

# High expression of B7-H3 and CD163 in cancer tissues indicates malignant clinicopathological status and poor prognosis of patients with urothelial cell carcinoma of the bladder

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**Abstract.** The objective of the present study was to investigate the association of B7-H3 expression and cluster of differentiation (CD)163<sup>+</sup> tumor-associated macrophage (TAM) infiltration with clinicopathological parameters in urothelial cell carcinoma of the bladder (UCB), and to investigate their potential conjoint effects on progression of UCB. B7-H3 expression and CD163<sup>+</sup> TAM infiltration in tumor specimens from 134 consecutive patients that underwent radical cystectomy for UCB were tested using immunohistochemistry, followed by statistical analysis. In these 134 patients, B7-H3 expression and CD163<sup>+</sup> TAM infiltration in the bladder carcinoma tissues were significantly associated with an increased ratio of vascular invasion ( $P=0.009$ ;  $P=0.012$ ) and distant metastasis ( $P=0.015$ ;  $P=0.038$ ); however, they were not associated with gender, age, pathologic grade, tumor stage, recurrence or lymphatic metastasis. The results of  $\chi^2$  test analysis indicated that CD163<sup>+</sup> TAM infiltration and B7-H3 expression were positively correlated ( $\chi^2=20.714$ ;  $P<0.001$ ). Overall survival (OS) and progression-free survival (PFS) rates were significantly worsened by high B7-H3 expression ( $P=0.002$ ;  $P=0.020$ ). However, CD163<sup>+</sup> TAM infiltration was not associated with OS or PFS rate. Notably, the OS and PFS rates in patients with high B7-H3 expression or high CD163<sup>+</sup> TAM infiltration were significantly poorer than the patients with low B7-H3 expression ( $P<0.001$ ;  $P<0.001$ ) or low CD163<sup>+</sup> TAM infiltration ( $P=0.022$ ;  $P=0.017$ ) in the subgroup of 115 patients with muscle-invasive bladder cancer. The results of the present study indicate that B7-H3 expression level could be used as an independent prognostic indicator following radical cystectomy

for UCB and patients with high B7-H3 expression and high CD163<sup>+</sup> TAM infiltration experience a poorer prognosis.

## Introduction

Bladder cancer is the second most common malignancy of the urinary system, with urothelial cell carcinoma of the bladder (UCB) being the most common type of bladder cancer (1). It is estimated that 76,960 Americans were diagnosed with bladder cancer in 2016, and 16,390 of them will succumb the disease (1). Surgical resection is the primary treatment method for patients with muscle-invasive and recurrent non-muscle-invasive UCB. However, metastasis resulted in 90% of incidences of mortality caused by UCB following gradual cystectomy or transurethral resection, the underlying molecular mechanism of which remains poorly understood (2-7).

The initiation and progression of tumors is closely associated with immune dysfunction in the tumor microenvironment. B7-H3 is a member of the B7 family of molecules, and serves an immunoregulatory function between tumor and immune cells (8,9). B7-H3 is widely expressed in urological tumors and numerous other types of human malignancy, and is associated with tumor progression, metastasis, recurrence and other adverse clinical features (10-15).

Monocytes can enter tumor tissues through vessel walls, where they are able to differentiate into macrophages, designated tumor-associated macrophages (TAMs). TAMs are associated with tumor metastasis; conflicting studies regarding the positive or negative function of TAMs in survival outcomes exist for a number of human malignancies (16-19). This phenomenon may be partly due to the heterogeneity of TAMs and the tumor microenvironment, particularly the varied nature of immunoregulatory factors. It has been reported that TAMs affect tumor angiogenesis, invasion and patient prognosis in various types of cancer, including bladder cancer (3-5). TAMs have been divided into two phenotypes: M1, the pro-inflammatory response, characterized by cells with surface markers cluster of differentiation (CD)80, CD8 or major histocompatibility complex II; and M2, the anti-inflammatory response, characterized by cells with surface markers CD20, CD30, CD16, or SLAM and responsible for tissue repair; it has been reported that the M2 phenotype affects the microvessels, pathological

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outcome, tumor grade and invasion of UCB (6). The cluster of differentiation 163 (CD163) antibody has been reported to be an M2-polarized-specific cell-surface receptor (6,7).

In the present study, which has 10 years of follow up data, the level of B7-H3 expression and the density of CD163<sup>+</sup> TAM infiltration were investigated in postoperative tumor specimens from 134 patients undergoing radical cystectomy (RC) for UCB, and the associations with various clinicopathological features were analyzed. Survival analysis was performed to determine the prognostic significance of B7-H3 expression and the association of CD163<sup>+</sup> TAMs with the postoperative survival of patients, and to evaluate the potential effects of these factors on the progression of UCB.

## Materials and methods

**Specimen collection.** Data and tumor and adjacent non-tumor tissue samples were obtained during surgical resection from 134 consecutive patients (67.2±6.89 years old, 115 males and 19 females) who were followed-up following radical cystectomy (RC) for UCB in the Urology Department, Fourth Hospital of Hebei Medical University (Hebei, China), between March 2004 and October 2005, were analyzed. The criteria for RC were: Muscle-invasive bladder carcinoma, recurrent Ta/T1 stage non-muscle-invasive bladder carcinoma and carcinoma *in situ* refractory to transurethral resection with intravesical chemotherapy. Patients were classified according to the methodology described in previous studies (3,6). Patients who did not manifest signs of tumor metastasis, as evidenced by cross-sectional imaging, and had no histories of preoperative radiotherapy or chemotherapy and neo-adjuvant chemotherapy were included.

**Ethics statement.** The use of human samples and the research involving human participants in this study was approved by the Ethics Committee of the Fourth Hospital of Hebei Medical University (Hebei, China). All participants provided written informed consent prior to participation in the present study.

**Immunohistochemistry and scoring.** Specimens were fixed using 10% formalin at room temperature for 4 h, followed by paraffin embedding. Samples were sliced into 5  $\mu$ m thick sections. The latter were dewaxed in xylene at 60°C, and hydrated with serial dilutions of ethanol (100, 95, 90, 85 and 75%), heated in 1 mmol/l EDTA (pH 8.0) to 121°C, cooled to 90°C, and incubated for 5 min. The sections were submerged in 3% H<sub>2</sub>O<sub>2</sub>, followed by deionized water for 10 min to eliminate endogenous peroxidase activity, and blocked with goat serum at 37°C for 30 min (Origene Technologies, Inc., Beijing, China) for 30 min. Sections were incubated with primary mouse anti-human B7-H3 antibody (1:500; cat. no., ab134161; Abcam, Cambridge, UK) or mouse anti-human CD163 antibody (1:100; cat. no., GTX42364, EDHu-1; Bio-Rad Laboratories, Inc., Hercules, CA, USA) at 4°C overnight. The sections were incubated with horseradish peroxidase-conjugated goat anti-mouse Immunoglobulin G (1:500; cat. no., TA130004, Origene Technologies, Inc.) as the secondary antibody at room temperature for 30 min, and rendered with diaminobenzidine for 3-5 min. Subsequently, sections were counterstained with 5% hematoxylin at room temperature for 10 min, dehydrated in ethanol at room

temperature (75 for 30 sec, 85 for 30 sec, 90 for 30 sec, 95 for 30 sec and 100% for 10 sec), cleared in 100% xylene for 30 sec, and mounted on coverslips. PBS was used to replace the primary antibody as the negative control.

A total of 5 random fields of view on each stained slide were viewed by light microscopy at magnification, x400, with the positive rate of every 100 tumor cells counted each view, and the immunohistochemical results were analyzed according to the tumor cells positively-dyed percentage (percentage=number of positively dyed cells in each 100 cells counted) and strength evaluation. The scoring method of B7-H3 expression was based on the stained area and intensity of staining. The frequencies of positive cells were categorized into low, medium and high groups that represented <33%, 34-67% and >67% positively-stained cells, respectively (20). Sections exhibiting low B7-H3 expression were categorized as having negative/weak staining intensity, whereas sections exhibiting high B7-H3 expression were categorized as samples with moderate and strong staining intensity, as described previously (20).

The density of CD163<sup>+</sup> TAM infiltration was based on the number of CD163<sup>+</sup> cells per field (magnification, x200). Quantification was performed as described previously (21), as follows: CD163<sup>+</sup> TAM infiltration of stroma and islets was scored I-III (score I, low CD163<sup>+</sup> TAM infiltration; score II, moderate CD163<sup>+</sup> TAM infiltration; score III, high CD163<sup>+</sup> TAM infiltration). Weak (score I), moderate (score II) and strong (score III) CD163<sup>+</sup> TAM infiltrations are presented in this article. Statistical analyses were performed using the following groupings: Low and high CD163<sup>+</sup> TAM infiltrations.

**Statistical analysis.** Data were analyzed with SPSS software (version 19.0; IBM Corp., Armonk, NY, USA). Data are presented as the mean  $\pm$  standard error of the mean. Associations between B7-H3 expression or CD163<sup>+</sup> TAM infiltration and clinicopathological features were evaluated using  $\chi^2$  tests and Fisher's exact tests. The correlation between B7-H3 staining and CD163<sup>+</sup> TAM infiltration were analyzed with  $\chi^2$  test. Associations of survival and tumor progression with B7-H3 expression or CD163<sup>+</sup> TAM infiltration were estimated using the Kaplan-Meier method, and differences were assessed using log-rank tests. All tests were two sided and P<0.05 was considered to indicate a statistically significant difference.

## Results

**Associations of B7-H3 expression and CD163<sup>+</sup> TAM infiltration with clinicopathological features in UCB patients.** Representative images of weak, moderate, and strong intensity of B7-H3 expression are presented in Fig. 1. High B7-H3 positive expression was identified in 93/134 (69.4%) cases and low B7-H3 expression was observed in 41/134 (30.6%) cases (Table I). Notably, high B7-H3 expression was significantly associated with increased vascular invasion (69.9 vs. 46.3%; P=0.009) and distant metastasis (49.5 vs. 26.8%; P=0.015) compared with low B7-H3 expression. No significant associations were identified between B7-H3 expression and other pathological factors including gender, age, tumor grade, tumor stage, recurrence and lymph node metastasis (Table I).

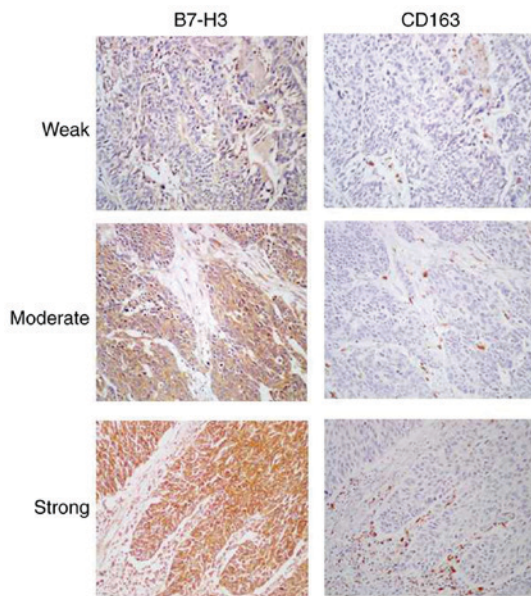


Figure 1. Images of B7-H3 expression and CD163<sup>+</sup> tumor-associated macrophage infiltration in urothelial cell carcinoma of the bladder tissues. Weak, moderate, and strong intensity of B7-H3 and CD163 expression are presented (magnification, x200). Left panels, expression levels of B7-H3 protein. Right panels, expression of CD163 protein in the same samples as the adjacent section. CD163, cluster of differentiation 163.

Representative images of weak, moderate and strong expression CD163<sup>+</sup> TAM infiltrations are presented in Fig. 1. Low CD163<sup>+</sup> TAM infiltration was identified in 75/134 cases (56.0%), and high infiltration was observed in 59/134 cases (44.0%) (Table I). High CD163<sup>+</sup> TAM infiltration was significantly associated with increased vascular invasion (74.6 vs. 53.3%,  $P=0.012$ ) and distant metastasis (52.5 vs. 34.7%,  $P=0.038$ ) compared with low CD163<sup>+</sup> TAM infiltration. No significant associations were identified between CD163<sup>+</sup> TAM infiltration and gender, age, tumor grade, tumor stage, recurrence and lymph node metastasis (Table I).

**Correlation between B7-H3 expression and CD163<sup>+</sup> TAM infiltration in UCB patients.** Among the 75 UCB patients exhibiting a low density of CD163<sup>+</sup> TAM infiltration, 35 (46.7%) exhibited a low level of B7-H3 expression. Of the 59 patients with a high density of CD163<sup>+</sup> TAM infiltration, 53 cases (89.8%) also exhibited a high level of B7-H3 expression. The results of Spearman's rank correlation analysis indicated that CD163<sup>+</sup> TAM infiltration and B7-H3 expression were positively correlated ( $r_s=0.393$ ,  $P<0.001$ , Table II).

**Association between B7-H3 expression or CD163<sup>+</sup> TAM infiltration and clinical outcomes in patients with UCB.** Kaplan-Meier survival analyses revealed that the estimated cancer overall survival (OS) rates at 1, 5 and 10 years following RC for patients with high B7-H3 expression were  $96.8\pm1.8$ ,  $23.9\pm4.9$  and  $13.6\pm6.6\%$ , respectively, compared with  $97.6\pm2.4$ ,  $40.2\pm7.8$  and  $27.8\pm8.2\%$  for patients with low B7-H3 expression, respectively. The estimated progression free survival (PFS) rates at 1, 5 and 10 years following RC for patients with high B7-H3 expression were  $68.5\pm4.8$ ,  $22.1\pm5.6$  and  $11.0\pm8.3\%$ , respectively, compared with  $90.1\pm4.7$ ,

$34.1\pm7.8$  and  $25.9\pm0.0\%$  for patients with low B7-H3 expression respectively. Thus, high B7-H3 expression was significantly associated with decreased OS ( $P=0.002$ ) and PFS ( $P=0.020$ ) in 134 patients with UCB (Fig. 2A and B). However, in this cohort of 134 patients, the density of CD163<sup>+</sup> TAM infiltration was irrelevant to the OS ( $P=0.074$ ) and PFS ( $P=0.090$ ) (Table III). Notably, the OS and PFS rates in patients with high B7-H3 expression or high CD163<sup>+</sup> TAM infiltration were significantly poorer than those in patients with low B7-H3 expression (OS,  $P<0.001$ ; PFS,  $P<0.001$ ; Table III; Fig. 2C and D) or low CD163<sup>+</sup> TAM infiltration (OS,  $P=0.022$ ; PFS,  $P=0.017$ ; Table III; Fig. 2E and F) in the subgroup of 115 UCB patients with muscle-invasive bladder cancer.

## Discussion

In this cohort of 134 patients with UCB, a positive correlation was identified between the level of B7-H3 expression and CD163<sup>+</sup> TAM infiltration, which were significantly associated with vascular invasion, distant metastasis and poor outcomes in the patients undergoing RC for UCB, indicating that B7-H3 may be involved in the progression of UCB with CD163<sup>+</sup> TAM infiltration, and that B7-H3 may be a novel immunotherapeutic target for patients with UCB.

B7-H3, a member of the B7 family, has been demonstrated to mediate the proliferation of CD4<sup>+</sup> and CD8<sup>+</sup> T cells, and to enhance interferon- $\gamma$  (IFN- $\gamma$ ) production (8). B7-H3 may confer protection from natural killer (NK)-cell-mediated cytotoxicity (22). However, other studies have indicated that B7-H3 is able to inhibit the proliferation of T cells and reduce the secretion of IFN- $\gamma$  and interleukin-2 (IL-2) (23,24). Although there is no consensus on the immunological or pathophysiological functions of B7-H3, aberrant B7-H3 expression has been demonstrated to be associated with tumor progression and poor prognosis in human urological neoplasms (13,14). The present study demonstrated that patients with UCB displaying high B7-H3 expression were more likely to exhibit vascular invasion and distant organ metastasis, and experience shorter OS and PFS times than patients displaying low B7-H3 expression. These findings differ from those obtained by Boorjian *et al* (25), who identified no association between B7-H3 expression and disease progression following cystectomy. These differences may be partly due to the higher proportion of muscle-invasive bladder cancer in the present cohort (85.8%) than in the Boorjian *et al* cohort (65.2%), thus the risk of postoperative tumor metastasis and mortality would be potentially increased in the present cohort of patients with UCB. Subsequently, survival analyses were conducted in the subgroup of muscle-invasive bladder cancer and it was identified that the OS and PFS rates for patients with high B7-H3 expression were significantly poorer than those for patients with low B7-H3 expression. B7-H3 may serve a pro-tumor function in patients with UCB and could be regarded as an independent indicator for malignant progression of UCB, particularly in patients with muscle-invasive bladder cancer, which, to the best of our knowledge, has not yet been reported. Additionally, B7-H3 expression was not associated with pathological features such as tumor grade, stage and lymph node metastasis in the present study, which is inconsistent with the findings of a previous study (26).



Table I. Association between B7-H3 or CD163<sup>+</sup> TAMs and clinicopathological features in 134 patients with UCB.

Feature	Patients, n%	B7-H3 expression		P-value	CD163 <sup>+</sup> TAM infiltration		P-value
		Low, n (%)	High, n (%)		Low, n (%)	High, n (%)	
Age, years				0.067			0.634
<65	53 (39.6)	21 (51.2)	32 (34.4)		31 (41.3)	22 (37.3)	
≥65	81 (60.4)	20 (48.8)	61 (65.6)		44 (58.7)	37 (62.7)	
Gender				0.330			0.855
Male	115 (85.8)	37 (90.2)	78 (83.9)		64 (85.3)	51 (86.4)	
Female	19 (14.2)	4 (9.8)	15 (16.1)		11 (14.7)	8 (13.6)	
Tumor stage				0.202			0.220
Ta/CIS/T1	19 (14.1)	8 (19.5)	11 (11.8)		12 (16.0)	7 (11.9)	
T2	58 (43.3)	21 (51.2)	37 (39.8)		36 (48.0)	22 (37.3)	
T3	40 (29.9)	9 (22.0)	31 (33.3)		21 (28.0)	19 (32.2)	
T4	17 (12.7)	3 (7.3)	14 (15.1)		6 (8.0)	11 (18.6)	
Tumor grade				0.201			0.177
Low	27 (20.1)	11 (26.8)	16 (17.2)		12 (16.0)	15 (25.4)	
High	107 (79.9)	30 (73.2)	77 (82.8)		63 (84.0)	44 (74.6)	
Lymph node metastasis				0.185			0.364
Yes	40 (29.9)	9 (22.0)	31 (33.3)		20 (26.7)	20 (33.9)	
No	94 (70.1)	32 (78.0)	62 (66.7)		55 (73.3)	39 (66.1)	
Recurrence				0.306			0.192
Yes	71 (53.0)	19 (46.3)	52 (55.9)		36 (48.0)	35 (59.3)	
No	63 (47.0)	22 (53.7)	41 (44.1)		39 (52.0)	24 (40.7)	
Distant metastasis				0.015			0.038
Yes	57 (42.5)	11 (26.8)	46 (49.5)		26 (34.7)	31 (52.5)	
No	77 (57.5)	30 (73.2)	47 (50.5)		49 (65.3)	28 (47.5)	
Vascular invasion				0.009			0.012
Yes	84 (62.7)	19 (46.3)	65 (69.9)		40 (53.3)	44 (74.6)	
No	50 (37.3)	22 (53.7)	28 (30.1)		35 (46.7)	15 (25.4)	

CD, cluster of differentiation; TAM, tumor-associated macrophage.

Table II. Correlation between B7-H3 expression and CD163<sup>+</sup> TAM infiltration in UCB patients.

CD163 <sup>+</sup> TAM infiltration	Patients, n	B7-H3 expression		$\chi^2$	P-value
		Low, n 41	High, n 93		
Low	75	35	40	20.714	<0.001
High	59	6	53		

CD, cluster of differentiation; Tam, tumor-associated macrophage.

Numerous growth factors, cytokines, chemokines and enzymes released by TAMs promote tumor progression and poor survival rates (16,27,28). CD163<sup>+</sup> TAM infiltration was correlated with high-stage and –grade disease, and associated with the formation of micro vessels, pathological outcome, tumor grade and invasiveness of UCB (3,6,21). Furthermore, the expression of CD163 was markedly associated with local

expression of IL-6 and IL-10, which function as mediators to induce CD163 expression *in vitro* (21). The association between M2 TAM infiltration and histologically advanced UCB was confirmed in 46 patients (21). In the present study, 134 patients were treated with RC for UCB, of which 115 patients had muscle-invasive UCB. The levels of vascular invasion and distant organ metastasis were increased significantly in the

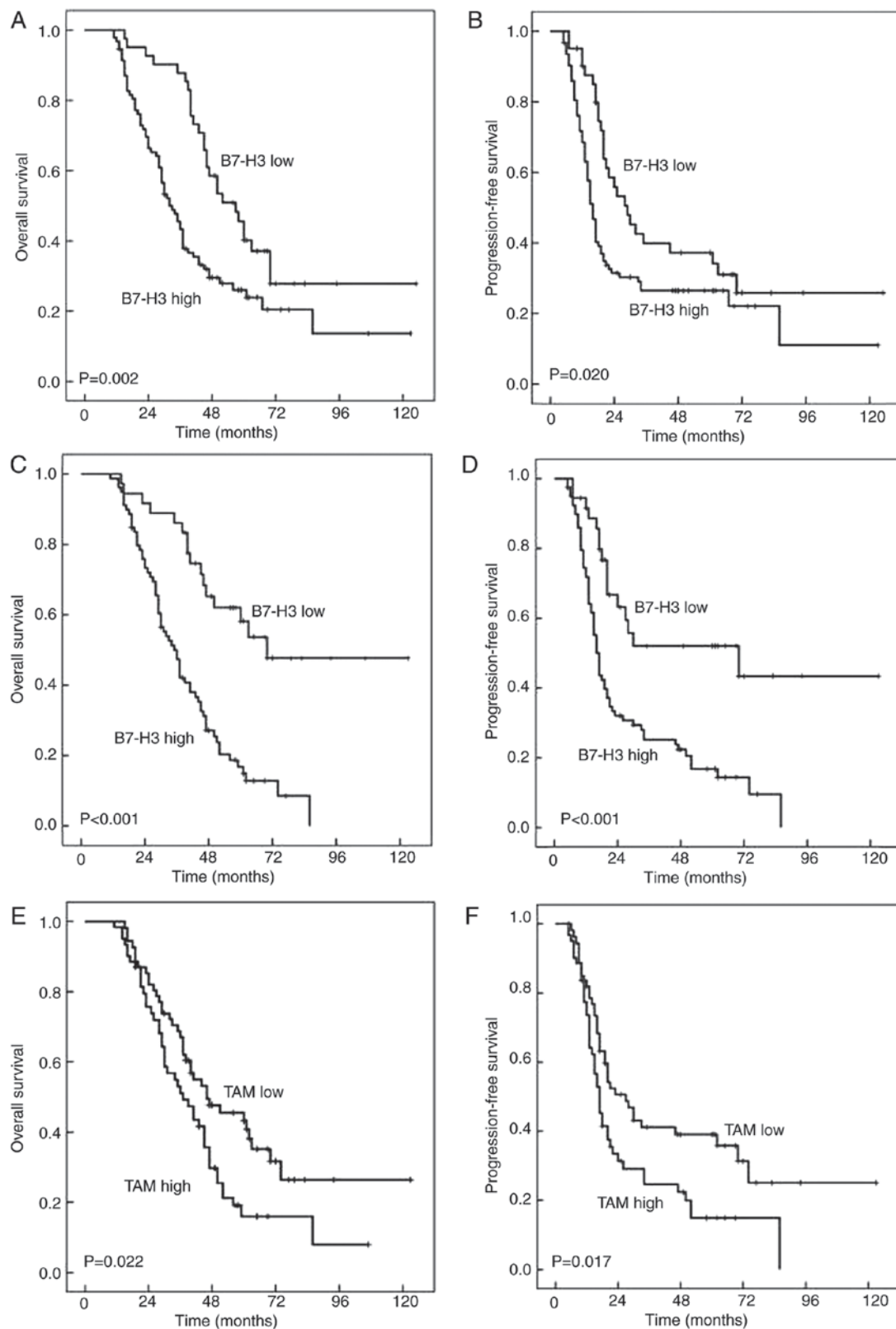


Figure 2. Association of B7-H3 expression and CD163<sup>+</sup> TAM infiltration with OS and PFS in patients with UCB. (A) OS rates with B7-H3 expression in cohort of 134 patients. Shorter OS times were observed in patients with high B7-H3 expression compared with low B7-H3 expression (P=0.002). (B) PFS rates with B7-H3 expression in cohort of 134 patients. Shorter PFS times were observed in patients with high B7-H3 expression than that in low B7-H3 expression (P=0.020). (C) OS rates with B7-H3 expression in the subgroup of patients with muscle-invasive bladder cancer. Shorter OS times were observed in patients with high B7-H3 expression compared with low B7-H3 expression (P<0.001). (D) PFS rates with B7-H3 expression in the subgroup of patients with muscle-invasive bladder cancer. Shorter PFS times were observed in patients with high B7-H3 expression compared with low B7-H3 expression (P<0.001). (E) OS rates with CD163<sup>+</sup> TAM infiltration in the subgroup of patients with muscle-invasive bladder cancer. Shorter OS times were observed in patients with high CD163<sup>+</sup> TAM infiltration compared with low CD163<sup>+</sup> TAM infiltration (P=0.022). (F) PFS rates with CD163<sup>+</sup> TAM infiltration in the subgroup of patients with muscle-invasive bladder cancer patients. Shorter OS times were observed in patients with high CD163<sup>+</sup> TAM infiltration compared with low CD163<sup>+</sup> TAM infiltration (P=0.017). CD163, cluster of differentiation 163; OS, overall survival; PFS, progression-free survival; UCB, urothelial cell carcinoma of the bladder; TAM, tumor-associated macrophages.

Table III. OS and PFS rates at different levels of B7-H3 expression and CD163<sup>+</sup> TAM infiltration in patients with UCB.

A, All patients (n=134)									
Feature	n	OS, % (±SE)			P-value	PFS, % (±SE)			P-value
		1 year	5 years	10 years		1 year	5 years	10 years	
B7-H3 expression					0.002				0.020
High	93	96.8 (1.8)	23.9 (4.9)	13.6 (6.6)		68.5 (4.8)	22.1 (5.6)	11.0 (8.3)	
Low	41	97.6 (2.4)	40.2 (7.8)	27.8 (8.2)		90.1 (4.7)	34.1 (7.8)	25.9 (8.0)	
CD163 <sup>+</sup> TAM infiltration					0.074				0.090
High	59	98.3 (1.7)	22.0 (5.8)	16.5 (6.4)		65.5 (6.2)	23.7 (5.6)	15.8 (7.5)	
Low	75	97.3 (1.9)	35.3 (5.8)	22.1 (6.1)		82.6 (4.4)	32.2 (5.7)	22.8 (6.2)	

## B, Patients with muscle-invasive bladder cancer (n=115)

Feature	n	OS, % (±SE)			P-value	PFS, % (±SE)			P-value
		1 year	5 years	10 years		1 year	5 years	10 years	
B7-H3 expression					<0.001				<0.001
High	79	98.7 (1.3)	14.9 (4.5)	0 (0.0)		71.8 (5.1)	16.8 (4.6)	0 (0.0)	
Low	36	100 (0.0)	58.1 (8.7)	47.7 (9.9)		94.4 (3.8)	52.0 (9.2)	43.4 (11.0)	
CD163 <sup>+</sup> TAM infiltration					0.022				0.017
High	54	100 (0.0)	15.9 (5.5)	8.0 (6.3)		73.6 (6.1)	14.9 (5.3)	0 (0.0)	
Low	61	98.4 (1.6)	43.2 (6.7)	26.4 (7.6)		81.9 (4.9)	35.8 (6.8)	25.1 (8.1)	

OS, overall survival; PFS, progression-free survival; SE, standard error; CD163, cluster of differentiation; TAM, tumor-associated macrophage; UCB, urinary carcinoma of the bladder.

subgroup exhibiting high CD163<sup>+</sup> TAM infiltration compared with the patients with low CD163<sup>+</sup> TAM infiltration, whereas no association was observed between the two subgroups for OS rate, PFS rate and other clinicopathological features, including gender, tumor grade, stage and lymph node metastasis. However, in the subgroup of 115 patients with muscle-invasive bladder cancer, it was identified that the OS and PFS rates in patients with high CD163<sup>+</sup> TAM infiltration were significantly poorer than in patients with low infiltration. These findings reveal that CD163<sup>+</sup> TAM infiltration may be useful for prognostic prediction of survival in patients with UCB, indicating a possible pro-tumor function for CD163<sup>+</sup> TAM infiltration in the malignant progression of UCB, which has not been accurately described in a large cohort with a high proportion of patients with muscle-invasive bladder cancer.

The rates of vascular invasion and distant metastasis were significantly elevated in the subgroup of patients with high B7-H3 expression or high CD163<sup>+</sup> TAM infiltration. The results of  $\chi^2$  test analysis revealed that there was a positive correlation between CD163<sup>+</sup> TAM infiltration and B7-H3 expression, indicating that B7-H3 and TAMs may be involved in the initiation and malignant progression of UCB and jointly exhibit immunosuppressive effects on the tumor microenvironment. Pro-tumor TAM polarization facilitated by over-expression of B7-H3 expression maybe involved in the B7-H3/signal transducer and activation of transcription 3 signaling pathway (29).

As a novel co-regulatory molecule, the immunological function of B7-H3 remains controversial, which may reflect the potential presence of two receptors for B7-H3 with opposing co-regulatory functions on the proliferation of CD4<sup>+</sup> T cells and induction of cytotoxic T cells (8,24). B7-H3 and TAMs in UCB may be involved in the adaptive immune response to tumors mediated by CD4<sup>+</sup> T cells, particularly T helper cells, in addition to their potential interaction with other tumor-infiltrating immune cells, such as regulatory T cells, CTLs and NK cells. Additionally, it is hypothesized that the aberrant expression of B7-H3 or CD163<sup>+</sup> TAM infiltration resulting from the complicated UCB tumor microenvironment may attract peripheral circulating monocytes to UCB tissues, priming them to induce the polarization of macrophages to pro-tumor TAMs (6-15,22-29). These pro-tumor TAMs are characterized by production of epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), matrix metalloproteinases (MMPs) and high levels of IL-10, IL-17 and transforming growth factor- $\beta$  (TGF- $\beta$ ) (18,30). These cytokines markedly promote angiogenesis, stromal invasion and metastasis, and suppress adaptive antitumor immune responses (18,30). Therefore, it is hypothesized that B7-H3 expression and CD163<sup>+</sup> TAM infiltration maybe effective predictors of tumor progression and prognosis, particularly in patients with muscle-invasive bladder cancer.

The present study represents a valuable evaluation of the prognostic significance of B7-H3 and CD163<sup>+</sup> TAM infiltration

in UCB; however, the present study is not devoid of limitations. Although the identification of CD163<sup>+</sup> TAM infiltration has been demonstrated to be associated with a poor prognosis in several types of cancer, including UCB (30-32), CD163 is also expressed by a significant portion of the malignant cells in tumors and lymph nodes (7,21,32,33). Additionally, TAMs were not mechanically divided into M1-type and M2-type phenotype macrophages, as was performed in previous studies (2-7,14-18). Therefore, continued study of the effects of B7-H3 and CD163<sup>+</sup> TAM infiltration with other macrophage phenotypes in the progression of UCB are required in larger series and different cohorts.

To conclude, the expression levels of the co-stimulatory molecule B7-H3 and CD163<sup>+</sup> TAM infiltration were investigated in tumor specimens following RC for UCB. Patients with high B7-H3 expression and high CD163<sup>+</sup> TAM infiltration exhibited malignant progression and poor survival rates, particularly those with muscle-invasive bladder cancer. It was also identified that B7-H3 expression level maybe used as a valuable prognostic indicator following RC for UCB. B7-H3 and CD163<sup>+</sup> TAM infiltration may function as prognostic indicators and, with additional study, may be a novel immunotherapeutic target for patients with UCB.

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#### Availability of data and materials

All data generated or analyzed during this study are included in this published article.

#### Authors' contributions

ZX and BS conceived and designed the experiments. ZX, LW, JT, HM and PL performed the experiments. LW, JT, HM and PL analyzed the data. ZX, LW, JT, HM, PL and BS contributed reagents, materials and analytical tools. ZX, LW and BS wrote the paper.

#### Ethics approval and consent to participate

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The use of human samples and the research involving human participants in this study was approved by the Ethics Committee of the Fourth Hospital of Hebei Medical University (Hebei, China). All participants provided written informed consent prior to participation in the present study.

#### Consent for publication

The patients, or legal guardians, have provided written informed consent for the publication of any associated data and accompanying images.

#### Competing interests

The authors declare that they have no competing interests.

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