that MALT1 can induce immune escape and after treatment with PD-1 inhibitors, $CD8^+$ T cells were activated, which attenuated the immune escape, leading to a decrease in MALT1 levels (21). Therefore, MALT1 was decreased after treatment of PD-1 inhibitors in patients with advanced melanoma. Furthermore, it was demonstrated that MALT1 levels were associated with an ECOG PS score of 1, a total tumor size >5 cm and TNM stage IV in patients with advanced melanoma receiving PD-1 inhibitor monotherapy. This may be explained by the potential for MALT1 to facilitate melanoma cell proliferation and metastasis to aggravate the disease conditions, leading to a higher ECOG PS score, larger tumor size and higher TNM stage (33).

Benefiting from the development of PD-1 inhibitors, the median OS of patients with advanced melanoma has been prolonged to more than 35 months (16,34,35). However, certain patients still lack therapeutic responses, and the corresponding markers that reflect therapeutic responses to PD-1 inhibitors are still limited (17). In the current study, it was demonstrated that MALT1 levels at T_1 were negatively associated with overall therapeutic response, ORR and DCR, and its level at T_2 was also negatively associated to overall therapeutic response and ORR in patients with advanced melanoma receiving PD-1 inhibitor monotherapy. The possible reasons may be as follows: i) MALT1 may impair the activation of $CD8^+$ T cells, which reduces the effect of PD-1 inhibitors and resulted in a poor therapeutic response to this treatment (21); and ii) MALT1 may maintain the immune-suppressive function of Treg cells in the tumor microenvironment, which facilitates immune escape and reduces the efficacy of PD-1 inhibitors (36). Taken together, MALT1 levels were associated with a poor therapeutic response to PD-1 inhibitors in patients with advanced melanoma.

The estimation of survival of patients with solid cancers receiving PD-1 inhibitors using their MALT1 levels was assessed by a previous study, which reported that high MALT1 levels before and after PD-1 inhibitor-based treatment was associated with a poor PFS and OS in patients with mCRC (32).



	Median PFS (months)	95% CI
Patients with advanced melanoma	7.8	1.0-14.6

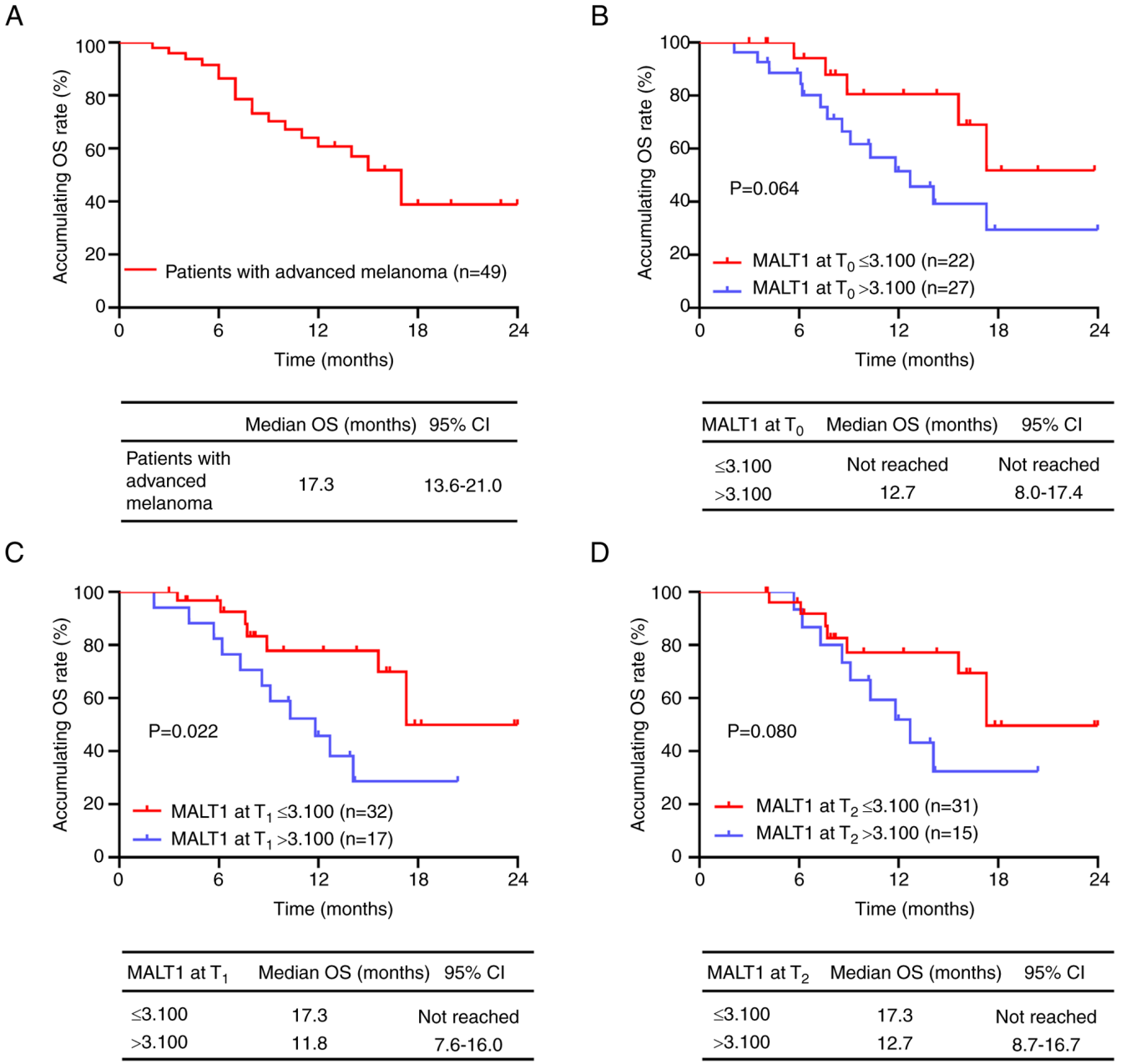
MALT1 at T_0	Median PFS (months)	95% CI
≤3.100	13.8	Not reached
>3.100	3.1	2.1-4.1

MALT1 at T_1	Median PFS (months)	95% CI
≤3.100	13.8	6.7-20.9
>3.100	3.1	2.7-3.3

MALT1 at T_2	Median PFS (months)	95% CI
≤3.100	10.2	3.2-17.2
>3.100	3.1	2.2-4.0

Figure 2. Association between MALT1 levels at T_0, T_1 and T_2, and PFS in patients with advanced melanoma receiving programmed cell death-1 inhibitor monotherapy. (A) Accumulating PFS rate. Association between MALT1 levels at (B) T_0, (C) T_1 and (D) T_2 with PFS. MALT1, mucosa-associated lymphoid tissue 1; T_0, before treatment; T_1, 2 months after treatment; T_2, 4 months after treatment; PFS, progression-free survival; CI, confidence interval.

The current study demonstrated that MALT1 levels at T_0 and T_1 were associated with a shortened PFS, and its level at T_1 was associated with a poor OS in patients with advanced melanoma receiving PD-1 inhibitor monotherapy. Multivariate Cox regression analysis further suggested that MALT1 levels at T_0 independently estimated poor PFS, and MALT1 levels at T_1 independently estimated worse OS in patients with advanced melanoma receiving PD-1 inhibitor monotherapy. The possible reasons may be as follows: i) MALT1 may facilitate the progression of melanoma, leading to poor survival (33); and ii) MALT1 may enhance the immune escape, leading to reduced PD-1 inhibitor efficacy and ultimately contributing to a worse prognosis (21,36). Therefore, MALT1 levels were associated with poor survival in patients with advanced melanoma receiving PD-1 inhibitor monotherapy. Notably, in patients with a MALT1 level >3.100, the median PFS and OS values at T_2 were increased compared with those at T_1. We hypothesize that the potential reason is that 3 patients were not analyzed at T_2, including 2 patients who experienced disease progression or death, and 1 patient who was lost to follow-up, which affected the results. Moreover, after searching relevant studies, only one previous study was identified that classified MALT1 into high and low levels based on its median value (2.529) in patients with mCRC receiving PD-1 inhibitors (32). This study reported that MALT1 >2.529 was independently associated with a shorter PFS in these patients with a HR of 1.981 (32). In comparison, the HR of MALT1 >3.100 for predicting PFS was higher in the present study, calculated to be 2.248. Therefore, according to the findings of the aforementioned previous study and the present study, MALT1 >3.100 may possess an improved effect for predicting poor prognosis in patients with several cancers who receive PD-1



	Median OS (months)	95% CI
Patients with advanced melanoma	17.3	13.6-21.0

MALT1 at T_0	Median OS (months)	95% CI
≤3.100	Not reached	Not reached
>3.100	12.7	8.0-17.4

MALT1 at T_1	Median OS (months)	95% CI
≤3.100	17.3	Not reached
>3.100	11.8	7.6-16.0

MALT1 at T_2	Median OS (months)	95% CI
≤3.100	17.3	Not reached
>3.100	12.7	8.7-16.7

Figure 3. Association between MALT1 levels at T_0, T_1 and T_2, and OS in patients with advanced melanoma receiving programmed cell death-1 inhibitor monotherapy. (A) Accumulating OS rate. Association between MALT1 levels at (B) T_0, (C) T_1 and (D) T_2 with OS. MALT1, mucosa-associated lymphoid tissue 1; T0, before treatment; T_1, 2 months after treatment; T_2, 4 months after treatment; OS, overall survival; CI, confidence interval.

inhibitors (32). However, both 2.529 and 3.100 were relative expression levels; therefore, their significance for clinical applications may be limited.

There are several limitations of the current study. Firstly, the sample size was small, which may have induced low statistical power. Secondly, the number of HCs was unmatched to the number of patients with advanced melanoma receiving PD-1 inhibitor monotherapy. This may have affected the results. Thirdly, the type of PD-1 inhibitors was not unified; thus, the generalization of the findings of the present study should be further validated.

In summary, MALT1 levels were decreased after PD-1 inhibitor treatment, and a high level estimated a poor therapeutic response and unsatisfactory survival in patients with advanced melanoma receiving PD-1 inhibitor monotherapy. Clinically, the present study proposes that MALT1 may serve as a potential marker to predict the prognosis of patients with advanced melanoma receiving PD-1 inhibitor monotherapy. Notably, using 3.100 as a threshold value for MALT1, and detecting it after 2 months of PD-1 inhibitor treatment yields a satisfactory predictive ability of MALT1 for prognosis in patients with advanced melanoma. MALT1 level detection may help physicians improve the management of patients with advanced melanoma receiving PD-1 inhibitor monotherapy; however, the prognostic role of MALT1 in patients with advanced melanoma receiving other treatments, and whether 3.100 could serve as the clinical normal level of MALT1, should be validated by further large-scale studies.

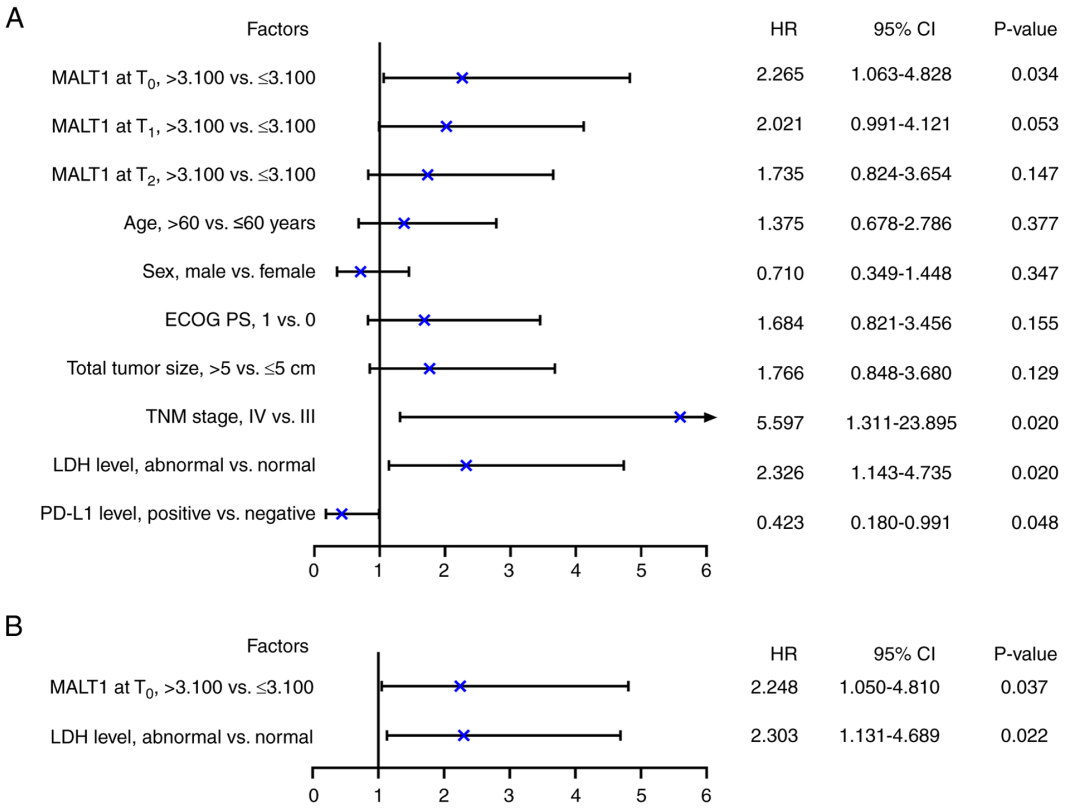


Figure 4. Independent factors associated with PFS in patients with advanced melanoma receiving programmed cell death-1 inhibitor monotherapy. (A) Univariate and (B) backward stepwise multivariate Cox regression analyses of PFS. PFS, progression-free survival; MALT1, mucosa-associated lymphoid tissue 1; T_0, before treatment; T_1, 2 months after treatment; T_2, 4 months after treatment; Eastern Cooperative Oncology Group Performance Status; TNM, tumor-node-metastasis; LDH, lactate dehydrogenase; PD-L1, programmed cell death-ligand 1; HR, hazard ratio; CI, confidence interval.

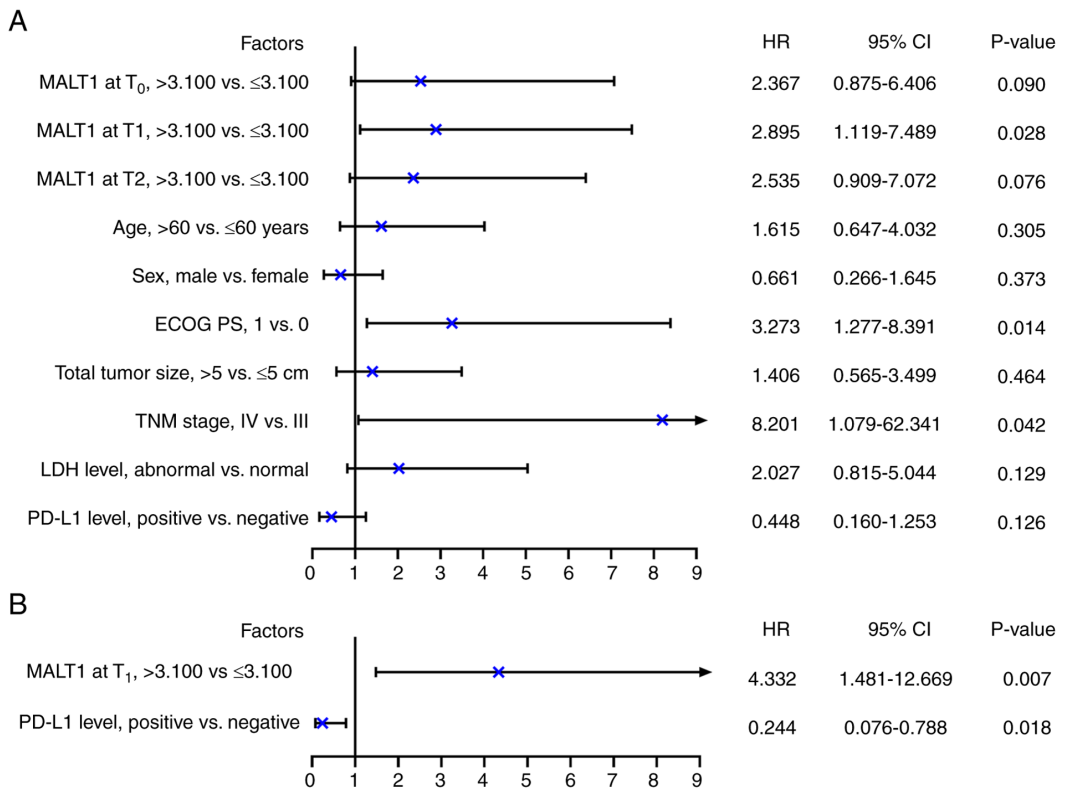


Figure 5. Independent factors associated with OS in patients with advanced melanoma receiving programmed cell death-1 inhibitor monotherapy. (A) Univariate and (B) backward stepwise multivariate Cox regression analyses of OS. OS, overall survival; MALT1, mucosa-associated lymphoid tissue 1; T_0, before treatment; T_1, 2 months after treatment; T_2, 4 months after treatment; Eastern Cooperative Oncology Group Performance Status; TNM, tumor-node-metastasis; LDH, lactate dehydrogenase; PD-L1, programmed cell death-ligand 1; HR, hazard ratio; CI, confidence interval.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

YT conceived and designed the present study. XM performed the experiments. KZ and JC collected and analyzed the experimental data. JY, ZG and GM were responsible for the interpretation of the data. YT and XM confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the Affiliated Hospital of Hebei Engineering University (Handan, China; approval no. 2018K037; February 17, 2018). The patients with advanced melanoma and healthy controls provided written informed consent at the time of enrolment.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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