

**Original Article: Clinical Investigation****Presence of CD133-positive circulating tumor cells predicts worse progression-free survival in patients with metastatic castration-sensitive prostate cancer**Yunjie Yang,<sup>1,2,†</sup> Zheng Liu,<sup>1,2,†</sup> Qifeng Wang,<sup>2,3</sup> Kun Chang,<sup>1,2</sup> Junyu Zhang,<sup>1,2</sup> Dingwei Ye,<sup>1,2</sup> Yunyi Kong,<sup>2,3</sup> and Bo Dai<sup>1,2</sup> <sup>1</sup>Department of Urology, Fudan University Shanghai Cancer Center, Shanghai, <sup>2</sup>Department of Oncology, Shanghai Medical College, Fudan University, Shanghai, and <sup>3</sup>Department of Pathology, Fudan University Shanghai Cancer Center, Shanghai, China**Abbreviations & Acronyms**

ADT = androgen deprivation therapy  
ALP = alkaline phosphatase  
AR = androgen receptor  
CI = confidence interval  
CRPC = castration-resistant prostate cancer  
CSPC = castration-sensitive prostate cancer  
CTC = circulating tumor cell  
DAPI = 6-diamidino-2-phenylindole  
ECOG PS = Eastern Cooperative Oncology Group performance status  
EDTA = ethylene diamine tetraacetic acid  
EMT = epithelial-mesenchymal transition  
HR = hazard ratio  
ISET = isolation by size of epithelial tumor cells  
LDH = lactate dehydrogenase  
MRI = magnetic resonance imaging  
NE = neuroendocrine  
OS = overall survival  
PBS = phosphate buffer saline  
PFS = progression-free survival  
PSA = prostate-specific antigen

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**Objective:** To investigate the clinical significance of the expression of the stemness marker CD133 in circulating tumor cells of newly diagnosed metastatic castration-sensitive prostate cancer patients.

**Methods:** For this study, 104 metastatic castration-sensitive prostate cancer patients treated at the Fudan University Shanghai Cancer Center from September 2015 to February 2017 were considered. After enrollment, the patients received androgen deprivation therapy (bicalutamide + goserelin). Circulating tumor cells were isolated and identified using the CanPatrol system, which can identify not only traditional epithelial markers but also mesenchymal markers in cells that have undergone epithelial mesenchymal transition. CD133 was used to characterize the circulating tumor cells. The primary endpoint of this research was to evaluate progression to castration resistance.

**Results:** Among the 104 patients enrolled, 89 patients were circulating tumor cell positive at baseline, and the median circulating tumor cell count was four. The median follow-up was 24 months, and at the end of follow-up, the proportion of patients who progressed to castration-resistant prostate cancer in the CTC+CD133+ group was 93.3%, which was significantly higher than that of the circulating tumor cell negative group (73.3%) and the CTC+CD133− group (75.0%), with  $P = 0.043$ . After follow-up, progression-free survival for CTC+CD133+, CTC+CD133−, and circulating tumor cell patients was 10.0, 13.0, and 14.0 months, respectively, with  $P = 0.022$ . Univariate and multivariate analyses also confirmed that the characterization of circulating tumor cells using CD133 can independently predict progression-free survival in metastatic castration-sensitive prostate cancer patients after receiving androgen deprivation therapy ( $P = 0.042$ ; hazard ratio 1.396).

**Conclusion:** Baseline CTC+CD133+ was a poor independent prognostic factor for metastatic castration-sensitive prostate cancer patients to progress to castration-resistant prostate cancer after receiving androgen deprivation therapy.

**Key words:** CD133, circulating tumor cells, progression-free survival, prostate cancer, time to castration resistance.

**Introduction**

Prostate cancer is one of the malignant tumors with a high morbidity and mortality in men worldwide.<sup>1</sup> In past treatment of prostate cancer, first-generation androgen deprivation agents such as bicalutamide and flutamide were used during the castration-sensitive stage; after the patient progressed to castration resistance, second-generation anti-androgen drugs such as abiraterone or docetaxel chemotherapy were used. However, in recent years, because of studies such as STAMPEd, LATITUDE, and TITAN, in which drugs such as docetaxel, abiraterone, and apalutamide have been used during the castration-sensitive stage and have greatly prolonged OS in patients, we have focused our research on CSPC.<sup>2–5</sup> However, previous studies related to prostate cancer have mostly focused on CRPC, and it is certainly unclear whether these prospective studies have determined risk factors for prognosis; whereas studies on CSPC, especially those regarding new predictive markers using liquid biopsies, have rarely been studied. Therefore, we carried out this study to determine our own poor prognostic factors.

Liquid biopsies are being used increasingly in the early diagnosis of tumors, evaluation of treatment effects, and early monitoring of recurrence because it is noninvasive and can be performed in real time.<sup>6,7</sup> Measurement of CTCs is widely used and has been employed in the evaluation of therapeutic effects and recurrence monitoring in CRPC as well as the differential diagnosis and evaluation of metastatic tumor burden in CSPC.<sup>8–11</sup> CD133 has often been described as an antigen on the surface of both stem cells and cancer stem cells. A previous study showed that CD133 is highly overexpressed at the mRNA and protein levels in a multitude of patients possessing progressive prostate cancer with an AR-negative, NE marker-positive (AR–/NE+) phenotype.<sup>12</sup> Moreover, previous studies show that the epitope the antibody recognizes on CD133 is not highly expressed in early stages of prostate cancer or healthy tissues, demonstrating its promise as a targetable marker for patients with late-stage progressive prostate cancer.<sup>13</sup> Previous studies have shown that in CRPC patients, the expression of CD133 molecules in CTC is positively correlated with the expression of AR-V7 and Ki-67, indicating that CRPC patients with CD133-positive CTCs have higher tumor malignancy and proliferation ability.<sup>14,15</sup> However, it is relatively rare to combine the identification of CTCs with CD133 expression in CSPC patients. In this study, the CanPatrol system based on the ISET method was used to determine the count of CTCs and the CD133 expression in newly diagnosed CSPC patients, and combined with the time to CRPC, we were able to identify patients with greater tumor burden and provide guidance for the individualized treatment of mCSPC patients.

## Methods

### Patients, treatment, and follow-up

The current study considered newly diagnosed mCSPC patients who were treated at the Fudan University Shanghai Cancer Center from September 2015 to February 2017. The inclusion criteria of this study are listed below: 18–80 years old; histologically confirmed prostate adenocarcinoma patients by prostate biopsy or transurethral resection of the prostate; have not had surgery and radiotherapy for the primary lesion, systemic chemotherapy, or endocrine therapy; and metastatic lesions were determined by bone scan and/or pelvic MRI. The study also included 10 healthy male volunteers as controls, of which five cases were negative controls, and five cases were injected with a CD133-expressing liver cancer cell line SMMC-7721 in their peripheral blood as positive controls. This study was approved by the Ethics Committee of our hospital, and the IRB numbers are 1602156-3 and 1602156-3-1602; all patients enrolled provided informed consent. This study was registered on ClinicalTrials.gov, registration number: NCT 02723526.

The patients received ADT after enrollment: goserelin 3.6 mg subcutaneously, monthly and bicalutamide 50 mg orally, once per day. Complete blood count, PSA, testosterone, and liver and kidney function levels were measured once a month; a whole-body bone scan and pelvic MRI were performed every 6 months. The primary endpoint of this study was to estimate PFS, which was calculated from ADT

initiation to the occurrence of castration resistance. The definition of castration resistance was as follows: PSA progression, which was defined as when the testosterone concentration is at the castrated level (<1.7 nmol/L), PSA rises 3 times in a row, 1 week apart, and all are higher than the lowest value by >50%; or radiological progression (the original lesions progress or the appearance of new bone or soft tissue lesions, according to the Response Evaluation Criteria in Solid Tumors standard).

### Identification of CTCs and expression of CD133

After the enrollment of patients and healthy volunteers, 5 mL of peripheral blood was collected into an EDTA anticoagulant tube from each person by venipuncture and the samples were stored at 4°C for ≤4 h, and further, CTCs were separated and characterized using the CanPatrol™ system (SurExam, Guangzhou, China). The CanPatrol CTC enrichment technique mainly uses the ISET methodology combined with marker expression to isolate and identify CTCs; it can identify CTCs expressing EpCAM and can also identify CTCs that arise from EMT; thus, the CanPatrol system can detect CTCs more comprehensively than the traditional CellSearch system.<sup>16</sup>

The basic methodology of the CanPatrol system to separate and classify CTCs was as follows: first, red blood cell lysis solution (10 mM KHCO<sub>3</sub>, 0.1 mM EDTA, and 154 mM NH<sub>4</sub>Cl [Sigma, St. Louis, MO, USA] in deionized water) was applied to remove erythrocytes in peripheral blood; PBS containing 4% formaldehyde (Sigma) was then applied to resuspend the remaining cells for 5 min. The cell suspension was subsequently transferred to the filtration system, which included a calibrated membrane with 8 μm diameter pores (SurExam), a manifold vacuum plate with valves (SurExam), an E-Z 96 vacuum manifold (Omega, Norcross, GA, USA), and a vacuum pump (Auto Science, Tianjin, China). The pump valve was pumped with at least 0.08 MPa.<sup>16</sup>

The count and characterization of CTCs with the CanPatrol system were carried out using a multiplex RNA in situ hybridization assay on the basis of the expression of tumor markers (epithelial and mesenchymal markers) and a stemness marker CD133. EpCAM and CK8/18/19 were used as epithelial markers, and vimentin and twist were used as mesenchymal markers. Leukocyte biomarker CD45 and cell nuclei marker 40, DAPI were applied to detect and classify CTCs.

The count and characterization of CTCs were performed with an automatic fluorescent microscope Imager Z2 (Zeiss, Jena, Germany) after DAPI nuclear staining and in situ hybridization of markers, as described above. A circulating cell was considered to be a CTC under the following conditions: the cell nuclear marker DAPI-positive, leukocyte marker CD45-negative, and positive for epithelial and/or mesenchymal tumor markers. CTCs can be divided into CD133+ and CD133– CTCs on the basis of CD133 expression.

### Statistical analysis

The correlation analyses were assessed with the Mann–Whitney *U* test between two groups, and the Kruskal–Wallis *H*

test for multigroup analysis. Kaplan–Meier method and log-rank test were used to calculate survival curves between groups in this study. Multivariate analyses of Cox regression model were conducted to estimate HR.  $P < 0.05$  (two tailed) was considered statistically significant. SPSS 22.0 (SPSS Inc., Chicago, IL, USA) was used to conduct statistical analyses.

## Results

### Baseline characteristics and CTC counts

A total of 104 newly diagnosed mCSPC patients were considered in this study. The median age of included patients was 67 years; the median PSA level was 196.15 ng/mL. All 104 patients had bone metastases, among which 29 patients had limb bone metastases, and six patients had visceral metastases. The other baseline conditions of enrolled patients are shown in Table 1.

**Table 1** Baseline characteristics and primary outcomes of 104 patients

Characteristics and outcomes	<i>n</i> = 104	100%
Age at baseline, years		
Median (range)	67 (51–79)	
Baseline PSA, ng/mL		
Median (range)	196.15 (5.77–5011.90)	
Gleason score at biopsy		
<9	41	39.4
≥9	63	60.6
ECOG PS		
0–1	92	88.5
2	12	11.5
Site of metastasis		
Bone	104	100
Limb bone	29	27.9
Viscera	6	5.8
Extent of metastasis		
Median (range)	10 (5–16)	
LDH, U/L		
Median (range)	179.0 (49.0–630.0)	
ALP, U/L		
Median (range)	120.5 (23.0–2519.2)	
Hemoglobin, g/L		
Median (range)	132.0 (90.0–172.0)	
Albumin, g/L		
Median (range)	43.6 (33.0–49.0)	
Baseline CTC count per 5 mL		
Median (range)	4 (0–35)	
0	15	14.4
1–4	54	51.9
5–10	30	28.8
>10	5	4.8
PSA nadir after 6 months of ADT, ng/mL		
<0.2	29	27.9
0.2–4.0	41	39.4
>4.0	34	32.7
Proportion of PSA decline after 6 months of ADT		
0–50%	2	1.9
50–90%	11	10.6
>90%	91	87.5
Progression to CRPC at the end of follow-up		
Yes	86	82.7
No	18	17.3

The median number of baseline CTCs in 104 patients was four, of which 15 were CTC– (subsequently referred to as the CTC– group), 44 of 89 CTC+ patients were CD133– (hereafter referred to as the CD133– group), and 45 were CD133+ (subsequently referred to as the CD133+ group). The median (range) of positive rates of these patients is 33% (13–100%). The baseline conditions of PSA level, Gleason score, and number of metastases in the three groups of patients were not statistically different (see Table 2 for details).

The typical images of CTCs of mCSPC patients detected by the CanPatrol system and the SMMC-7721 cell line expressing CD133 molecules mixed into the peripheral blood of five healthy control volunteers are shown in Figure 1. No CTCs were detected in the peripheral blood of the five negative control volunteers.

### Comparison of outcomes between patients in different CTC subgroups

All patients received ADT treatment (goserelin + bicalutamide) after being enrolled in this study. The median time of follow-up was 24 months, with a range of 18–32 months. At the end of follow-up, 86 (82.7%) patients progressed to CRPC. The proportion of the CD133+ group that progressed to CRPC was 93.3%, which was significantly higher than that of the CTC– group (73.3%) and the CD133– group (75.0%), with a  $P$  value of 0.043.

Additionally, there were 53.3% of patients in the CD133+ group with PSA values that were below 4.0 ng/mL after 6 months of ADT treatment, which was significantly lower than 70.5% in the CD133– group and 100% in the CTC– group, and the  $P$  value was 0.003.

Figure 2 shows the survival curves of 104 newly diagnosed mCSPC patients according to the different groups. Figure 2a shows the survival curves of different CTC and CD133 status patients; median PFS for patients in the CD133+, CD133–, and CTC– groups was 10.0, 13.0, and 14.0 months, respectively, with a  $P$  value of 0.022. Figure 2b shows that for patients with metastatic lesions >10 and ≤10, median PFS was 10.0 and 13.0 months ( $P = 0.015$ ). Median PFS for patients with limb bone metastasis or not was 10.0 and 13.0 months, which is shown in Figure 2c ( $P = 0.014$ ).

### Multivariate analysis of factors affecting PFS

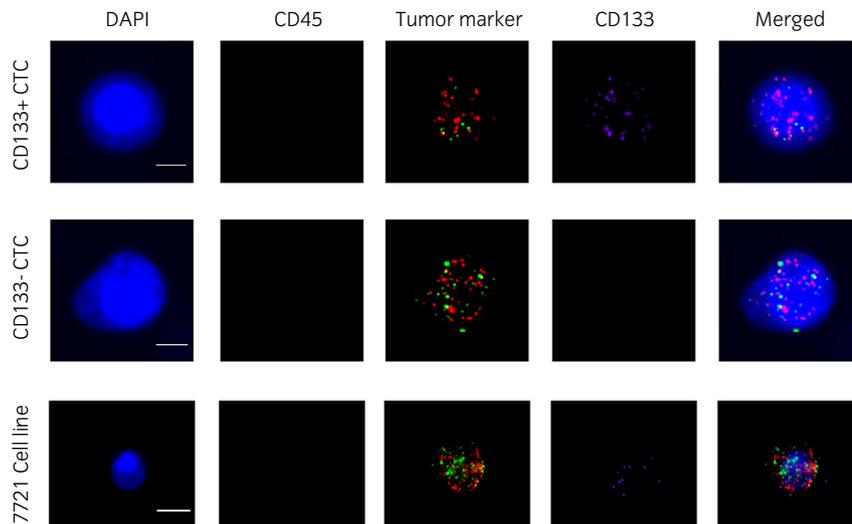
Table 3 reveals the relationship among patient baseline variables and PFS. Univariate analysis showed that limb bone metastases, metastatic lesions >10, and baseline CD133+ CTCs were factors associated with poor prognosis after ADT in mCSPC patients; further multivariate analysis demonstrated that baseline CD133+ CTCs were the only independent factor affecting time to CRPC from CSPC after ADT, with a  $P$  value of 0.042 and a HR of 1.396 (95% CI 1.012–1.927).

## Discussion

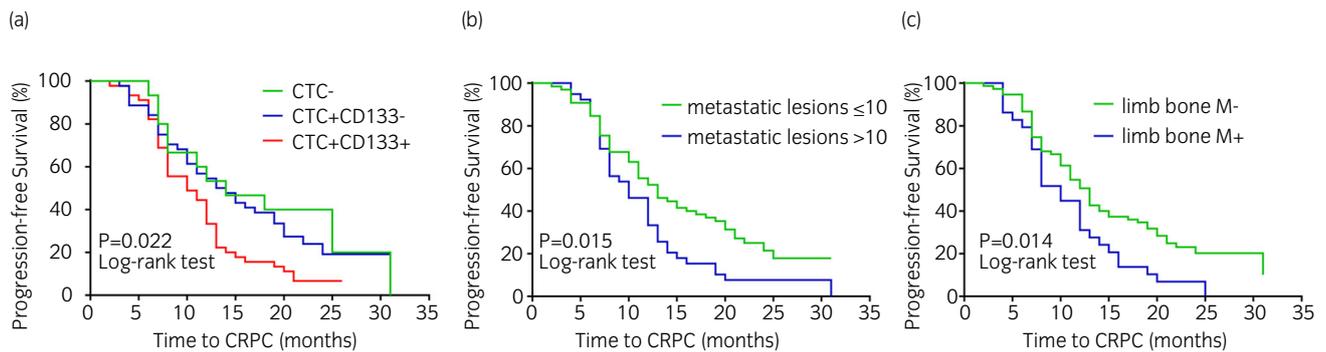
In this study, we focused on mCSPC patients, a recently emerging research area, combined with CTC detection and characterization to explore the efficacy of ADT treatment and

**Table 2** Baseline characteristics according to patients with different status of CTCs

Baseline characteristics and outcomes	CTC– <i>n</i> = 15; <i>n</i> (%)	CTC+CD133– <i>n</i> = 44; <i>n</i> (%)	CTC+CD133+ <i>n</i> = 45; <i>n</i> (%)	<i>P</i>
Age, years				0.464
≤67	6 (40.0)	22 (50.0)	26 (51.9)	
>67	9 (60.0)	22 (50.0)	19 (48.1)	
Baseline PSA, ng/mL				0.730
PSA <196.15	8 (53.3)	20 (45.5)	24 (53.3)	
PSA >196.15	7 (46.7)	24 (54.5)	21 (46.7)	
Gleason score				0.990
<9	6 (40.0)	17 (38.6)	18 (40.0)	
≥9	9 (60.0)	27 (61.4)	27 (60.0)	
ECOG PS				0.782
0–1	14 (93.3)	39 (88.6)	39 (86.7)	
2	1 (6.7)	5 (11.4)	6 (13.3)	
Metastatic lesions				0.233
≤10 lesions	10 (66.7)	31 (70.5)	24 (53.3)	
>10 lesions	5 (33.3)	13 (29.5)	21 (46.7)	
Limb bone metastasis				0.519
No	12 (80.0)	33 (75.0)	30 (66.7)	
Yes	3 (20.0)	11 (25.0)	15 (33.3)	
LDH, U/L				0.731
Normal (≤250)	13 (86.7)	36 (81.8)	35 (77.8)	
Elevated (>250)	2 (13.3)	8 (18.2)	10 (22.2)	
ALP, U/L				0.285
Normal (≤160)	8 (53.3)	32 (72.7)	27 (60.0)	
Elevated (>160)	7 (46.7)	12 (27.3)	18 (40.0)	
Hemoglobin, g/L				0.822
Normal (≥120)	12 (80.0)	35 (79.5)	38 (81.7)	
Low (<120)	3 (20.0)	9 (20.5)	17 (15.6)	
Albumin, g/L				0.267
Normal (≥40)	12 (80.0)	37 (84.1)	42 (93.3)	
Low (<40)	3 (20.0)	7 (15.9)	3 (6.7)	
PSA nadir within the first 6 months of ADT				0.003
≤4.0 ng/mL	15 (100)	31 (70.5)	24 (53.3)	
>4.0 ng/mL	0 (0)	13 (29.5)	21 (46.7)	
Progression to CRPC				0.043
No	4 (26.7)	11 (25.0)	3 (6.7)	
Yes	11 (73.3)	33 (75.0)	42 (93.3)	



**Fig. 1** Typical images of CD133 status of CTCs detected by CanPatrol CTC enrichment technique in peripheral blood from metastatic CSPC patients and for SMMC-7721 cell cultures spiked into blood specimens of healthy volunteers. Nuclei were stained with DAPI (blue); CD45 was applied as a leukocyte biomarker; epithelial markers (EpCAM, CK8/18/19: red dots) and mesenchymal markers (twist and vimentin: green dots) were used as markers of tumor cells. Scale bar, 5  $\mu$ m.



**Fig. 2** Kaplan–Meier analysis of PFS in 104 newly diagnosed metastatic CRPC patients according to different groups. (a) The survival curves of different CTCs and CD133 status patients; median PFS for patients in the CD133+, CD133–, and CTC– groups was 10.0, 13.0, and 14.0 months, respectively, with a *P* value of 0.022. For patients of metastatic lesions >10 and ≤10, median PFS was 10.0 and 13.0 months (b; *P* = 0.015). Median PFS for patients with limb bone metastasis or not was 10.0 and 13.0 months (c; *P* = 0.014).

**Table 3** Univariate and multivariate analyses for patient baseline variables affecting time to CRPC

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	<i>P</i>	HR (95% CI)	<i>P</i>
Age (>67 vs ≤67)	1.107 (0.723–1.696)	0.640		
Baseline PSA, ng/mL (>196 vs ≤196)	1.362 (0.889–2.087)	0.156		
Gleason score (≥9 vs <9)	1.034 (0.670–1.594)	0.881		
ECOG PS (2 vs 0–1)	1.754 (0.928–3.313)	0.084		
Metastatic sites (>10 vs ≤10)	1.665 (1.080–2.567)	0.021	1.350 (0.815–2.238)	0.244
Limb bone metastasis (yes vs no)	1.722 (1.091–2.719)	0.020	1.331 (0.778–2.275)	0.296
LDH, U/L (>250 vs ≤250)	1.568 (0.929–2.647)	0.092		
ALP, U/L (>160 vs ≤160)	1.293 (0.838–1.997)	0.246		
Hemoglobin, g/L (<120 vs ≥120)	1.553 (0.922–2.614)	0.098		
Albumin, g/L (<40 vs ≥40)	1.349 (0.729–2.495)	0.341		
CTCs count (>4 vs ≤4)	0.783 (0.496–1.235)	0.292		
CTCs status (CTC+CD133+ vs CTC– and CTC+CD133–)	1.762 (1.145–2.712)	0.010	1.396 (1.012–1.927)	0.042

PFS in mCSPC patients. The results showed that patients who were CTC+CD133+ had markedly shorter PFS than those who were CTC– or CTC+CD133–, which suggests that mCSPC patients who are baseline CTC+CD133+ are more likely to develop a progressive disease.

Previous studies have investigated the prognostic factors in CSPC patients. In a retrospective population-based study of 3556 mCSPC patients conducted in Canada, researchers found that in multivariate analysis, the efficacy of ADT was correlated with neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and albumin, hemoglobin, and PSA decrease.<sup>17</sup> Briones *et al.* studied the survival of mCSPC patients after receiving docetaxel or abiraterone combination therapy and found that age at diagnosis of mCSPC, PSA90 at 3 months, and LATITUDE risk classification are prognostic factors of PFS.<sup>18</sup>

However, these traditional markers all have their own limitations. Gleason score mainly reflects the degree of malignancy of the primary lesion and cannot reflect tumor burden in the whole body of metastatic prostate cancer patients. PSA levels at ADT initiation and other potential baseline characteristics related not only to disease burden but also to

physical conditions, for instance, organ function, complications, and nutrition. As for PSA nadir and time to PSA nadir, these indicators can only be obtained after a period of treatment and cannot ascertain the tumor burden before the start of treatment. In the current study, univariate analysis showed that, except for CTC characterization, metastatic lesions and limb bone metastasis were both statistically significant, whereas in multivariate analysis, metastatic lesions and limb bone metastasis did not reach significance, which illustrates that the prognostic role of traditional markers is not certain.

As an important component of a liquid biopsy, CTCs have become increasingly important in the study of tumor prognosis because of their safety, noninvasiveness, and ability to dynamically monitor the treatment response.<sup>6,7</sup> However, for prostate cancer in particular, CTC-related research concentrated mostly on CRPC; only a handful of research focused on CSPC. Okegawa *et al.* found that CTC count was a significant parameter for predicting the effect of ADT in multivariate analyses when the cutoff was set at 5 CTCs.<sup>9</sup> Goodman *et al.* enumerated CTCs in 33 consecutive patients using the Cell-Search platform, and the results showed that only baseline CTCs were independent predictors of progression to CRPC,

and the cutoff was 3 cells.<sup>10</sup> Folkersma detected CTCs in 30 mCSPC patients, and the results revealed a CTC cutoff value of 4 as an independent prognostic variable for PFS.<sup>11</sup>

However, the eligibility criteria and therapeutic regimens in the above three studies were not uniform; furthermore, CTCs, which were used as prognostic markers in the three studies, also had different cutoff values. Moreover, after several years of rapid development of the liquid biopsy, it is no longer enough to conduct a simple CTC enumeration study, and simultaneous detection of the number of CTCs and the expression of certain genes or markers in prognostic studies has become a recent research hotspot. Antonarakis *et al.* explored the clinical significance of CTC determination (+ vs -) and AR-V7 detection (+ vs -) in 202 CRPC patients starting abiraterone or enzalutamide therapy, and for the outcomes of PSA response, PFS, and OS in the overall cohort, patients of CTC- were the best, patients of CTC+AR-V7- were intermediate, and patients of CTC+AR-V7+ were the worst.<sup>19</sup> In a previous study, we also found that the estimated value of concordance probability could be improved from 0.716 to 0.889 when stem cell gene expression was added to the prognostic model with CTC counts; additionally, we detected the expression of EMT-related genes (*TWIST1* and *VIM*), which may lead to EMT of CTCs.<sup>20</sup> Recent studies have found that in the process of being released into the blood from the primary lesion, among the CTCs that have undergone EMT, loss of EpCAM has occurred. Furthermore, the CTCs in which EMT has occurred play a more critical role in local invasion and distant metastasis of the tumor.<sup>21-23</sup> More importantly, CTCs that have undergone EMT cannot be detected using the CellSearch system that is widely applied.<sup>24</sup>

Thus, we designed this study to characterize CTCs in peripheral blood of mCSPC patients utilizing the established and documented CanPatrol CTC enrichment technique. The main steps and principles of CanPatrol CTC enrichment technique to detect CTCs have been described in detail above; the system can not only identify CTCs expressing EpCAM but also detect CTCs with EMT (CTCs that express mesenchymal markers vimentin and twist); thus, the CanPatrol CTC system can detect CTCs more comprehensively than the traditional CellSearch system.<sup>25-27</sup> At the same time, the use of multiplex in situ hybridization technology can further evaluate the CD133 expression. After follow-up, the median times for CTC+CD133+, CTC+CD133-, and CTC- patients to progress to CRPC were 10.0, 13.0, and 14.0 months, respectively ( $P = 0.022$ ). Moreover, multivariate analyses revealed that the characterization of CTCs using CD133 as a marker was the only independent adverse prognostic factor for mCSPC patients progressing to CRPC after receiving ADT treatment. This indicates that baseline CTC+CD133+ patients have a greater tumor burden and may develop CRPC in a shorter period of time. Therefore, if our results could be externally validated, we would suggest that CTC+CD133+ mCSPC patients should have a stricter follow-up schedule to monitor disease progression.

There are also some limitations in this study, such as small sample size, failure to dynamically monitor CTC counts after treatment, and the primary endpoint of this study is CRPC instead of patient death. Since the main purpose of this study

was to find indicators reflecting tumor burden in patients, the CTC counts after treatment were not dynamically monitored. At the same time, the treatment choices of patients after progression to CRPC were not uniform; therefore, the endpoint of this study was determined as castration resistance after ADT treatment instead of patient death.

In conclusion, we found that CTC characterization based on the stem cell marker CD133 can predict the PFS in mCSPC patients who have received ADT treatment. This study may help in determining individualized treatment for mCSPC patients, but further research with more patients and a longer follow-up time is needed.

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## Author contributions

Yunjie Yang: data curation, formal analysis, methodology, writing – original draft and review & editing. Zheng Liu: data curation, formal analysis, methodology, writing – original draft and review & editing. Qifeng Wang: resources and validation. Kun Chang: methodology and software. Junyu Zhang: data curation, methodology and software. Dingwei Ye: resources. Yunyi Kong: conceptualization, funding acquisition, supervision, writing – review & editing. Bo Dai: conceptualization, funding acquisition, project administration, supervision, Writing – review & editing.

## Conflict of interest

None declared.

## Approval of the research protocol by an Institutional Reviewer Board

This study was approved by the Ethics Committee of Fudan University Shanghai Cancer Center, and the IRB number is 1602156-3/1602156-3-1602.

## Informed consent

All patients enrolled provided informed consent.

## Registry and the Registration No. of the study/trial

This study was registered on ClinicalTrial.gov, registration number: NCT 02723526.

## Animal studies

N/A.

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