Int J Clin Exp Pathol 2015;8(9):11510-11516
www.ijcep.com /ISSN:1936-2625/IJCEP0012292

Original Article

High CD4+ T cell density is associated with poor prognosis in patients with non-muscle-invasive bladder cancer

Qinglei Zhang[1,2*], Chongli Hao[3*], Guangzhou Cheng[2], Lei Wang[2], Xiang Wang[2], Chang Li[4], Juhui Qiu[1], Kejia Ding[1]

[1]Department of Urology, Shandong Provincial Hospital Affiliated to Shandong University, Jinan, Shandong, China; [2]Department of Urology, Tengzhou Central People's Hospital, Zaozhuang, Shangdong, China; [3]Department of Oncology, Tengzhou Central People's Hospital, Zaozhuang, Shangdong, China; [4]Department of Pathology, Tengzhou Central People's Hospital, Zaozhuang, Shangdong, China. [*]Equal contributors.

Received July 2, 2015; Accepted August 25, 2015; Epub September 1, 2015; Published September 15, 2015

Abstract: Purpose: The aim of this study was to investigate the clinical significance of CD4+ T cells in non-muscle-invasive bladder cancer (NMIBC) tissues *in situ*. Methods: Immunohistochemistry was used to examine the distribution of CD4+ T cells in 131 NMIBC tissues. Kaplan-Meier analysis and Cox proportional hazards regression models were applied to estimate overall survival (OS) and recurrence-free survival (RFS). Results: NMIBC patients were divided into two groups based on the median frequency of CD4+ T cells (median, 1/×400 high resolution). On univariate analysis, CD4+ T cell density was inversely associated with overall survival ($P = 0.01$). In those patients with high CD4+ T density, 5-year OS rates was only 77%, compared with 86% in those with low density, respectively. Although CD4+ T cell density showed no prognostic significance for RFS ($P = 0.36$), 5-year RFS rates of patients with high CD4+ T density (58%) was lower than those of patients with low CD4+ T density (65%, respectively). By multivariate analysis, tumor infiltrating CD4+ T cell density emerged as an independent prognostic factor for OS (HR, 2.75; $P = 0.004$). In addition, no association was found between CD4+ T cell density and any clinicopathological variables ($P > 0.05$). Conclusion: Our findings suggest that CD4+ T cells could potentially serve as a poor prognostic marker for patients with NMIBC.

Keywords: CD4, T cells, prognosis, non-muscle-invasive bladder cancers (NMIBC)

Introduction

High-risk Non-muscle-invasive bladder cancer (NMIBC) is clinically characterized by high recurrence rates and represents one of the most costly cancers [1]. Bacillus Calmette-Guerin immune therapy has been used to prevent recurrence in patients with NMIBC for more than 40 years [2-4]. Although little is known about the exact mechanism for the effectiveness of Bacillus Calmette-Guerin immune therapy in NMIBC, induction of a local T cell-mediated immune response seems to be the most likely cause [5].

Tumor-infiltrating T cells may be an indicator of the host immune response to tumor and an attractive target for immunotherapy [6, 7]. Most emphasis to date has been focused on CD8+ T cells, as they can be directly cytotoxic to tumor cells and their abundance inside tumors appears to indicate better prognosis [8]. Previous studies have shown that CD8+ T cells infiltration is associated with improved disease outcome in human bladder cancer [9] and other various tumors, including ovarian, renal, lung and colorectal carcinoma [10-13]. In contrast, the complex ways of CD4+ T cells influencing tumor immunity are not fully understood [8, 14]. In mice models, tumor specific CD4+ T cells can reject myeloma by activating macrophages [15]. CD4+ T cells can also be more efficient than CD8+ T cells at rejecting solid tumors [16]. In fact, CD4+ T cells can either display anti- or pro-tumoral activity [17-19]. However, little is known about the clinical significance of CD4+ T cells in patients with NMIBC.

Herein, by using a new rabbit anti-human CD4 antibody, we investigated the prognostic signifi-

cance of CD4+ T cells in 131 patients with NMIBC. The results showed that CD4+ T cells could predict a poor prognosis in human bladder cancer.

Materials and methods

Patients and tissue specimens

Tissue specimens were obtained from 131 patients who had pathologically confirmed NMIBC, at Tengzhou central people's hospital of Shandong province during January 2003 and December 2009. No patient had a distant metastasis or received anticancer therapies prior to surgery. All tumors were graded according to the World Health Organization 2004 classification and staged according to the TNM classification (6th edition, 2002). Specimens were obtained immediately after surgical resection and fixed in 10% neutral formalin, then paraffin embedded and used for histological assays. This study was approved by the Ethics Committee of our institution, following the ethical guidelines of the Declaration of Helsinki, with informed consent in writing obtained from every patient.

The clinicopathologic and follow-up data of patients were prospectively collected. All patients were evaluated every 3 months during the first year, every 6 months during the second year, and annually thereafter. Follow-up visits consisted of a history, physical examination, and routine biochemical analyses. Ultrasonography of the abdomen, urography, and chest X-rays were performed at 3, 6 and 12 months post operatively, and then annually unless otherwise clinically indicated. Abdominal/pelvic CT scans were performed 6 months postoperatively and annually thereafter. Overall survival (OS) was defined as the interval between surgery and death or between surgery and the last observation for surviving patients. Recurrence-free survival (RFS) was defined as the interval between surgery and recurrence or between surgery and the last observation for patients without recurrence.

Immunohistochemistry

Formalin-fixed and paraffin-embedded samples were cut into 4-μm sections, which then were processed for immunohistochemistry as previously described [20]. Briefly, paraffin sections were first deparaffinized and hydrated, then endogenous peroxidase activity was blocked by incubating the slides in 3% hydrogen peroxide containing double distilled water. Antigen retrieval was performed by microwave treatment in citrate buffer (pH 6.0). Sections were blocked with normal sera from the same species from which secondary antibodies were derived. After overnight incubation at 4°C with antibody against CD4 (1:500 dilution, Sino Biological Inc., Beijing, China), or control antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), sections were incubated with secondary antibody conjugated to horseradish peroxidase (Envision + Dual Link Kit, DAKO) for 30 min at 37°C. The enzymatic reactions were developed peroxidase and 3,3'-diaminobenzidine tetrahydrochloride. Sections were counterstained with hematoxylin (Zymed Laboratories, San Francisco, CA, USA) and mounted in non-aqueous mounting medium.

Evaluation of immunohistology

Tissue sections were analyzed by two independent observers who were blinded to the clinical outcome. To evaluate the density of tissue-infiltrating CD4+ T cells, tissue sections were screened at a low-power field (100×) and the five most representative fields were selected for analysis at 400× magnification (0.07 mm^2 per field) using a Nikon DS-Fi2 CCD camera (Nikon, Tokyo, Japan) that was installed on a Nikon Eclipse *80i* microscope (Nikon, Tokyo, Japan). The infiltrating cells per field were enumerated manually and counts were expressed as means ± SEM. Because macrophages occasionally stain with CD4 antibody, CD4+ T cells with apparent morphological appearance of macrophages were excluded from the count. A significant linear correlation existed between the counts of two independent observers and the average count of the two investigators was used in subsequent analyses to minimize inter-observer variability.

Statistical analyses

Statistical analyses were performed using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). Cumulative survival time was calculated using the Kaplan-Meier method and was analyzed by the log-rank test. A multivariate Cox proportional hazards model was used to estimate the adjusted hazard ratios and 95% confi-

Table 1. Clinicopathological characteristics of non-muscle-invasive bladder cancer patients

Variable	No.
No. of patients	131
Age, years (median, range)	62 (20-90)
Sex (male/female)	113/18 (86.3%/13.7%)
Tumor size (≤ 3 cm/> 3 cm)	108/23 (82.4%/17.6%)
Tumor number (unifocal/multifocal)	89/42 (67.9%/32.1%)
Tumor stage (Ta/T1)	31/100 (23.7%/76.3%)
Histological grade (low/high)	94/37 (71.8%/28.2%)

dence intervals (CIs), and to identify independent prognostic factors. For categorical analyses, the median value was used as a cut-off to dichotomize continuous variables (for clinical applications). The relationships between categorical variables were analyzed using χ^2 tests. For such comparisons, two-tailed *P*-values < 0.05 were considered statistically significant.

Results

Study population

Clinicopathological characteristics of the 131 NMIBC patients are presented in **Table 1**. Median age of patients was 60 yr, 86.3% were male, 82.4% had tumors < 3 cm, 67.9% had multifocal disease, 23.7% had Ta stage, and 71.8% had low histological grade. The median follow-up for living patients was 76.9 months (range, 6.4-137.3 months). Of 131 patients, 37 (28.2%) patients died and 59 (45%) patients had evidence of disease recurrence during this follow-up period. The OS and RFS rates were 97% and 78% at 2 year, 79% and 62% at 5 years, respectively.

Survival analysis

On univariate analysis (**Table 2**), sex, tumor size, tumor number and tumor stage showed no prognostic significance for OS and RFS. In contrast, age was predictor for OS ($P < 0.001$) and histological grade was predictor for RFS ($P = 0.04$). For further analysis, the counting data of CD4+ T cells were divided into 2 groups: high density and low density. Kaplan-Meier survival curves were then plotted to investigate further the association with survival (**Figure 1**). The log-rank statistic was used to compare survival rates. There was an inverse correlation between CD4+ T cell density and OS ($P = 0.01$, **Figure 1A**). Patients with higher CD4+ T cell density had significantly shorter OS (median 75.9 months) than those with lower CD4+ T cell density (median 82.4 months). In those patients with a CD4+ T density above the median, 5-year and 10-year OS rates were only 77% and 54%, compared with 86% and 71% in those below the median, respectively. Although CD4+ T cell density showed no prognostic significance for RFS ($P = 0.36$, **Figure 1B**), 5-year and 10-year RFS rates of patients with a CD4+ T density above the median (58% and 32%, respectively) were lower than those of patients with a CD4+ T density below the median (65% and 54%, respectively). However, no association was found between CD4+ T cell density and clinicopathological variables including age, sex, tumor size, tumor number, tumor stage and histological grade (**Table 3**).

We next assessed whether CD4+ T cell density could serve as independent predictor of OS. A multivariate Cox proportional hazards analysis was performed, and those variables that were associated with survival by univariate analysis were adopted as covariates (**Table 4**). The density of CD4+ T was an independent prognostic factor for OS (HR, 2.75; $P = 0.004$). Moreover, age remained associated with OS (HR, 5.169; $P < 0.001$).

Discussion

The available prognostic tool that was based on clinicopathological variables, such as the European Organisation for Research and Treatment of Cancer (EORTC) risk tables, had insufficient discriminative ability to accurately predict the risk of disease recurrence and progression at the level of the individual patient [21]. New biomarkers are needed to predict the outcome of bladder cancer, in addition to commonly used clinicopathological parameters [22].

Biomarkers may help to identify patients with NMIBC at higher risk for disease progression [23]. However, to date, such biomarkers have remained substantially limited. In this study, we found high CD4+ T cell density was an independent predictor of poor OS for patients with NMIBC. These findings identify CD4+ T cells as a potential prognostic marker that may help

Table 2. Univariate analysis of the factors associated with survival and recurrence

	Overall survival			Recurrence-free survival		
Variable	HR	95% CI	P	HR	95% CI	P
Age, years (> 62/≤ 62)	4.843	2.283-10.277	< 0.001	0.888	0.527-1.495	0.654
Sex (female/male)	0.762	0.27-2.153	0.608	1.127	0.554-2.292	0.742
Tumor size (> 3 cm/≤ 3 cm)	1.8	0.849-3.819	0.125	0.912	0.448-1.856	0.8
Tumor number (multifocal/unifocal)	0.856	0.414-1.771	0.675	1.556	0.919-2.633	0.1
Tumor stage (T1/Ta)	0.604	0.285-1.281	0.189	0.812	1.449-1.47	0.492
Histological grade (high/low)	1.615	0.831-3.14	0.158	1.75	1.025-2.988	0.04
CD4+ T cells (high/low)	2.487	1.248-4.955	0.01	1.43	0.856-2.389	0.172

Abbreviations: HR, hazard ratio; CI, confidence interval; NA, not applicable. Note: Univariate and multivariate analysis. Cox proportional hazards regression model. HR > 1, risk for death increased; HR < 1, risk for death reduced.

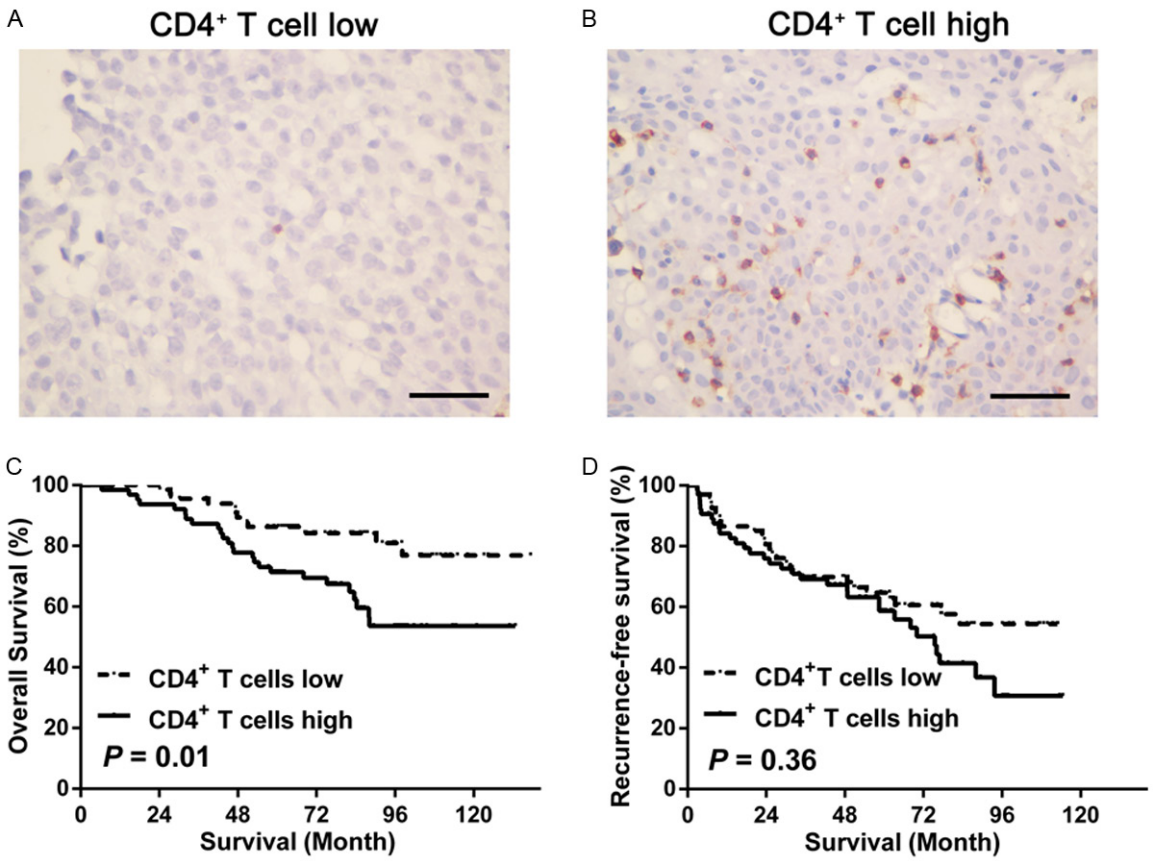

Figure 1. Prognostic significance of CD4+ T cells in NMIBC (n = 131). A, B: Representative pictures of CD4+ T cells. Patients were divided into two groups according to the median number of CD4+ T cells per ×400 field (median = 1). Bars, 50 μm. C, D: Cumulative overall survival and recurrence-free survival curves were calculated by the Kaplan-Meier method and analyzed by the log-rank test. *Dash lines*, low group; *Solid lines*, high group.

guide individual therapeutic management of patients with NMIBC.

The adaptive immune system may play an important role in the tumor progression [14, 24, 25]. Improved clinical outcome has been associated with the presence of intratumoral T cells in ovarian, renal, colorectal, and hepatocellular carcinoma [8-13]. By contrast, other reports have demonstrated an inverse clinical correlation between regulatory T cell density and the local immune control of tumors from patients with ovarian, breast, and hepatocellular carcinomas [27-29]. Herein, we observed

Table 3. Associations between CD4+ T cell density and the clinicopathological characteristics

	CD4+ T cell density			
Variable	Low	High	R	P
No. of patients	67 (51%)	64 (49%)		
Age, years			-0.01	0.907
≤ 62	37 (55%)	36 (56%)		
> 62	30 (45%)	28 (44%)		
Sex			0.009	0.917
Male	58 (87%)	55 (86%)		
Female	9 (13%)	9 (14%)		
Tumor size			-0.009	0.914
≤ 3 cm	55 (82%)	53 (83%)		
> 3 cm	12 (18%)	11 (17%)		
Tumor number			0.016	0.858
Unifocal	46 (69%)	43 (67%)		
Multifocal	21 (31%)	21 (33%)		
Tumor stage			-0.031	0.728
Ta	15 (22%)	16 (25%)		
T1	52 (78%)	48 (75%)		
Histological grade			-0.003	0.977
Low	48 (72%)	46 (72%)		
High	19 (28%)	18 (28%)		

Table 4. Multivariate analysis of the factors associated with survival

Variable	HR	95% CI	P
Age, years (> 62/≤ 62)	5.169	2.43-10.992	< 0.001
CD4+ T cells (High/Low)	2.75	1.375-5.497	0.004

Note: Multivariate analysis. Cox proportional hazards regression model. Variables associated with survival by univariate analyses were adopted as covariates in multivariate analyses. HR > 1, risk for death increased; HR < 1, risk for death reduced.

that the density of CD4+ T cells was higher in NMIBC tissues, a high number of CD4+ T cells was associated with poor OS of NMIBC patients. Although CD4+ T cell density had no association with RFS, patients with higher CD4+ T cell density had a 10-year median RFS rate 1. 7 times shorter than patients with lower CD4+ T cell density. These results suggested that the recurrence rate sustained increasing duing the long time follow-up period and CD4+ T cells may play important role in promoting bladder cancer recurrence and progression. Notably, the anti-CD4 antibody shows robust immunoreactivity in bladder formalin-fixed, paraffin-embedded tissues that can be treated with diverse antigen retrieval methods with good inter-observer reproducibility (data not shown). Therefore, immunohistochemical detection of CD4+ T cells could represent a novel prognostic indicator for NMIBC.

CD4+ T cells play crucial roles for host defense and immune-mediated disease by their ability to differentiate into specialized subpopulations, such as Th1 cells, Th2 cells, Th17 cells and regulatory T cells [27]. Among them, Th1 cells are strongly associated with good clinical outcome for almost all cancer types [8]. Regulatory T cells, characterized by the CD4+ CD25+ FOXP3+ phenotype, are believed to dampen T-cell immunity and to be the main obstacle tempering immunotherapy [7]. In the present study, we found that all CD4+ T cells could be an independent adverse predictor for patients with NMIBC. A possible explanation for the detrimental effect of CD4+ T cells on patient prognoses is that protumoral T cell subpopulations (regulatory T cells) might be more prevalent than those with antitumoral phenotypes (e.g. Th1 cells) in NMIBC tissues. Testing this hypothesis may be the subject of further investigations.

There are some limitations in this study. Firstly, the samples selected from a single institution, and the number of samples enrolled may not enough for subgroup analysis. Subsequently, this is a retrospective study. Last, the information on chemotherapy or radiotherapy is inadequate to draw a conclusion about the potential role of CD4+ T cells expression to therapeutic sensitivity.

In conclusion, our results demonstrate that CD4+ T cells are associated with a poor prognosis in NMIBC. These results may provide a novel independent predictor for prognosis, and suggest that a more detailed characterization of distinct CD4+ T cell subpopulations might provide an opportunity to eliminate protumoral CD4+ T cells or harness antitumoral CD4+ T cells in human cancers.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Kejia Ding, Department of Urology, Shandong Provincial Hospital Affiliated to Shandong University, 324 Jingwu Road,

Jinan 250021, Shandong, China. E-mail: kejiading-001@sina.com

References

[1] Babjuk M, Burger M, Zigeuner R, Shariat SF, van Rhijn BW, Compérat E, Sylvester RJ, Kaasinen E, Böhle A, Palou Redorta J, Rouprêt M; European Association of Urology. Eau guidelines on non-muscle-invasive urothelial carcinoma of the bladder: Update 2013. Eur Urol 2013; 64: 639-653.

[2] Lamm DL, Blumenstein BA, Crissman JD, Montie JE, Gottesman JE, Lowe BA, Sarosdy MF, Bohl RD, Grossman HB, Beck TM, Leimert JT, Crawford ED. Maintenance bacillus calmette-guerin immunotherapy for recurrent ta, t1 and carcinoma in situ transitional cell carcinoma of the bladder: A randomized southwest oncology group study. J Urol 2000; 163: 1124-1129.

[3] Shelley MD, Kynaston H, Court J, Wilt TJ, Coles B, Burgon K, Mason MD. A systematic review of intravesical bacillus calmette-guerin plus transurethral resection vs transurethral resection alone in ta and t1 bladder cancer. BJU Int 2001; 88: 209-216.

[4] Gontero P, Bohle A, Malmstrom PU, O'Donnell MA, Oderda M, Sylvester R, Witjes F. The role of bacillus calmette-guerin in the treatment of non-muscle-invasive bladder cancer. Eur Urol 2010; 57: 410-429.

[5] Redelman-Sidi G, Glickman MS, Bochner BH. The mechanism of action of bcg therapy for bladder cancer--a current perspective. Nat Rev Urol 2014; 11: 153-162.

[6] Finn OJ. Cancer immunology. N Engl J Med 2008; 358: 2704-2715.

[7] Curiel TJ. Tregs and rethinking cancer immunotherapy. J Clin Invest 2007; 117: 1167-1174.

[8] Fridman WH, Pages F, Sautes-Fridman C, Galon J. The immune contexture in human tumours: Impact on clinical outcome. Nat Rev Cancer 2012; 12: 298-306.

[9] Sharma P, Shen Y, Wen S, Yamada S, Jungbluth AA, Gnjatic S, Bajorin DF, Reuter VE, Herr H, Old LJ, Sato E. Cd8 tumor-infiltrating lymphocytes are predictive of survival in muscle-invasive urothelial carcinoma. Proc Natl Acad Sci U S A 2007; 104: 3967-3972.

[10] Sato E, Olson SH, Ahn J, Bundy B, Nishikawa H, Qian F, Jungbluth AA, Frosina D, Gnjatic S, Ambrosone C, Kepner J, Odunsi T, Ritter G, Lele S, Chen YT, Ohtani H, Old LJ, Odunsi K. Intraepithelial cd8+ tumor-infiltrating lymphocytes and a high cd8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. Proc Natl Acad Sci U S A 2005; 102: 18538-18543.

[11] Nakano O, Sato M, Naito Y, Suzuki K, Orikasa S, Aizawa M, Suzuki Y, Shintaku I, Nagura H, Ohtani H. Proliferative activity of intratumoral cd8(+) t-lymphocytes as a prognostic factor in human renal cell carcinoma: Clinicopathologic demonstration of antitumor immunity. Cancer Res 2001; 61: 5132-5136.

[12] Kawai O, Ishii G, Kubota K, Murata Y, Naito Y, Mizuno T, Aokage K, Saijo N, Nishiwaki Y, Gemma A, Kudoh S, Ochiai A. Predominant infiltration of macrophages and cd8(+) t cells in cancer nests is a significant predictor of survival in stage iv nonsmall cell lung cancer. Cancer 2008; 113: 1387-1395.

[13] Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, Tosolini M, Camus M, Berger A, Wind P, Zinzindohoué F, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH, Pagès F. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 2006; 313: 1960-1964.

[14] Disis ML, Bernhard H, Jaffee EM. Use of tumour-responsive t cells as cancer treatment. Lancet 2009; 373: 673-683.

[15] Corthay A, Skovseth DK, Lundin KU, Røsjø E, Omholt H, Hofgaard PO, Haraldsen G, Bogen B. Primary antitumor immune response mediated by cd4+ t cells. Immunity 2005; 22: 371-383.

[16] Perez-Diez A, Joncker NT, Choi K, Chan WF, Anderson CC, Lantz O, Matzinger P. Cd4 cells can be more efficient at tumor rejection than cd8 cells. Blood 2007; 109: 5346-5354.

[17] Bromwich EJ, McArdle PA, Canna K, McMillan DC, McNicol AM, Brown M, Aitchison M. The relationship between t-lymphocyte infiltration, stage, tumour grade and survival in patients undergoing curative surgery for renal cell cancer. Br J Cancer 2003; 89: 1906-1908.

[18] Peguillet I, Milder M, Louis D, Vincent-Salomon A, Dorval T, Piperno-Neumann S, Scholl SM, Lantz O. High numbers of differentiated effector cd4 t cells are found in cancer patients and correlate with clinical response after neo-adjuvant therapy of breast cancer. Cancer Res 2014; 74: 2204-2216.

[19] Zhang Q, Jia Q, Deng T, Song B, Li L. Heterogeneous expansion of CD4+ tumor-infiltrating t-lymphocytes in clear cell renal cell carcinomas. Biochem Biophys Res Commun 2015; 458: 70-76.

[20] Wang B, Shaoxu W, Zeng H, Liu Z, Dong W, He W, Chen X, Dong X, Zheng L, Lin T, Huang J. CD103 tumor-infiltrating lymphocytes predict a favorable prognosis in urothelial cell carcinoma of the bladder. J Urol 2015; 194: 556-562.

[21] Sylvester RJ. How well can you actually predict which non-muscle-invasive bladder cancer patients will progress? Eur Urol 2011; 60: 431-433, discussion 433-434.

[22] Kaufman DS, Shipley WU, Feldman AS. Bladder cancer. Lancet 2009; 374: 239-249.
[23] Zuiverloon TC, Nieuweboer AJ, Vekony H, Kirkels WJ, Bangma CH, Zwarthoff EC. Markers predicting response to bacillus calmette-guerin immunotherapy in high-risk bladder cancer patients: A systematic review. Eur Urol 2012; 61: 128-145.
[24] Zou W. Regulatory t cells, tumour immunity and immunotherapy. Nat Rev Immunol 2006; 6: 295-307.
[25] Wilke CM, Kryczek I, Wei S, Zhao E, Wu K, Wang G, Zou W. Th17 cells in cancer: Help or hindrance? Carcinogenesis 2011; 32: 643-649.
[26] Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, Evdemon-Hogan M, Conejo-Garcia JR, Zhang L, Burow M, Zhu Y, Wei S, Kryczek I, Daniel B,Gordon A, Myers L, Lackner A, Disis ML, Knutson KL, Chen L, Zou W. Specific recruitment of regulatory t cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med 2004; 10: 942-949.
[27] Bates GJ, Fox SB, Han C, Leek RD, Garcia JF, Harris AL, Banham AH. Quantification of regulatory t cells enables the identification of high-risk breast cancer patients and those at risk of late relapse. J Clin Oncol 2006; 24: 5373-5380.
[28] Gao Q, Qiu SJ, Fan J, Zhou J, Wang XY, Xiao YS, Xu Y, Li YW, Tang ZY. Intratumoral balance of regulatory and cytotoxic t cells is associated with prognosis of hepatocellular carcinoma after resection. J Clin Oncol 2007; 25: 2586-2593.
[29] Nakayamada S, Takahashi H, Kanno Y, O'Shea JJ. Helper t cell diversity and plasticity. Curr Opin Immunol 2012; 24: 297-302.