ORIGINAL ARTICLE

Prostate Cancer

High IL-23+ cells infiltration correlates with worse clinical outcomes and abiraterone effectiveness in patients with prostate cancer

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Individualized treatment of prostate cancer depends on an accurate stratification of patients who are sensitive to various treatments. Interleukin-23 (IL-23) was reported to play a significant role in prostate cancer. Here, we aimed to explore the clinical value of IL-23-secreting (IL-23+) cells in prostate cancer patients. We evaluated interleukin-23A (IL-23A) expression in The Cancer Genome Atlas database and retrospectively enrolled 179 treatment-naïve metastatic prostate cancer patients diagnosed in our institute between June 2012 and December 2014. IL-23+ cells were stained and evaluated via immunohistochemistry. Further, survival and multivariate Cox regression analyses were conducted to explore the prognostic value of IL-23+ cells. We found that IL-23A expression correlated with disease progression, while IL-23+ cells were clearly stained within prostate cancer tissue. Patients with higher Gleason scores and multiple metastatic lesions tended to have more IL-23+ cell infiltration. Further analyses showed that patients with higher levels of IL-23+ cells had significantly worse overall survival (hazard ratio [HR] = 2.996, 95% confidence interval [95% CI]: 1.812–4.955; P = 0.001) and a higher risk of developing castration resistance (HR = 2.725, 95% CI: 1.865–3.981; P = 0.001). Moreover, subgroup analyses showed that when patients progressed to a castration-resistant status, the prognostic value of IL-23+ cells was observed only in patients treated with abiraterone instead of docetaxel. Therefore, we showed that high IL-23+ cell infiltration is an independent prognosticator in patients with metastatic prostate cancer. IL-23+ cell infiltration may correlate with abiraterone effectiveness in castration-resistant prostate cancer patients.
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INTRODUCTION

Prostate cancer remains a leading cause of cancer incidence in males and mortality worldwide.[1] In China, both the incidence rate and mortality rate of prostate cancer are significantly increasing.[2,3] Unlike localized prostate cancer, metastatic prostate cancer is considered not amenable to surgical resection, making androgen deprivation therapy (ADT) the cornerstone of treatment for metastatic disease.[4,5] Until recently, a series of important studies, such as CHAARTED (chemohormonal therapy in metastatic hormone-sensitive prostate cancer), STAMPEDE (addition of docetaxel, zoledronic acid, or both to first-line long-term hormone therapy in prostate cancer), and LATITUDE (abiraterone acetate plus prednisone in patients with newly diagnosed high-risk metastatic castration-sensitive prostate cancer), highlighted the treatment benefits of abiraterone and docetaxel in the field of metastatic prostate cancer.[6–8] However, the response rate to these treatments is heterogeneous, and screening treatment-sensitive patients is urgent for individualized therapy.[9,10]

Although prostate cancer has long been considered a "cold" tumor that lacks immune cells and tends to have an immunosuppressive tumor microenvironment, some immune-related interleukins play a significant role in tumor progression and castration resistance.[11,12]

Calcinotto *et al.*[13] showed that interleukin-23 (IL-23) was secreted by myeloid-derived suppressor cells (MDSCs) in prostate cancer, and IL-23 could then activate the androgen receptor (AR) pathway in tumor cells, leading to castration resistance. We previously reported the effect of IL-23 in another type of urological malignancy, renal cell carcinoma (RCC), and found that high IL-23 expression correlated with a poor prognosis in patients with RCC.[14] We have demonstrated that IL-23 secreted by tumor-associated macrophages could promote regulatory T cells (Treg) functions and cause immune suppression in kidney cancer.[14] These studies illustrated the pro-tumor mechanism of IL-23 in urologic cancer; thus, we sought to explore the clinical significance of IL-23-secreting (IL-23+) cells in prostate cancer patients.

In this study, we evaluated IL-23A expression using The Cancer Genome Atlas (TCGA) database and then enrolled 179 treatment-naïve metastatic prostate cancer patients to establish a tissue microarray. IL-23+ cells were stained and evaluated via immunohistochemistry. Survival analyses suggested that patients with higher IL-23+ cells tended to have shorter overall survival (OS) and castration-resistant prostate cancer (CRPC)-free survival. Further multivariate analyses confirmed that IL-23+ cells could act as an independent prognosticator. In addition, after patients progressed to castration-resistant status,

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IL-23+ cell infiltration could significantly stratify patients who had worse sensitivity to abiraterone, suggesting a more complicated role of IL-23+ cells in prostate cancer.

PATIENTS AND METHODS

Study population

This study was a retrospective study of patients with metastatic prostate cancer diagnosed between June 2012 and December 2014 at Fudan University Shanghai Cancer Center, Shanghai, China. The inclusion criteria were as follows: (1) histologically diagnosed with prostate adenocarcinoma without evidence of neuroendocrine differentiation; (2) previously untreated at the time of diagnosis; (3) had enough tumor tissues; and (4) had complete clinicopathological and follow-up information for further analyses. After screening, 179 eligible patients were included in this study.

Metastatic diseases were evaluated and confirmed according to bone scintigraphy scan, imaging examinations (computed tomography [CT] or magnetic resonance imaging [MRI]), or both. Oligometastatic disease was defined as ≤5 bone or extrapelvic lymph node metastases and no visceral metastases. Patients were diagnosed based on either prostate needle biopsy or transurethral resection of the prostate (TURP), and other clinicopathological factors, such as serum prostate-specific antigen (PSA), hemoglobin, albumin, alkaline phosphatase (ALP), and lactate dehydrogenase (LDH), were obtained at the time of diagnosis.

After pathologic diagnoses, all patients were treated with first-generation antiandrogen agents, such as bicalutamide and flutamide as ADT. Abiraterone, docetaxel, and any other second-generation antiandrogen therapy were not considered until patients progressed to castration-resistant disease. During the follow-up, PSA and testosterone were tested monthly for the first 6 months, every 3 months for the next 2 years, and every 6 months thereafter. Imaging examinations, including CT (chest, abdomen, and pelvis) and bone scans, were performed every 6 months or when patients had symptomatic progression or PSA increase. CRPC status was confirmed when patients had either biochemical progression or radiological progression according to the description in guidelines.[5] Patients were treated with ADT, such as gonadotropin-releasing hormone agonists (including goserelin, leuprolide, and triptorelin) combined with first-generation antiandrogen agents, either bicalutamide or flutamide. When progression to CRPC, eligible patients were treated with either abiraterone or docetaxel, along with castration. Some patients were treated with VP-16, estramustine phosphate, and drugs tested in other clinical trials, and we categorized these patients into the other treatment group. In addition, a few patients did not receive any treatment due to personal reasons. All follow-up information was updated in August 2020, and the median of the follow-up period was 40 (interquartile range: 23–62) months.

The endpoints of interest were OS and CRPC-free survival. OS was defined as the time from the date of disease pathological diagnosis to the date of death or the last follow-up. CRPC-free survival was calculated from the date of pathologic diagnoses to the date of CRPC development or the last follow-up. This study was approved by the Ethics Committee and Institutional Review Board of Fudan University Shanghai Cancer Center (No. 050432-4-1911D), and written consent was obtained from each patient. Reporting Recommendations for Tumour Marker Prognostic Studies (REMARK) criteria were applied in this study.[15]

TCGA data collection

TCGA prostate adenocarcinoma (TCGA-PRAD) cohort provides comprehensive mRNA expression, clinicopathological information, and follow-up data. We downloaded TCGA-PRAD data via R software (R Foundation for Statistical Computing, Vienna, Austria) in December 2020. IL-23A expression was evaluated as fragments per kilobase of exon per million fragments mapped (FPKM), and the cutoff value was determined by X-tile 3.6.1 (Yale University, New Haven, CT, USA). Briefly, X-tile software could visualize different survival curves by each potential cutoff point and determine the optimal cutoff point by assessing the strength of the relationship between a biomarker and clinical outcomes.[16]

Immunohistochemistry and evaluation

A tissue microarray (TMA) was constructed, and immunohistochemistry was performed as we previously reported.[17] Generally, slides were heated at 60°C overnight and then deparaffinized with an ethanol series. Antigen retrieval was performed by incubating slides in boiled citrate buffer (pH 6.0) for 30 min. An anti-IL-23 antibody (ab190356, clone number EPR5585[N], a target for IL-23A, diluted 1:300; Abcam, Cambridge, UK) was applied as the primary antibody, and a DAB detection system (abs9210, Absin, Beijing, China) was used to explore positive staining. Stained slides were analyzed under an Olympus BX51 microscope (Olympus, Tokyo, Japan). One experienced urologic pathologist (YYK; Fudan University Shanghai Cancer Center) who was blinded to the clinical and follow-up data counted IL-23+ cells. Specifically, the number of IL-23+ cells was counted in four representative high-power fields (HPFs) for each sample, and the mean value of these four was adopted as the IL-23+ cell count. The cutoff value was determined by X-tile 3.6.1.

Statistical analyses

Analysis of variance (ANOVA) was performed to compare IL-23A expression among different groups. Student's t-test was conducted to compare the levels of IL-23+ cells between two groups. Associations between two variables were analyzed using the Chi-square or Fisher's exact test for categorical variables and the Mann–Whitney U test for continuous variables. Survival curves were compared by log-rank test. Cox proportional hazard regression models were established to perform univariate and multivariate analyses. All tests were two-sided, and statistical significance was defined as $P < 0.05$. Statistical analyses were performed using IBM SPSS Statistics version 21.0 (IBM Corp., Armonk, NY, USA).

RESULTS

IL-23+ cells correlated with disease progression, especially in metastatic disease

To explore the prognostic value of IL-23+ cells, we first evaluated IL-23A expression in the TCGA-PRAD cohort ($n = 495$). As shown in **Figure 1a** and **1b**, IL-23A expression positively correlated with pT stage and the Gleason score ($P = 0.032$ and $P = 0.002$, respectively). **Supplementary Table 1** lists the main clinical and pathological information of TCGA-PRAD patients.

Next, we divided TCGA-PRAD patients into high ($n = 337$) and low ($n = 158$) IL-23A expression groups. The cutoff value was determined as 0.72 FPKM using X-tile software. As listed in **Supplementary Table 1**, IL-23A expression positively correlated with pT stage, pN stage, and the Gleason score (all $P < 0.001$). Further survival analyses also showed that patients with higher IL-23A expression tended to have worse OS and CRPC-free survival (log-rank $P = 0.046$ and log-rank $P = 0.019$, respectively; **Figure 1c** and **1d**). However, we noticed that the survival curves in both **Figure 1c** and **1d** were nearly crossed, diminishing the reliability of the survival curves. We also found that clinical events, such as death and CRPC status, were not correlated with IL-23A

Table 1: Patient baseline characteristics and associations with interleukin-23+ cell infiltration

Factor	All patients	IL-23+ cell infiltration		aP
		Low (<9.5 cells per HPF; n=106)	High (≥9.5 cells per HPF; n=73)	
Age at surgery (year), median (IQR)	68 (63–74)	68 (62–73)	69 (63–75)	0.743b
PSA at diagnosis (ng ml⁻¹), median (IQR)	153 (100–474)	153 (100–547)	125 (95–425)	0.542b
PSA at diagnosis (ng ml⁻¹), n (%)				0.956
≤100	74 (41.3)	44 (41.5)	30 (41.1)	
>100	105 (58.7)	62 (58.5)	43 (58.9)	
Hemoglobin (g l⁻¹), median (IQR)	134 (122–144)	135 (122–145)	133 (124–142)	0.588b
Albumin (g l⁻¹), median (IQR)	43 (40–46)	43 (40–46)	43 (40–45)	0.366b
Alkaline phosphatase (IU l⁻¹), median (IQR)	120 (80–211)	124 (80–219)	112 (77–178)	0.403b
Lactate dehydrogenase (IU l⁻¹), median (IQR)	171 (151–192)	171 (145–188)	172 (158–206)	0.126b
Clinical M stage, n (%)				0.496
M1a	11 (6.1)	8 (7.6)	3 (4.2)	
M1b	161 (90.0)	93 (87.7)	68 (93.2)	
M1c	7 (3.9)	5 (4.7)	2 (2.7)	
Gleason score, n (%)				0.370
10	23 (12.8)	10 (9.4)	13 (17.8)	
9	97 (54.2)	58 (54.7)	39 (53.4)	
8	48 (26.8)	33 (31.1)	15 (20.5)	
≤7	11 (6.2)	5 (4.7)	6 (8.2)	
Nerve invasion, n (%)				0.090
Present	82 (45.8)	43 (40.6)	39 (53.4)	
Absent	97 (54.2)	63 (59.4)	34 (46.6)	
Oligometastasis, n (%)				0.667
Yes	77 (43.0)	47 (44.3)	30 (41.1)	
No	102 (57.0)	59 (55.7)	43 (58.9)	
Visceral metastasis, n (%)				0.702
Yes	7 (3.9)	5 (4.7)	2 (2.7)	
No	172 (96.1)	101 (95.3)	71 (97.3)	
Source of tissue, n (%)				0.557
TURP	86 (48.0)	49 (46.2)	37 (50.7)	
Biopsy	93 (52.0)	57 (53.8)	36 (49.3)	
Treatment after progressionc, n (%)				0.796
Abiraterone	35 (30.7)	16 (29.1)	19 (32.2)	
Docetaxel	28 (24.6)	15 (27.3)	13 (22.0)	
Other	22 (19.3)	9 (16.4)	13 (22.0)	
None	29 (25.4)	15 (27.3)	14 (23.7)	
Type of ADT, n (%)				0.863
Castration + bicalutamide	156 (87.2)	92 (86.8)	64 (87.7)	
Castration + flutamide	23 (12.8)	14 (13.2)	9 (12.3)	
Events, n (%)				
Death	66 (36.9)	27 (25.5)	39 (53.4)	<0.001
CRPC	114 (63.7)	55 (51.9)	59 (80.8)	<0.001

aFisher's exact test was used when data did not meet the requirement of Chi-square test. bMann–Whitney U test. cAmong 179 patients, 114 patients progressed to CRPC and had records of treatment after progression, while other 65 patients were not applicable. IL-23: interleukin-23; HPF: high-power field; IQR: interquartile range; PSA: prostate-specific antigen; TURP: transurethral resection of the prostate; ADT: androgen deprivation therapy; CRPC: castration-resistant prostate cancer

expression according to **Supplementary Table 1** (*P* = 0.054 and *P* = 0.161, respectively). We blamed these phenomena for insufficient events (death: 2.0%, CRPC: 21.8%) in the TCGA-PRAD cohort. In addition, most cases in TCGA-PRAD were early disease with pT2–3 (96.6%). These results from the TCGA-PRAD cohort suggested that the prognostic value of IL-23+ cells might be more distinct in advanced prostate cancer.

We then examined 205 metastatic prostate cancer patients diagnosed and treated in our institute between 2012 and 2014. During IHC staining and evaluation, 10 cases with insufficient tissue for evaluation were excluded. We also excluded another 16 patients because of tissue detachment during staining. Therefore, 179 patients were included in the subsequent analyses (**Figure 2a**). The baseline clinicopathological characteristics of the study population are listed in **Table 1**.

As illustrated in **Figure 2b**, IL-23+ cells were clearly stained within prostate cancer tissue. After the evaluation, we found that patients with higher Gleason scores (9–10) tended to have more IL-23+ cell infiltration than those with Gleason scores ≤ 8 (*P* = 0.044; **Figure 2c**). Moreover, patients with multiple metastatic lesions also had higher IL-23+ cell counts than patients with oligometastases (*P* = 0.004; **Figure 2d**). From these results, IL-23+ cell infiltration might correlate with disease progression in metastatic disease.
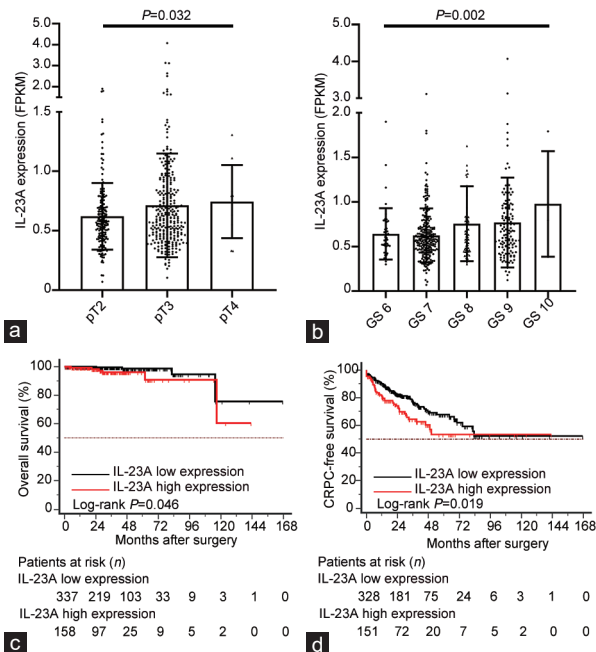
Figure 1: IL-23A expression correlated with disease progression. (**a**) Comparison of IL-23A expression according to pT stage in the TCGA-PRAD cohort. Data are shown as the mean±s.d. and were compared with ANOVA. (**b**) Association of IL-23+ cells with GS subgroups. Data are shown as the mean±s.d. and were compared with ANOVA. Kaplan–Meier plots for (**c**) overall survival and (**d**) CRPC-free survival in the TCGA-PRAD cohort according to IL-23A expression. Sixteen cases without CRPC-free survival time were excluded in **d**. IL-23: interleukin-23; GS: Gleason score; TCGA-PRAD: The Cancer Genome Atlas prostate adenocarcinoma; ANOVA: analysis of variance; CRPC: castration-resistant prostate cancer; s.d.: standard deviation; FPKM: fragments per kilobase of exon per million fragments mapped.

To further evaluate the clinical significance of IL-23+ cells, we divided 179 patients into an IL-23+ cell high infiltration group ($n = 73$, 40.8%) and a low infiltration group ($n = 106$, 59.2%). The cutoff value was determined to be 9.5 cells per HPF using X-tile software. As shown in **Table 1**, patients with higher IL-23+ cell infiltration had worse clinical outcomes, such as death and castration resistance (both $P < 0.001$). Other clinicopathological factors and treatment options did not statistically correlate with IL-23+ cell infiltration (all $P > 0.05$; **Table 1**).

Higher IL-23+ cell levels correlated with a worse prognosis in metastatic disease

We then conducted survival analyses to explore the prognostic value of IL-23+ cells in metastatic prostate cancer. As shown in **Figure 3a** and **3b**, patients in the high IL-23+ cell infiltration group had shorter OS and CRPC-free survival than patients in the low IL-23+ cell group (both log-rank $P = 0.001$). In addition, subgroup survival analyses also confirmed this prognostic value of IL-23+ cell infiltration in both oligometastatic and multimetastatic patients (**Supplementary Figure 1**).

Further univariate analyses revealed that high IL-23+ cell infiltration was associated with a higher risk of death and CRPC status (hazard ratio [HR] = 3.128, 95% confidence interval [CI]: 1.904–5.139, $P = 0.001$; HR = 2.832, 95% CI: 1.946–4.121, $P = 0.001$, respectively). Other potential prognosticators, such as the Gleason score and nerve invasion, also presented higher risk in both OS and CRPC-free survival models (**Figure 3c** and **3d**, respectively). It should be mentioned that patients with oligometastases had lower risk of CRPC than patients with multimetastases (HR = 0.656, 95% CI: 0.447–0.963, $P = 0.031$; **Figure 3d**), which was consistent with previous studies.[18,19] However, this reduction in risk did not gain statistical significance in the OS model (HR = 0.755, 95% CI: 0.456–1.250, $P = 0.274$; **Figure 3c**).
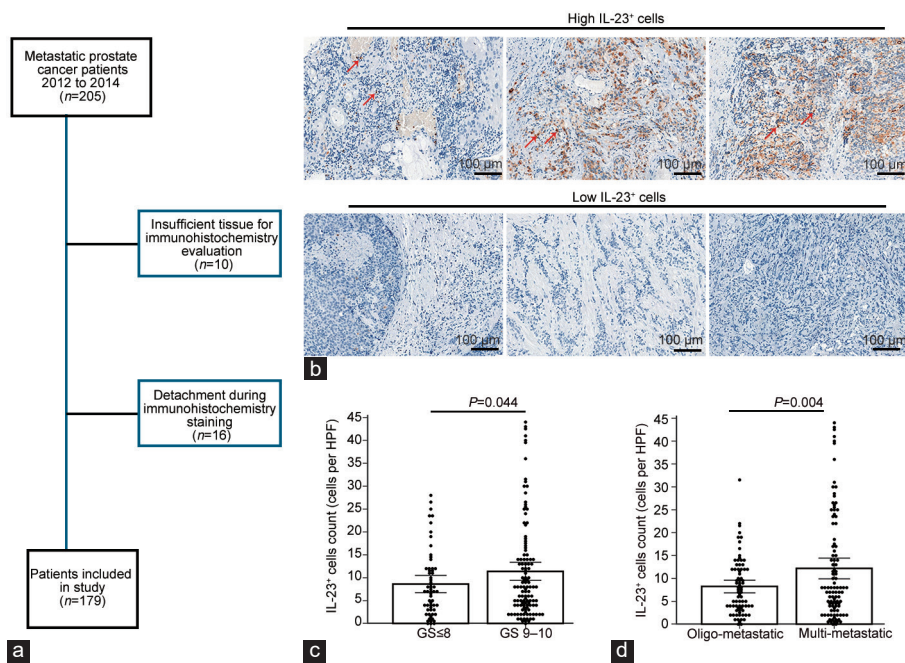

Figure 2: The presence of IL-23+ cells in metastatic prostate cancer tissues. (**a**) The flowchart of study population enrolment. (**b**) Representative pictures of IL-23+ cells in metastatic prostate cancer tissues under a high-power field (high and low infiltration of IL-23+ cells). Red arrows indicate stained IL-23+ cells. Scale bars=100 μm. (**c**) Association of IL-23+ cells with GS subgroups. (**d**) Comparison of IL-23+ cells in oligo-metastatic with multi-metastatic patients. Barplots display mean±s.d., data are compared using Student's *t*-test. IL-23: interleukin-23; GS: Gleason score; s.d.: standard deviation; HPF: high-power field.

Table 2: Multivariate cox regression analyses of clinicopathological features and interleukin-23+ cells for overall survival and castration-resistant prostate cancer-free survival

Factor	HR (95% CI)	[a]P
Overall survival		
PSA at diagnosis (ng ml−1, >100 *vs* ≤100)	1.443 (0.833–2.499)	0.191
Gleason score	1.221 (0.876–1.700)	0.238
Nerve invasion (present *vs* absent)	1.532 (0.925–2.538)	0.097
Oligo-metastasis (yes *vs* no)	0.899 (0.523–1.546)	0.701
IL-23+ cells (high *vs* low)	2.996 (1.812–4.955)	0.001
CRPC-free survival		
PSA at diagnosis (ng ml−1, >100 *vs* ≤100)	1.234 (0.829–1.836)	0.300
Gleason score	1.403 (1.063–1.851)	0.017
Nerve invasion (present *vs* absent)	1.529 (1.050–2.228)	0.027
Oligometastasis (yes *vs* no)	0.742 (0.499–1.104)	0.141
IL-23+ cells (high *vs* low)	2.725 (1.865–3.981)	0.001

[a]All HR and 95% CI were calculated from 1000 bootstrap samples protected from overfitting. IL-23: interleukin-23; CRPC: castration-resistant prostate cancer; HR: hazard ratio; CI: confidence interval; PSA: prostate-specific antigen

A multivariate Cox model was applied to further evaluate the prognostic value of IL-23+ cell infiltration. As presented in **Table 2**, IL-23+ cell infiltration was confirmed to be an independent prognosticator for both OS and CRPC-free survival (HR = 2.996, 95% CI: 1.812–4.955, *P* = 0.001; HR = 2.725, 95% CI: 1.865–3.981, *P* = 0.001, respectively). Other potential prognosticators, including the Gleason score and nerve invasion, also showed prognostic value in CRPC-free survival (**Table 2**). Taken together, high IL-23+ cells could independently predict the prognoses of patients with metastatic prostate cancer.

IL-23+ cells correlated with abiraterone effectiveness during CRPC
As listed in **Table 1**, during the follow-up period, 63.7% of patients progressed to CRPC. After progression, 30.7% of them received abiraterone, and 24.6% received docetaxel. Surprisingly, we found that in patients treated with abiraterone, IL-23+ cells could still predict worse clinical outcome (log-rank *P* = 0.018; **Figure 4a**). However, this prognostic value was not found in patients treated with docetaxel (log-rank *P* = 0.134; **Figure 4b**).

DISCUSSION
Emerging immunotherapies, such as immune checkpoint inhibitors (ICIs), exert striking therapeutic effects on many solid tumors, including renal cell carcinoma and muscle-invasive bladder cancer.[20,21] In the field of prostate cancer, ICIs have not shown such significant progress.[22] Some have argued that prostate cancer is a "cold" tumor and thus prostate cancer might be less sensitive to ICIs.[23] A recent review concluded that the tumor immune environment, which consists of tumor-infiltrating immune cells and cytokines, may affect the efficacy of various antitumor treatments.[24] Therefore, accurate risk stratification of patients should consider these immune cells and cytokines.

IL-23 is generally produced by myeloid cells and contributes to various immune-mediated inflammatory diseases, such as inflammatory bowel disease, psoriasis, and psoriatic arthritis.[25] Anti-IL-23 therapy, such as guselkumab, was recently approved by the Food and Drug Administration (FDA) to treat psoriatic arthritis,[25,26] suggesting a potent immune regulation ability of IL-23. In cancer immunology, we previously reported that IL-23 produced by tumor-associated macrophages could promote the immunosuppressive function of Tregs and thus induce immune evasion in kidney cancer.[14] IL-23 was also reported to enhance the androgen receptor pathway in prostate cancer tissue and result in castration resistance.[13]

In this study, we first evaluated IL-23A expression in the TCGA-PRAD cohort and found that IL-23A might correlate with disease progression. We then collected metastatic prostate cancer patients and found that IL-23 was clearly stained as a cell within prostate cancer tissues (**Figure 2b**). As reported in previous studies,[25] the source of IL-23 varies, and we set the definition of IL-23+ cells for further study. Consistent with previous studies, we then showed that higher IL-23+ cell infiltration correlated with shorter OS and CRPC-free survival (**Figure 3**). Oligometastatic prostate cancer is considered an intermediate state between localized and multi-metastatic disease.[18,19] We did find that oligometastatic patients had lowers risk of death and the development of castration resistance (**Figure 3c** and **3d**); however, we also found that the prognostic value of IL-23+ cells remained the same in both oligometastatic and multimetastatic patients (**Supplementary Figure 1**), suggesting a relatively minor role of IL-23+ cells in tumor cell metastasis.

According to the latest guidelines, abiraterone and docetaxel are considered the first-line treatments in metastatic patients who progress to metastatic castration-resistant prostate cancer (mCRPC).[5] In our study population, patients who progressed to CRPC disease were also treated with abiraterone (30.7%) or docetaxel (24.6%), as shown in **Table 1**. To our surprise, the prognostic value of IL-23+ cells was only present in patients treated with abiraterone other than docetaxel (**Figure 4**). A previous study showed that IL-23 could promote the AR pathway in tumor cells,[13] while abiraterone could inhibit the synthesis of androgen, especially reducing androgen derived from tumor cells.[27] We hypothesized that patients with higher IL-23+ cell infiltration might have higher AR pathway activities, thus showing worse sensitivity to abiraterone and leading to shorter OS (**Figure 4a**). Detailed interactions between IL-23 and abiraterone require further experimental research.

As a significant immune regulatory cytokine, IL-23 has been reported to exert both pro- and antitumor functions. In a recent review by Mirlekar and Pylayeva-Gupta,[28] IL-23 could induce antitumor immunity by recruiting other proinflammatory cytokines and enhancing cytotoxic immune cells; however, IL-23 could also promote tumor progression by regulating the Th17 response and Treg functions.[14] The context-dependent nature of IL-23 function determined that its role in different types of tumors required a more detailed mechanistic study. In prostate cancer, a recent study showed that IL-23 could activate the AR pathway in tumor cells, leading to castration resistance and displaying its protumor role.[13] Considering its immune regulatory functions, IL-23 might interact with other immune cells and tumor cells to affect the tumor microenvironment in a manner dependent on AR signaling. Another important study by van Dessel *et al*.[29] identified eight gene clusters based on whole-genome sequencing in mCRPC patients, suggesting a complicated gene landscape in prostate cancer. Therefore, the role of IL-23 in prostate cancer might also be distinct in different genotypes. Further study on immune regulatory cytokines, such as IL-23, might reveal tumor type dependence or even tumor genotype dependence, and their interactions with immune cells and different genotypes of tumor cells are our subsequent research direction.

There are several limitations to this study. First, this study had a retrospective design, and external validation is required. Second, we failed to evaluate IL-23+ cell infiltration in metastatic tissue and could not investigate the dynamic change in IL-23+ cells during follow-up. Third, the detailed functions and mechanisms of IL-23+ cells in metastatic prostate cancer remain to be further investigated.

To conclude, this study reveals that IL-23+ cells infiltrate prostate cancer tissues. Higher IL-23+ cell infiltration is an independent adverse prognosticator for OS and CRPC-free survival in patients with
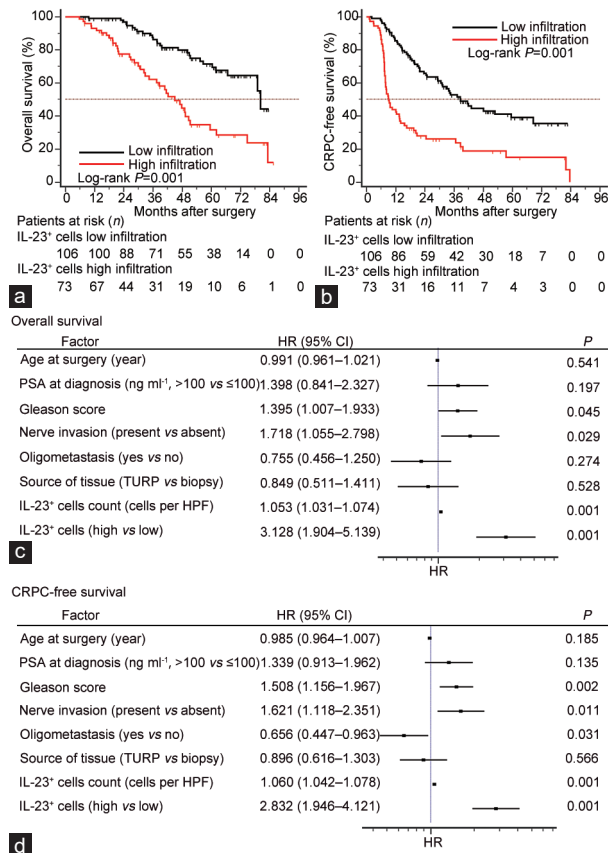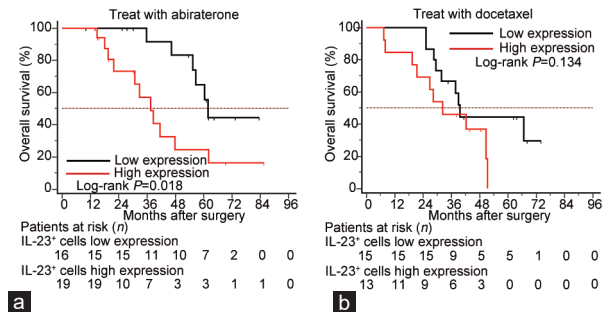
Figure 4: IL-23+ cells could predict abiraterone effectiveness in metastatic prostate cancer. Kaplan–Meier plots show the prognostic value of high and low IL-23+ cells in CRPC patients treated with (**a**) abiraterone and (**b**) docetaxel. IL-23: interleukin-23; CRPC: castration-resistant prostate cancer.

Figure 3: High IL-23+ cells could predict unfavourable prognosis in metastatic prostate cancer patients. Kaplan–Meier plots for (**a**) overall survival and (**b**) CRPC-free survival in patients with metastatic prostate cancer according to high and low IL-23+ cell infiltration. Forrest plots show various potential prognosticators for (**c**) overall survival and (**d**) CRPC-free survival in patients with metastatic prostate cancer. Cox proportional hazards regression models were used. IL-23: interleukin-23; CRPC: castration-resistant prostate cancer; HR: hazard ratio; CI: confidence interval; PSA: prostate-specific antigen; TURP: transurethral resection of the prostate; HPF: high-power field.

study sponsors have no roles in the study design, collection, analysis, and interpretation of data.

Supplementary Information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

metastatic prostate cancer. IL-23+ cell infiltration could also correlate with the effectiveness of abiraterone when patients progress to CRPC.

AUTHOR CONTRIBUTIONS

ZL carried out the bioinformatics and immunohistochemistry analyses, interpreted the data, performed the statistical analyses, and drafted the manuscript. JYZ, YJY, KC, and QFW provided technical and material support. YYK reviewed the pathological slides and supervised the study. BD designed the study, helped to draft the manuscript, obtain funding, and supervised the study. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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REFERENCES

1 Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin* 2020; 70: 7–30.
2 Chen W, Zheng R, Baade PD, Zhang S, Zeng H, *et al*. Cancer statistics in China, 2015. *CA Cancer J Clin* 2016; 66: 115–32.
3 Qi D, Wu C, Liu F, Gu K, Shi Z, *et al*. Trends of prostate cancer incidence and mortality in Shanghai, China from 1973 to 2009. *Prostate* 2015; 75: 1662–8.
4 Attard G, Parker C, Eeles RA, Schroder F, Tomlins SA, *et al*. Prostate cancer. *Lancet* 2016; 387: 70–82.
5 Cornford P, van den Bergh RC, Briers E, Van den Broeck T, Cumberbatch MG, *et al*. EAU-EANM-ESTRO-ESUR-SIOG guidelines on prostate cancer. Part II-2020 update: treatment of relapsing and metastatic prostate cancer. *Eur Urol* 2021; 79: 263–82.
6 Sweeney CJ, Chen YH, Carducci M, Liu G, Jarrard DF, *et al*. Chemohormonal therapy in metastatic hormone-sensitive prostate cancer. *N Engl J Med* 2015; 373: 737–46.
7 James ND, Sydes MR, Clarke NW, Mason MD, Dearnaley DP, *et al*. Addition of docetaxel, zoledronic acid, or both to first-line long-term hormone therapy in prostate cancer (STAMPEDE): survival results from an adaptive, multiarm, multistage, platform randomised controlled trial. *Lancet* 2016; 387: 1163–77.
8 Fizazi K, Tran N, Fein L, Matsubara N, Rodriguez-Antolin A, *et al*. Abiraterone plus prednisone in metastatic, castration-sensitive prostate cancer. *N Engl J Med* 2017; 377: 352–60.
9 Litwin MS, Tan HJ. The diagnosis and treatment of prostate cancer: a review. *JAMA* 2017; 317: 2532–42.
10 Barata PC, Sartor AO. Metastatic castration-sensitive prostate cancer: abiraterone, docetaxel, or... *Cancer* 2019; 125: 1777–88.
11 Bilusic M, Madan RA, Gulley JL. Immunotherapy of prostate cancer: facts and hopes. *Clin Cancer Res* 2017; 23: 6764–70.
12 Kundu M, Roy A, Pahan K. Selective neutralization of IL-12 p40 monomer induces death in prostate cancer cells via IL-12-IFN-gamma. *Proc Natl Acad Sci U S A* 2017; 114: 11482–7.
13 Calcinotto A, Spataro C, Zagato E, Di Mitri D, Gil V, *et al*. IL-23 secreted by myeloid cells drives castration-resistant prostate cancer. *Nature* 2018; 559: 363–9.
14 Fu Q, Xu L, Wang Y, Jiang Q, Liu Z, *et al*. Tumor-associated macrophage-derived interleukin-23 interlinks kidney cancer glutamine addiction with immune evasion. *Eur Urol* 2019; 75: 752–63.
15 Sauerbrei W, Taube SE, McShane LM, Cavenagh MM, Altman DG. Reporting recommendations for tumor marker prognostic studies (REMARK): an abridged explanation and elaboration. *J Natl Cancer Inst* 2018; 110: 803–11.
16 Camp RL, Dolled-Filhart M, Rimm DL. X-tile: a new bio-informatics tool for biomarker assessment and outcome-based cut-point optimization. *Clin Cancer Res* 2004; 10: 7252–9.
17 Zhang J, Zhu Y, Wang Q, Kong Y, Sheng H, *et al*. Poliovirus receptor CD155 is up-regulated in muscle-invasive bladder cancer and predicts poor prognosis. *Urol Oncol* 2020; 38: 41.e11–8.
18 Foster CC, Weichselbaum RR, Pitroda SP. Oligometastatic prostate cancer: reality or figment of imagination? *Cancer* 2019; 125: 340–52.
19 Tosoian JJ, Gorin MA, Ross AE, Pienta KJ, Tran PT, *et al*. Oligometastatic prostate cancer: definitions, clinical outcomes, and treatment considerations. *Nat Rev Urol* 2017; 14: 15–25.
20 Motzer RJ, Escudier B, McDermott DF, George S, Hammers HJ, *et al*. Nivolumab versus everolimus in advanced renal-cell carcinoma. *N Engl J Med* 2015; 373: 1803–13.

21 Sharma P, Siefker-Radtke A, de Braud F, Basso U, Calvo E, *et al.* Nivolumab alone and with ipilimumab in previously treated metastatic urothelial carcinoma: checkmate 032 nivolumab 1 mg/kg plus ipilimumab 3 mg/kg expansion cohort results. *J Clin Oncol* 2019; 37: 1608–16.

22 Redman JM, Gulley JL, Madan RA. Combining immunotherapies for the treatment of prostate cancer. *Urol Oncol* 2017; 35: 694–700.

23 Fay EK, Graff JN. Immunotherapy in prostate cancer. *Cancers (Basel)* 2020; 12: 1752.

24 Fridman WH, Zitvogel L, Sautes-Fridman C, Kroemer G. The immune contexture in cancer prognosis and treatment. *Nat Rev Clin Oncol* 2017; 14: 717–34.

25 Teng MW, Bowman EP, McElwee JJ, Smyth MJ, Casanova JL, *et al.* IL-12 and IL-23 cytokines: from discovery to targeted therapies for immune-mediated inflammatory diseases. *Nat Med* 2015; 21: 719–29.

26 Mohanakrishnan R, Beier S, Deodhar A. IL-23 inhibition for the treatment of psoriatic arthritis. *Expert Opin Biol Ther* 2021. Doi: 10.1080/14712598.2021.1938538. [Online ahead of print].

27 de Bono JS, Logothetis CJ, Molina A, Fizazi K, North S, *et al.* Abiraterone and increased survival in metastatic prostate cancer. *N Engl J Med* 2011; 364: 1995–2005.

28 Mirlekar B, Pylayeva-Gupta Y. IL-12 family cytokines in cancer and immunotherapy. *Cancers (Basel)* 2021; 13: 167.

29 van Dessel LF, van Riet J, Smits M, Zhu Y, Hamberg P, *et al.* The genomic landscape of metastatic castration-resistant prostate cancers reveals multiple distinct genotypes with potential clinical impact. *Nat Commun* 2019; 10: 5251.

**Supplementary Table 1: The Cancer Genome Atlas cohort baseline characteristics and associations with interleukin-23A expression**

Factor	All patients	IL-23A expression		P
		Low (n=337) (<0.72 FPKM)	High (n=158) (≥0.72 FPKM)	
Age at surgery (year)				
Median (IQR)	61 (56–66)	61 (56–66)	63 (57–67)	0.060[a]
PSA at diagnosis (ng/ml)				
Median (IQR)	0.100 (0.030–0.113)	0.100 (0.030–0.100)	0.100 (0.030–0.300)	0.129[a]
Pathologic T stage				<0.001[b]
pT2a, n (%)	13 (2.6)	9 (2.7)	4 (2.5)	
pT2b, n (%)	10 (2.0)	7 (2.1)	3 (1.9)	
pT2c, n (%)	164 (33.1)	126 (37.4)	38 (24.1)	
pT3a, n (%)	157 (31.7)	116 (34.4)	41 (25.9)	
pT3b, n (%)	134 (27.1)	73 (21.7)	61 (38.6)	
pT4, n (%)	10 (2.0)	4 (1.2)	6 (3.8)	
Unknown, n (%)	7 (1.4)	2 (0.6)	5 (3.2)	
Pathologic N stage				<0.001[b]
pN0, n (%)	344 (69.5)	246 (73.0)	98 (62.0)	
pN1, n (%)	78 (15.8)	33 (9.8)	45 (28.5)	
Unknown, n (%)	73 (14.7)	58 (17.2)	15 (9.5)	
Clinical M stage				0.221[c]
M0, n (%)	453 (91.5)	307 (91.1)	146 (92.4)	
M1a, n (%)	1 (0.2)	1 (0.3)	0	
M1b, n (%)	1 (0.2)	0	1 (0.6)	
M1c, n (%)	1 (0.2)	0	1 (0.6)	
Unknown, n (%)	39 (7.9)	29 (8.6)	10 (6.3)	
Gleason score				<0.001[c]
10, n (%)	4 (0.8)	1 (0.3)	3 (1.9)	
9, n (%)	137 (27.7)	78 (23.1)	59 (37.3)	
8, n (%)	63 (12.7)	37 (11.0)	26 (16.5)	
7, n (%)	246 (49.7)	187 (55.5)	59 (37.3)	
6, n (%)	45 (9.1)	34 (10.1)	11 (7.0)	
Events				
Death, n (%)	10 (2.0)	4 (1.2)	6 (3.8)	0.054[b]
CRPC, n (%)	108 (21.8)	68 (20.2)	40 (25.3)	0.161[b]

[a]Mann-Whitney *U* test; [b]Chi-square test; [c]Fisher's exact test. TCGA: the Cancer Genome Atlas; IL-23: interleukin-23; FPKM: fragments per kilobase of exon per million fragments mapped; IQR: interquartile range; PSA: prostate specific antigen; CRPC: castration-resistant prostate cancer
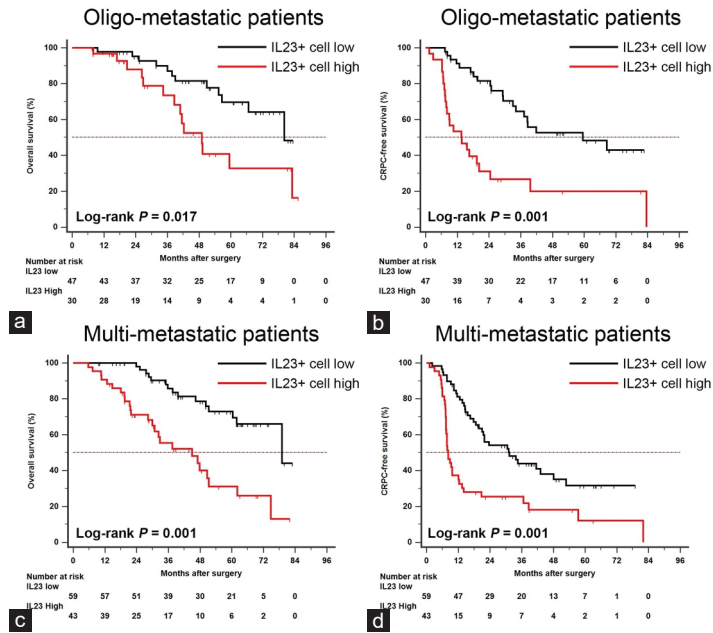
Supplementary Figure 1: The prognostic value of IL-23⁺ cells in different subgroups patients. (**a** and **b**) Kaplan–Meier plots for overall survival **(a)** and CRPC-free survival (**b**) in patients with oligometastatic prostate cancer. (**c** and **d**) Kaplan–Meier plots for overall survival (**c**) and CRPC-free survival (**d**) in patients with multi-metastatic prostate cancer according to IL-23⁺ cells infiltration. IL-23: interleukin-23; CRPC: castration-resistant prostate cancer.