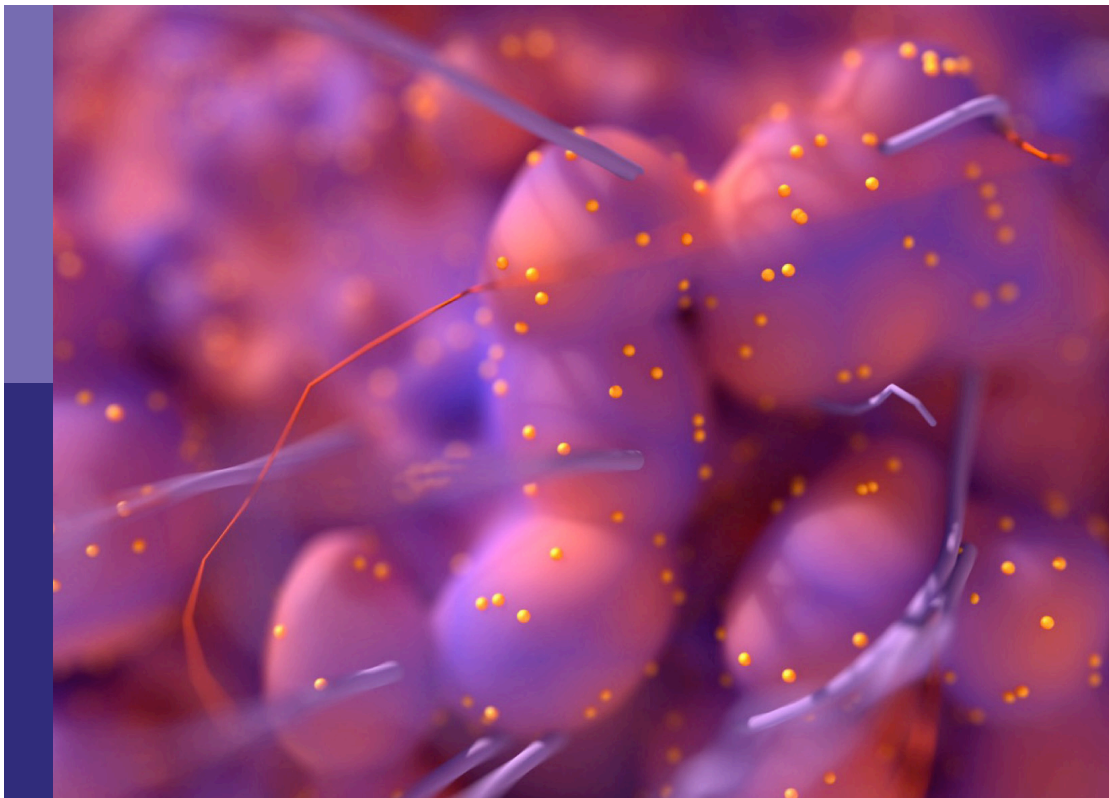
frontiers | Research Topics

Clinical and translational research in prostate cancer

Edited by
Ran Xu, Sifeng Qu and Dong Lin

Published in
Frontiers in Oncology



ISSN 1664-8714
ISBN 978-2-8325-4233-0
DOI 10.3389/978-2-8325-4233-0

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Clinical and translational research in prostate cancer

Topic editors

Ran Xu — Central South University, China

Sifeng Qu — Shandong University, China

Dong Lin — University of British Columbia, Canada

Citation

Xu, R., Qu, S., Lin, D., eds. (2024). *Clinical and translational research in prostate cancer*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-4233-0

Table of contents

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 13 January 2023
DOI 10.3389/fonc.2022.1059410

OPEN ACCESS

EDITED BY
Sifeng Qu,
Shandong University, China

REVIEWED BY
Yu Gao,
Chinese PLA General Hospital, China
Ming Zong,
Tongji University, China

*CORRESPONDENCE
Hongqian Guo
dr.ghq@nju.edu.cn
Junlong Zhuang
zhuangjl-2008@163.com
Linfeng Xu
linfengxu1107@hotmail.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Genitourinary Oncology, a section of the journal Frontiers in Oncology

RECEIVED 01 October 2022
ACCEPTED 28 December 2022
PUBLISHED 13 January 2023

CITATION
Wang Y, Zhang S, Huang H, Qiu X, Fu Y, Lyu X, Xu L, Zhuang J and Guo H (2023) A retrospective study to evaluate the effect of preoperative hormonal therapy on continence recovery.
Front. Oncol. 12:1059410.
doi: 10.3389/fonc.2022.1059410

A retrospective study to evaluate the effect of preoperative hormonal therapy on continence recovery

Yuwen Wang[1,2†], Shun Zhang[1,3†], Haifeng Huang[1,3†], Xuefeng Qiu[1,3], Yao Fu[4], Xiaoyu Lyu[1,3], Linfeng Xu[1,3*], Junlong Zhuang[1,3*] and Hongqian Guo[1,2,3*]

[1]Department of Urology, Affiliated Drum Tower Hospital, Medical School of Nanjing University, Nanjing, China, [2]Medical School of Southeast University Nanjing Drum Tower Hospital, Nanjing, China, [3]Institute of Urology, Nanjing University, Nanjing, China, [4]Department of Pathology, Affiliated Drum Tower Hospital, Medical School of Nanjing University, Nanjing, China

Objective: To evaluate whether different preoperative hormonal therapy options affect postoperative continence and to identify risk/protective factors for continence recovery.

Methods: This is a retrospective analysis of several clinical trials (NCT04356430, NCT04869371, NCT04992026 and NCT05406999). Data from patients treated with hormonal therapy followed by RARP were collected and analyzed. Continence was defined as 0 pad/day or one safety pad.

Results: The study included 230 patients with adequate information. The median time to continence recovery is 8 weeks. A total of 216 (93.9%) participants recovered to urinary continence within 12 months after surgery. 21 (9.1%) participants achieved immediate continence. 69, 85, 27 and 14 participants restored continence at 1 month, 1-3 month, 3-6 month, 6-12 month, accounting for 30.0%, 40.0%, 11.7% and 6.1% accordingly. No difference in continence recovery was found among different preoperative hormonal treatment options (p=0.821). Cox regression showed that membranous urethral length (MUL) was the only independent factor influencing urinary continence recovery either in the univariate analysis (OR=1.13, 95%CI: 1.04-1.22, p=0.002) or in the multivariate analysis (OR=1.12, 95%CI: 1.04-1.20, p=0.002). Different preoperative treatment options were not associated with urinary recovery. More advanced preoperative T stage (OR=0.46, 95%CI: 0.24-0.85, p=0.014) delayed the recovery of immediate continence. MUL was associated with continence restoring at 1 month (OR=1.20, 95%CI: 1.03-1.39, p=0.017), 3 month (OR=1.27, 95%CI: 1.07-1.51, p=0.006), 6 month (OR=1.34, 95%CI: 1.07-1.67, p=0.011) and 12 month (OR=1.36, 95%CI: 1.01-1.84, p=0.044).

Conclusion: There is no difference in postoperative continence recovery among ADT, ADT+Docetaxel and ADT+Abiraterone preoperative treatment options. More

advanced T stage indicated poor immediate continence recovery. Longer membranous urethral length was a promotional factor for both short-time and long-time continence recovery.

KEYWORDS

hormonal therapy, continence, prostatectomy, oligometastatic prostate cancer, locally advanced prostate cancer

1 Introduction

Urinary incontinence following robot-assisted radical prostatectomy (RARP) is a significant and perhaps under-reported consequence that substantially decreases quality of life (QOL) (1). Besides oncological outcomes after RARP in prostate cancer (PCa) patients, functional results have become another focus of attention (2). Emerging surgical techniques including bladder neck preservation, selective dorsal venous complex, nerve-sparing technique, and posterior musculofascial reconstruction as well as anterior restoration of the pelvis space were suggested by surgeons and showed promising improvements in restoring continence (3–7). However, there was 8% to 11% of patients still suffering from incontinence one year after RARP according to meta-analysis when the safety pad definition was used (7).

Androgen deprivation therapy (ADT) has been the standard of care for over 50 years for metastatic PCa (8). Combined hormonal therapy such as chemo-hormonal treatment and addition of new hormonal treatments (abiraterone, apalutamide, enzalutamide) was suggested by the STAMPEDE, CHAARTED and LATITUDE trials (9–11). Though lack of robust evidence, cytoreductive radical prostatectomy (CRP) in combination with hormonal therapy was hypothetically expected to reduce tumor burden, induce immune modulation and improved response to secondary treatment (12). A number of ongoing clinical trials might provide future evidence for the therapeutic effect of CRP (13–15).

The effects of neoadjuvant hormonal therapy (NHT) for high risk PCa have been a popular concern for many years, though the oncological results remained controversial. As for functional aspects, researchers found that neoadjuvant hormonal therapy resulted in immediate impairment of vitality and sexual quality of life (16). However, few studies looked into the effect on urinary continence. On the other hand, most of studies on postoperative continence excluded those previously treated with hormonal therapy due to potential bias, leaving this topic undiscovered (17–19).

Several clinical trials at our center focusing on hormonal therapy followed by RARP for localized, locally advanced and metastatic PCa with hundreds of participants were under way, which happened to be suitable for the continence research. The purpose of this study was to evaluate whether different preoperative hormonal therapy options affect postoperative continence and to identify risk/protective factors for incontinence.

Abbreviations: RARP, Robot-assisted radical prostatectomy; QOL, Quality of life; PCa, Prostate cancer; ADT, Androgen deprivation therapy; CRP, Cytoreductive prostatectomy; NHT, Neoadjuvant hormonal therapy; mpMRI, multi-parameter magnetic resonance imaging; 68Ga-PSMA PET, 68Ga-labeled molecular imaging with PET-targeted prostate-specific membrane antigen; LHRHa, Luteinizing hormone-releasing hormone analog; ePLND, Enlarged pelvic lymph node dissection; C-RARP, Conventional robot-assisted radical prostatectomy; RS-RARP, Retzius-sparing robot-assisted radical prostatectomy; DVCL, Dorsal venous complex ligation; PR, Posterior reconstruction; PSA, Prostate specific antigen; MUL, Membranous urethral length; BMI, Body mass index; SD, Standard deviation ; IQR, Interquartile range; ANOVA, Analysis of variance; CI, Confidence interval; OR, Odds ratio; NVB, Neurovascular bundle; PSM, Positive surgical margin.

2 Methods and materials

2.1 Patient selection

From December 2018 to May 2021, a total of 235 consecutive PCa patients from several phase 2 clinical trials (*ClinicalTrials.gov:* NCT04356430, NCT04869371, NCT04992026 and NCT05406999), who were treated with hormonal therapy followed by RARP were retrospectively collected. 230 well documented patients out of 235 patients were then included in the analysis. The study was approved by the Medical Ethics Committee of Nanjing Drum Tower Hospital, China.

According to the inclusion and exclusion criteria of the trials, patients with high risk localized (T1-2, N0, M0), locally advanced (T3-4, N0-1, M0) or oligometastatic PCa (no more than 5 metastatic lesions, no visceral metastasis) were all included in this analysis. All patients were diagnosed PCa with biopsy and went through careful examination including 1) Transrectal prostate ultrasonography; 2) prostate multi-parameter magnetic resonance imaging (mpMRI); 3) ECT plus CT scan of the whole abdomen or ^{68}Ga-labeled molecular imaging with PET-targeted prostate-specific membrane antigen (^{68}Ga-PSMA PET). Ultrasonography and mpMRI of prostate were carried out again after preoperative hormonal therapy to re-evaluated tumor conditions before surgery.

2.2 Preoperative therapy

The duration of preoperative hormonal therapy is 6 months. Regarding medication modality, it can be divided into the following three cases 1) ADT, hypodermic injection of luteinizing hormone-releasing hormone analog (LHRHa) every 12 weeks; 2) ADT

+Docetaxel, ADT with additional intravenous administration of docetaxel 75 mg/m^2 body surface area every 3 weeks for 6 cycles; 3) ADT+Abiraterone, ADT with additional daily 1000 mg of abiraterone acetate orally.

2.3 Surgical technique

The surgical technique was accomplished using da Vinci Surgical System. Robot-assisted radical prostatectomy (RARP) plus enlarged pelvic lymph node dissection (ePLND) within 2 weeks after the end of the therapy were performed by the same experienced surgeon (Dr. HG). Conventional robot-assisted radical prostatectomy (C-RARP), also known as anterior approach or Retzius-sparing robot-assisted radical prostatectomy (RS-RARP), also known as posterior approach was carefully chosen based on tumor conditions (tumor location, tumor stage and tumor lesion volume) and physical conditions (age and systematic complications). Other techniques, which could possibly improve continence were also applied including preserving maximal urethral length, dorsal venous complex ligation (DVCL) and posterior reconstruction (PR). Nerve sparing was not applied due to oncologic consideration.

2.4 Follow up and continence evaluation

Patients were discharged 4-6 days after surgery and the urinary catheter was removed on the 14th postoperative day. All patients were encouraged to practice Kegels exercise. The follow-up was continued until urinary continence, which was defined as 0 pad/day or one safety pad. Immediate continence was defined as continence within 7 days after the removal of catheter.

2.5 Data collection

Patients' data were extracted from their medical records. To be specific, basic information (age, BMI), information at initial diagnosis (PSA, TNM stage, biopsy Gleason, apex invasion or not), preoperative characteristics (membranous urethral length, PSA, prostate volume), preoperative therapy, surgery approach, post-surgery information (pathological T and N stage, surgical margin, post-surgery treatment) were collected. The membranous urethral length (MUL) (Figure 1) was measured on mpMRI.

2.6 Statistical analysis

Statistical analysis was realized by SPSS version 22.0 (IBM SPSS, Chicago, IL, USA). Continuous variables were presented as mean ± standard deviation (SD) or median and interquartile range (IQR). Categorical variables were reported as absolute frequency (percentage). One-way ANOVA (Analysis of Variance) or Kruskal-Wallis test was used to compare continuous variables between groups while Chi-square test or Fisher's exact test was used to compare categorical variables between groups. Cox regression as well as logistic regression analysis were then sequentially applied for univariate and multivariate analysis. Age, BMI, initial T stage, apex invasion, preoperative PSA, preoperative volume, membranous urethral length, preoperative T, preoperative therapy, surgery approach and post-surgery ADT were included as evaluated variables. $P < 0.05$ was considered statistically significant.

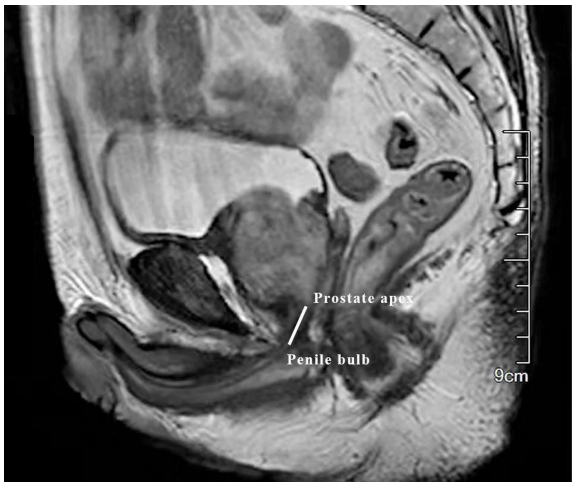


FIGURE 1
The measurement of membranous urethral length (MUL). The membranous urethral length (MUL) was measured from the prostate apex to penile bulb on the preoperative sagittal plane of T2-weighted MRI.

3 Results

Data from 235 consecutive PCa patients who received preoperative hormonal therapy followed by RARP were collected from December 2018 to May 2021. 5 patients were then excluded due to refusal of phone interview. The average age was 69.00 ± 6.90 years and the average BMI was 24.50 ± 2.94 kg/m^2. Participants' basic characteristics were summarized in Table 1.

The median time to continence recovery is 8 weeks. A total of 216 (93.9%) participants recovered to urinary continence within 12 months after surgery, leaving 14 (6.1%) not recovered at one year follow-up. 21 (9.1%) participants achieved immediate continence. 69, 85, 27 and 14 participants restored continence at 1 month, 1-3 month, 3-6 month, 6-12 month, accounting for 30.0%, 40.0%, 11.7% and 6.1% accordingly. More detailed information regarding time to continence recovery were illustrated in Table 2. No difference in continence recovery was found among different preoperative treatment options (p=0.821), as visualized in Figure 2.

After including evaluated variables in the Cox regression analysis (Table 3), it showed that membranous urethral length (MUL) was the only independent factor influencing recovery time of urinary continence either in the univariate analysis (OR=1.13, 95%CI: 1.04-1.22, p=0.002) or in the multivariate analysis (OR=1.12, 95%CI: 1.04-1.20, p=0.002). It turned out that different preoperative treatment options (ADT, ADT+Docetaxel and ADT+Abiraterone) were not associated with urinary recovery.

Deeper digging into the potential risk or protective factors for urinary continence at different time were then carried out using

10.3389/fonc.2022.1059410

TABLE 1 Summary of Characteristics.

	Total	ADT (N=45)	ADT+Docetaxel (N=50)	ADT+Abiraterone (N=135)	*p*
Basic characteristic					
Age, y, Mean ± SD	69.00 ± 6.90	70.58 ± 5.89	68.26 ± 7.28	68.75 ± 6.59	0.237
BMI, kg/m^{2,} Mean ± SD	24.50 ± 2.94	23.76 ± 3.01	24.40 ± 2.88	24.78 ± 3.13	0.358
Characteristic at initial diagnosis					
PSA, ng/ml, IQR	40.17 (18.94-75.13)	40.9 (13.70-56.99)	57.26 (19.76-100)	38.80 (19.46-69.76)	0.103
T stage, n(%)					0.756
T2	47 (20.4)	10 (22.2)	10 (20.0)	27 (20.0)	
T3a	54 (23.5)	9 (20.0)	14 (28.0)	31 (23.0)	
T3b	88 (38.3)	16 (35.6)	21 (42.0)	51 (37.8)	
T4	41 (17.8)	10 (22.2)	5 (10.0)	26 (19.3)	
N stage, n(%)					<0.001**
N0	149 (64.8)	40 (88.9)	50 (100.0)	59 (43.7)	
N1	81 (35.2)	5 (11.1)	0 (0.0)	76 (56.3)	
M stage, n(%)					<0.001**
M0	204 (88.7)	45 (100.0)	50 (100.0)	109 (80.7)	
M1	26 (11.3)	0 (0.0)	0 (0.0)	26 (19.3)	
ISUP, n(%)					0.845
1	40 (17.4)	10 (22.2)	10 (20.0)	20 (14.8)	
2	5 (2.2)	1 (2.2)	2 (4.0)	2 (1.5)	
3	53 (23.0)	10 (22.2)	13 (26.0)	30 (22.2)	
4	106 (46.1)	19 (42.2)	20 (40.0)	67 (49.6)	
5	26 (11.3)	5 (11.1)	5 (10.0)	16 (11.9)	
Apex invasion, n(%)					0.013*
Yes	103 (44.8)	23 (51.1)	30 (60.0)	50 (37.0)	
No	127 (55.2)	22 (48.9)	20 (40.0)	85 (63.0)	
Preoperative characteristic					
PSA, ng/ml, IQR	0.05 (0.01-0.19)	0.12 (0.04-0.41)	0.14 (0.03-0.53)	0.03 (0.01-0.10)	<0.001**
Prostate volume, ml, Mean ± SD	17.50 (13.60-22.73)	18.90 (14.50-25.25)	18.00 (14.60-23.05)	18.00 (14.63-23.05)	0.286
Membranous urethral length, mm, Mean ± SD	15.19 ± 1.87	14.77 ± 2.01	15.34 ± 2.03	15.28 ± 1.75	0.243
T stage, n(%)					0.170
T2	91 (39.6)	20 (44.4)	12 (24.0)	59 (43.7)	
T3a	61 (26.5)	14 (37.1)	16 (32.0)	31 (23.0)	
T3b	65 (28.3)	8 (17.8)	19 (38.0)	38 (28.1)	
T4	13 (5.7)	3 (6.7)	3 (6.0)	7 (5.2)	
Surgery					0.768
Anterior approach RARP	149 (64.8)	30 (66.7)	34 (68.0)	85 (63.0)	
Posterior approach RARP	81 (35.2)	15 (33.3)	16 (32.0)	50 (37.0)	

(Continued)

TABLE 1 Continued

	Total	ADT (N=45)	ADT+Docetaxel (N=50)	ADT+Abiraterone (N=135)	*p*
Post-surgery information					
Pathological T					0.302
T0	12 (5.2)	1 (2.2)	3 (6.0)	8 (5.9)	
T2	103 (44.8)	19 (42.2)	20 (40.0)	64 (47.4)	
T3a	61 (26.5)	18 (40.0)	13 (26.0)	30 (22.2)	
T3b	54 (23.5)	7 (15.6)	14 (28.0)	33 (24.4)	
Margin					0.918
Positive	46 (20.0)	9 (20.0)	9 (18.0)	28 (20.7)	
Negative	184 (80.0)	36 (80.0)	41 (82.0)	107 (79.3)	
Pathological N stage					0.352
N0	177 (77.0)	38 (84.4)	39 (78.0)	100 (74.1)	
N1	53 (23.0)	7 (15.6)	11 (22.0)	35 (25.9)	
Post-surgery ADT, n (%)					0.391
Yes	47 (20.4)	6 (13.3)	12 (24.0)	29 (21.5)	
No	183 (79.6)	39 (86.7)	38 (76.0)	106 (78.5)	

**p < 0.01
BMI, Body mass index; PSA, Prostate specific antigen; ISUP, International Society of Urological Pathology; RARP, Robot-assisted radical prostatectomy; ADT, Androgen deprivation therapy.

logistic regression (Supplementary Table 1). As for immediate continence, only preoperative T stage was correlated (OR=0.46, 95%CI: 0.24-0.85, p=0.014). MUL was associated with continence recovery at 1 month (OR=1.20, 95%CI: 1.03-1.39, p=0.017), 3 month (OR=1.27, 95%CI: 1.07-1.51, p=0.006), 6 month (OR=1.34, 95%CI: 1.07-1.67, p=0.011) and 12 month (OR=1.36, 95%CI: 1.01-1.84, p=0.044).

A portion of the patients continued to receive ADT shortly after surgery (Supplementary Table 2). To further investigate the potential role of postoperative hormonal therapy on continence recovery, subgroup analysis was carried out. Results showed that postoperative ADT delayed continence recovery in PCa patients previously treated with ADT+Docetaxel (p=0.005) while not in patients previously treated with ADT alone (p=0.232) or ADT+Abiraterone (p=0.805) (Supplementary Figure 1).

4 Discussion

The results of the study showed that 9.1% and 93.9% of patients restored continence immediately and 1 year after removal of catheter. No difference was found in postoperative continence recovery among ADT, ADT+Docetaxel and ADT+Abiraterone preoperative treatment options. More advanced T stage increased the risk of immediate incontinence and longer membranous urethral length (MUL) promoted continence at 1, 3, 6 and 12 month.

Radical prostatectomy leads to anatomical impairment to urethral sphincter complex, its surrounding tissue and innervation, which cause incontinence (20, 21). In addition, extensive dissection, neurovascular bundle (NVB) damage and postoperative fibrosis also impose a negative effect on post-prostatectomy continence recovery (20). We found that preoperative T was the only factor that affect

TABLE 2 Time to urinary continence.

	Absolute Number	Accumulated Number	Absolute Percentage (%)	Accumulated Percentage (%)
Immediate	21	21	9.1	9.1
1 month	69	90	30.0	39.1
3 month	85	175	40.0	76.1
6 month	27	202	11.7	87.8
12 month	14	216	6.1	93.9
>12month	14	230	6.1	100

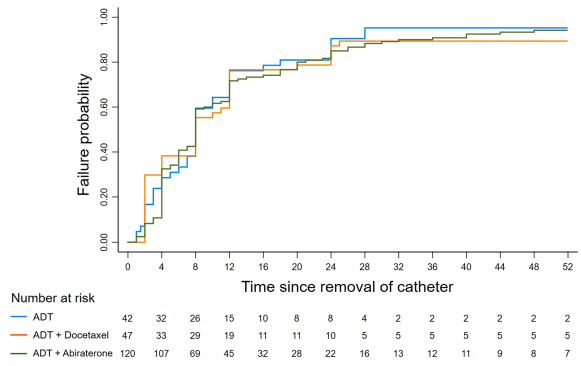


FIGURE 2
Kaplan-Meier failure graph of continence recovery. The Kaplan-Meier failure graph demonstrates continence results under different preoperative treatment conditions. No difference was found among the ADT, ADT + Docetaxel and ADT + Abiraterone groups (Log rank p=0.821).

immediate continence. Even after 6-month preoperative hormonal therapy, more than 60% of the participants were deemed to have extraprostatic invasion. The adhesions of adjacent tissues and loss of clear boundaries caused by advanced tumor stage forced the surgeon to extend the extrafascial dissection plane in order to reduce the rate of positive surgical margin (PSM). It's possible that extensive dissection posed a negative impact on continence.

Current evidence exhibited significant advantage of RS-RARP over C-RARP in terms of immediate continence recovery while not in long-term continence recovery (22–24). However, most of these researches have explicitly ruled out patients previously treated with preoperative hormonal treatment. Our results showed that surgical approaches (RS-RARP or C-RARP) did not affect immediate, short-time, median-time or long-time continence recovery. Though the matter of continence is influenced by patients' preoperative characteristics, surgeon experience, surgical techniques and methodological aspects such as continence definitions, tools used for data collection, and different follow-up intervals (7), it might be reasonable to suggest that RS-RARP is not superior as expected in this specific setting. One possible explanation is that the significant shrinkage of tumor volume after neoadjuvant therapy leads to the increase in maximum urethra length permissible to be retained. The increase in functional urethra length might eliminate the impact caused by different surgery approaches (RS-RARP or C-RARP), which is consistent with our conclusion that MUL is the most important factor influencing postoperative continence for patients with preoperative hormonal therapy. The RS-RARP would be less preferable if taking cancer control into account, as it was reported by several studies to increase the risk of PSM (22, 23).

Preoperative membranous urethral length (MUL) was shown to be a crucial factor influencing continence recovery, which is consistent with former researches (25, 26). The combined and coordinated function of smooth muscle fibers and the surrounding rhabdosphincter, which are two main components of membranous urethra contributes to maintaining the urethral closure pressure (27, 28). The longer MUL provide better urethral pressure profile, thus prompting continence recovery.

Though our research demonstrated that different preoperative treatment options did not make a difference on continence recovery, it did show that sustained postoperative hormonal therapy might impair continence recovery. The impact of occurent or previous hormonal therapy on postoperative continence still needs to be further discussed.

There are several limitations of this study. Firstly, this is a single center, single surgeon retrospective study. The learning curve and

TABLE 3 Cox regression model for urinary continence.

Factor	Univariate		Multivariate	
	OR (95% CI)	p	OR (95% CI)	p
Age	0.99 (0.97-1.01)	0.240	–	–
BMI	1.00 (0.95-1.06)	0.963	–	–
Initial T	0.88 (0.74-1.06)	0.184	–	–
Apex invasion	1.03 (0.78-1.36)	0.820	–	–
Preoperative PSA	0.99 (0.95-1.03)	0.632	–	–
Preoperative volume	1.00 (0.98-1.02)	0.844	–	–
Membranous urethral length	1.13 (1.04-1.22)	0.002**	1.12 (1.04-1.20)	0.002**
Preoperative T	1.05 (0.87-1.27)	0.590	–	–
Preoperative therapy				–
ADT + Docetaxel vs ADT	0.81 (0.52-1.26)	0.352	–	–
ADT + Abiraterone vs ADT	0.95 (0.66-1.38)	0.794	–	–
Surgery approach	1.04 (0.78-1.39)	0.781	–	–
Post-surgery ADT	0.79 (0.55-1.15)	0.781	0.76 (0.54-1.06)	0.109

**p < 0.01.
BMI, Body mass index; PSA, Prostate specific antigen; ADT, Androgen deprivation therapy; OR, Odds ratio; CI, Confidence Interval.

retrospective nature might undermine the general implication of the study. Secondly, the varying and complicated patients' characteristics might cause potential bias. Despite the limitations, this study provided evidence of the impact of different preoperative pharmacotherapy on postoperative urinary continence, which was scarcely ever mentioned in existing research.

5 Conclusion

In the study, 93.9% participants recovered to urinary continence within 12 months after surgery. There is no difference in postoperative continence recovery among ADT, ADT+Docetaxel and ADT+Abiraterone preoperative treatment options. More advanced T stage indicated poor immediate continence recovery. Longer membranous urethral length was a promotional factor for short-time and long-time continence recovery.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Medical Ethics Committee of Nanjing Drum Tower Hospital, China. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements. Written informed consent was not obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

JZ and YW designed and planned the study protocol. HG and LX supervised the work. YW, SZ and XL collected patients' clinical data while YF provided pathology reports. YW and HH performed the analysis, drafted the manuscript and designed the figures. JZ and XQ aided in interpreting the results and worked on the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was funded by the National Natural Science Foundation of China (81974394 to JZ, 82172639 to XQ, and 81972388 to HG), Natural Science Foundation of Jiangsu Province for Excellent Young Scholars (BK20200051 to JZ), Nanjing Medical Science and technique Development Foundation (GRX17127 to JZ), the Project of Invigorating Health Care through Science, Technology, and Education, Jiangsu Provincial Key Medical Discipline (Laboratory) (ZDXKB2016014 to HG).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.1059410/full#supplementary-material

References

1. Hoffman KE, Penson DF, Zhao Z, Huang LC, Conwill R, Laviana AA, et al. Patient-reported outcomes through 5 years for active surveillance, surgery, brachytherapy, or external beam radiation with or without androgen deprivation therapy for localized prostate cancer. *Jama* (2020) 323(2):149–63. doi: 10.1001/jama.2019.20675

2. Nyarangi-Dix JN, Görtz M, Gradinarov G, Hofer L, Schütz V, Gasch C, et al. Retzius-sparing robot-assisted laparoscopic radical prostatectomy: functional and early oncologic results in aggressive and locally advanced prostate cancer. *BMC Urol* (2019) 19 (1):113. doi: 10.1186/s12894-019-0550-9

3. Yanagida T, Koguchi T, Hata J, Yabe M, Sato Y, Akaihata H, et al. Current techniques to improve outcomes for early return of urinary continence following robot-assisted radical prostatectomy. *Fukushima J Med Sci* (2014) 60(1):1–13. doi: 10.5387/fms.2013-25

4. Abdul Rouf M, Kumar V, Agarwal A, Sharma M, Rawat SK, Taneja R. Effect of a novel technique of posterior reconstruction of pubourethralis on 'early' return of continence after robot assisted radical prostatectomy (RARP): A comparative study. *Urologia* (2021) 88(4):315–20. doi: 10.1177/0391560321100752

5. Hashimoto T, Yoshioka K, Gondo T, Hasama K, Hirasawa Y, Nakashima J, et al. The impact of lateral bladder neck preservation on urinary continence recovery after robot-assisted radical prostatectomy. *J Endourol* (2018) 32(1):40–5. doi: 10.1089/end.2017.0459

6. Sridhar AN, Abozaid M, Rajan P, Sooriakumaran P, Shaw G, Nathan S, et al. Surgical techniques to optimize early urinary continence recovery post robot assisted radical prostatectomy for prostate cancer. *Curr Urol Rep* (2017) 18(9):71. doi: 10.1007/s11934-017-0717-4

7. Ficarra V, Novara G, Rosen RC, Artibani W, Carroll PR, Costello A, et al. Systematic review and meta-analysis of studies reporting urinary continence recovery after robot-assisted radical prostatectomy. *Eur Urol* (2012) 62(3):405–17. doi: 10.1016/j.eururo.2012.05.045

8. Pagliarulo V, Bracarda S, Eisenberger MA, Mottet N, Schröder FH, Sternberg CN, et al. Contemporary role of androgen deprivation therapy for prostate cancer. *Eur Urol* (2012) 61(1):11–25. doi: 10.1016/j.eururo.2011.08.026

9. James ND, de Bono JS, Spears MR, Clarke NW, Mason MD, Dearnaley DP, et al. Abiraterone for prostate cancer not previously treated with hormone therapy. *New Engl J Med* (2017) 377(4):338–51. doi: 10.1056/NEJMoa1702900

10. Fizazi K, Tran N, Fein L, Matsubara N, Rodriguez-Antolin A, Alekseev BY, et al. Abiraterone plus prednisone in metastatic, castration-sensitive prostate cancer. *New Engl J Med* (2017) 377(4):352–60. doi: 10.1056/NEJMoa1704174

11. James ND, Sydes MR, Clarke NW, Mason MD, Dearnaley DP, Spears MR, 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 (London England)* (2016) 387(10024):1163–77. doi: 10.1016/S0140-6736(15)01037-5

12. Veeratterapillay R, Goonewardene SS, Barclay J, Persad R, Bach C. Radical prostatectomy for locally advanced and metastatic prostate cancer. *Ann R Coll Surgeons Engl* (2017) 99(4):259–64. doi: 10.1308/rcsann.2017.0031

13. UNLoMC Tg. *Impact of radical prostatectomy as primary treatment in patients with prostate cancer with limited bone metastases (g-RAMPP): US national library of medicine clinical trials.gov* (2015). Available at: https://clinicaltrials.gov/ct2/show/NCT02454543.

14. ISRCTN58401737 rI. *Local cytoreductive treatments for men with newly diagnosed metastatic prostate cancer in addition to standard of care treatment: ISRCTN registry* (2018). Available at: https://www.isrctn.com/ISRCTN58401737.

15. UNLoMC Tg. *Testing radical prostatectomy in Chinese men with prostate cancer and oligoMetastases to the bone: US national library of medicine clinical trials.gov; 2019*. Available at: https://www.clinicaltrials.gov/ct2/show/NCT03988686

16. Gay HA, Michalski JM, Hamstra DA, Wei JT, Dunn RL, Klein EA, et al. Neoadjuvant androgen deprivation therapy leads to immediate impairment of vitality/hormonal and sexual quality of life: results of a multicenter prospective study. *Urology* (2013) 82(6):1363–8. doi: 10.1016/j.urology.2013.06.062

17. Huang J, Wang Y, An Y, Liao Y, Qiu M. Impact of diabetes mellitus on urinary continence recovery after radical prostatectomy: a systematic review and meta-analysis. *Urol J* (2020) 18(2):136–43. doi: 10.22037/uj.v16i7.5750

18. Lee Z, Lee M, Keehn AY, Asghar AM, Strauss DM, Eun DD. Intermediate-term urinary function and complication outcomes after robot-assisted simple prostatectomy. *Urology* (2020) 141:89–94. doi: 10.1016/j.urology.2020.04.055

19. Nyarangi-Dix JN, Tosev G, Damgov I, Reimold P, Aksoy C, Hatiboglu G, et al. Recovery of pad-free continence in elderly men does not differ from younger men undergoing robot-assisted radical prostatectomy for aggressive prostate cancer. *World J Urol* (2020) 38(2):351–60. doi: 10.1007/s00345-019-02797-5

20. Heesakkers J, Farag F, Bauer RM, Sandhu J, De Ridder D, Stenzl A. Pathophysiology and contributing factors in postprostatectomy incontinence: A review. *Eur Urol* (2017) 71(6):936–44. doi: 10.1016/j.eururo.2016.09.031

21. Walz J, Epstein JI, Ganzer R, Graefen M, Guazzoni G, Kaouk J, et al. A critical analysis of the current knowledge of surgical anatomy of the prostate related to optimisation of cancer control and preservation of continence and erection in candidates for radical prostatectomy: An update. *Eur Urol* (2016) 70(2):301–11. doi: 10.1016/j.eururo.2016.01.026

22. Barakat B, Othman H, Gauger U, Wolff I, Hadaschik B, Rehme C. Retzius sparing radical prostatectomy versus robot-assisted radical prostatectomy: Which technique is more beneficial for prostate cancer patients (MASTER study)? a systematic review and meta-analysis. *Eur Urol Focus* (2021) 8(4):1060–71. doi: 10.1016/j.euf.2021.08.003

23. Tai TE, Wu CC, Kang YN, Wu JC. Effects of retzius sparing on robot-assisted laparoscopic prostatectomy: a systematic review with meta-analysis. *Surg endosc* (2020) 34(9):4020–9. doi: 10.1007/s00464-019-07190-2

24. Yee CH, Liu AQ, Chiu PKF, Teoh JYC, Hou SSM, Ng CF. A propensity score-matching study on retzius-sparing robotic-assisted radical prostatectomy: Evidence of continence advantage in the early learning curve. *Asian J Surg* (2021) 45(7):1403–7. doi: 10.1016/j.asjsur.2021.09.013

25. Mungovan SF, Sandhu JS, Akin O, Smart NA, Graham PL, Patel MI. Preoperative membranous urethral length measurement and continence recovery following radical prostatectomy: A systematic review and meta-analysis. *Eur Urol* (2017) 71(3):368–78. doi: 10.1016/j.eururo.2016.06.023

26. Lin D, O'Callaghan M, David R, Fuller A, Wells R, Sutherland P, et al. Does urethral length affect continence outcomes following robot assisted laparoscopic radical prostatectomy (RALP)? *BMC Urol* (2020) 20(1):8. doi: 10.1186/s12894-020-0578-x

27. Strasser H, Pinggera GM, Gozzi C, Horninger W, Mitterberger M, Frauscher F, et al. Three-dimensional transrectal ultrasound of the male urethral rhabdosphincter. *World J Urol* (2004) 22(5):335–8. doi: 10.1007/s00345-004-0416-x

28. Strasser H, Ninkovic M, Hess M, Bartsch G, Stenzl A. Anatomic and functional studies of the male and female urethral sphincter. *World J Urol* (2000) 18(5):324–9. doi: 10.1007/s003450000145

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 28 February 2023
DOI 10.3389/fonc.2023.1126752

OPEN ACCESS

EDITED BY
Ran Xu,
Second Xiangya Hospital, Central South University, China

REVIEWED BY
Di Gu,
First Affiliated Hospital of Guangzhou Medical University, China
Haolong Li,
University of California, San Francisco, United States

*CORRESPONDENCE
Jun Chen
chenjunxinxiang@163.com

†These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION
This article was submitted to Genitourinary Oncology, a section of the journal Frontiers in Oncology

RECEIVED 18 December 2022
ACCEPTED 13 February 2023
PUBLISHED 28 February 2023

CITATION
Liang H, Liu Y, Guo J, Dou M, Zhang X, Hu L and Chen J (2023) Progression in immunotherapy for advanced prostate cancer.
Front. Oncol. 13:1126752.
doi: 10.3389/fonc.2023.1126752

Progression in immunotherapy for advanced prostate cancer

Hao Liang[1†], Yang Liu[1,2†], Jiao Guo[3], Maoyang Dou[1], Xiaoyi Zhang[1], Liyong Hu[1] and Jun Chen*

[1]Department of Urology, Qilu Hospital of Shandong University, Jinan, Shandong, China, [2]Department of Urology, Weifang People's Hospital, Weifang Medical University, Weifang, Shandong, China, [3]Department of Immunology, School of Basic Medical sciences, Weifang Medical University, Weifang, Shandong, China

Prostate cancer is one of the most common malignant cancers of the male genitourinary system and has high morbidity and mortality. Currently, treatment modalities for localized prostate cancer focus mainly on radical prostatectomy or radical radiation therapy. Some patients still experience disease recurrence or progression after these treatments, while others are already at an advanced stage or have metastases at the time of diagnosis. With the continuous development and progress of medicine in recent years, immunotherapy has become a revolutionary cancer treatment, and has achieved remarkable accomplishments in the treatment of hematologic malignancies. A variety of immunotherapies have also appeared in the field of advanced prostate cancer treatment, including therapeutic vaccines and immune checkpoint therapies. Despite the discrepancy between the results of some immunotherapy studies, immunotherapy for prostate cancer has shown some initial success, especially in combination immunotherapies. Currently, immunotherapy is mainly used in advanced prostate cancer, especially in patients with metastatic castration-resistant prostate cancer. However, with the development of more clinical trials of immunotherapy, more evidence will be provided supporting the rational application of immunotherapy in the future.

KEYWORDS

prostate cancer, immunotherapy, vaccine therapy, targeted therapy, combination therapy

1 Introduction

Prostate cancer is one of the most common cancers among men worldwide, with an incidence of 1.4 million new cases per year. Approximately ten million men currently have prostate cancer worldwide, of which about 700,000 have metastasis, causing about 400,000 deaths each year (1, 2).

Current guidelines recommend radical prostatectomy or radiation therapy for early-stage localized prostate cancer (3–6). Some patients still experience disease recurrence or progression after treatment (7). For hormone-sensitive prostate cancer that responds to endocrine therapy, androgen-deprivation therapy (ADT) is typically maintained. Patients

have a high response rate when they are initially treated with ADT, but long-term ADT leads to drug resistance. Androgen receptor (AR) amplification, AR mutation, AR splice variation, and the emergence of compensatory pathways are possible resistance mechanisms. Studies have shown that within 1–3 years of ADT, most patients experience progression of the cancer to metastatic castration-resistant prostate cancer (mCRPC), which defines patients who are at an advanced stage of the disease (8). The 5-year survival rate for patients with mCRPC is approximately 30% (9). Compared with conventional examinations such as CT and bone scan, the new PSMA-PET/CT and FDG-PET/CT have higher sensitivity for metastases, especially in patients with lower PSA levels (10–12). The advancement of imaging technology has further increased the number of mCRPC patients. Currently, a variety of drugs have been approved for the treatment of patients with mCRPC, such as the new generation of AR signaling inhibitors, chemotherapy drugs, bone-targeted therapy drugs, and poly-ADP-ribose polymerase (PARP) inhibitors (13). However, mCRPC remains an incurable fatal disease. In recent years, many new drugs have been approved for the treatment of hormone-sensitive prostate cancer (HSPC), and reports of cross-drug resistance in mCRPC patients have attracted wide attention (14), prompting us to explore a new, safer, and more effective cancer treatment. Immunotherapy for malignancies has achieved exciting results and a series of exploratory studies on immunotherapy for prostate cancer have been conducted. Immunotherapy enhances the immune system's ability to recognize and kill cancer cells by regulating the autoimmune system, improving the antigen presentation ability, destroying the inhibitory tumor microenvironment, and reducing the apoptosis of effector cells to achieve the purpose of anti-tumor therapy (15–17). Prostate cancer has unique tumor characteristics compared to other tumors. First, it expresses multiple tumor-associated antigens: e.g., prostate-specific antigen (PSA), prostate-specific membrane antigen (PSMA), and prostate stem cell antigen (PSCA), which provide a reliable therapeutic target for prostate cancer immunotherapy (18–20). Second, the relatively "inert" tumor growth characteristics of prostate cancer also provide an extended window for cancer immunotherapy to establish an effective immune response. However, prostate cancer is a "cold" tumor that lacks immune cell infiltration (21). The low number of lymphocytes and the predominance of immunosuppressive components in the tumor microenvironment may limit the efficacy of immunotherapy (22, 23).

We review current research advances, clinical applications, and the risks and challenges related to prostate cancer immunotherapy. Most of these studies have been conducted in patients with advanced prostate cancer represented by mCRPC, so this will help us to understand some of the latest progress in the field of immunotherapy for advanced prostate cancer.

2 Therapeutic vaccine

There are various types of vaccines for prostate cancer treatment currently available, including cellular vaccines, viral vaccines, DNA vaccines, and other classifications, and in this section, we will present several representative vaccines.

Sipuleucel-T: first introduced in April 2010 and was the first therapeutic cancer vaccine approved by the Food and Drug Administration (FDA), primarily for use in asymptomatic or minimally symptomatic patients with mCRPC (24). The vaccine utilizes leukocyte isolation technology to isolate monocytes from the peripheral blood of the patient, which are cocultured *in vitro* with a recombinant fusion protein (PA2024) of prostatic acid phosphatase (PAP) and colony-stimulating factor (GM-CSF). granulocyte-macrophage. PA2024 stimulates the maturation of monocytes into dendritic cells that specifically present PAP. Dendritic cells activate PAP-specific cytotoxic T cells in patients after transfusion, enhancing their ability to recognize and kill prostate tumor cells. The results of the IMPACT phase III clinical trial (NCT00065442) demonstrated a survival benefit of Sipuleucel-T (25), compared to the placebo group, it prolonged the median overall survival (OS) of mCRPC patients by 4.1 months (median OS: 25.8 vs 21.7 months) and reduced the risk of death by 22%, hazard ratio (HR): 0.78, 95% CI: 0.61–0.98. This is consistent with another study showing an OS benefit of 4.5 months with Sipuleucel-T (26). However, there was no improvement in the time to disease progression. The study observed a more significant benefit in patients with low tumor load, suggesting a more significant OS benefit with early use of Sipuleucel-T in mCRPC patients. Similarly, an inverse association between PSA level and OS benefit was also seen in PROCEED study (27). Sipuleucel-T also exhibits a satisfactory safety profile, with studies reporting common adverse events (AEs) such as chills, fever, headache, muscle pain, and flu-like symptoms, which were associated with cytokine release after infusion. 65.2% of AEs were G1-G2, and most symptoms lasted no more than 2 days. Only 0.9% of the patients did not complete the infusion because of infusion-related adverse reactions (21). Several studies have recently been conducted to explore combination therapy regimens of Sipuleucel-T to analyze the most significant therapeutic benefit of Sipuleucel-T (28). It is still uncertain whether combination therapy can provide more benefit to specific groups, and we will introduce it in the subsequent combination therapy section of the article.

PROSTVAC: PROSTVAC is composed of a heterologous prime-boost regimen using two different live poxviral-based vectors: PROSTVAC-V, a recombinant vaccinia virus, and PROSTVAC-F, a recombinant fowlpox virus. The two vectors contain transgenes for human PSA and three costimulatory molecules (TRICOM: b7.1, LFA-3, ICAM-1). In phase II clinical trials (29), PROSTVAC prolonged median OS by 8.5 months and reduced the risk of death by 44% compared with placebo control, and corrected data expanded the survival benefit (median OS: 26.2 vs 16.3 months) and the survival advantage (HR=0.50) (30). Regarding safety, most AEs reported by PROSTVAC were local injection reactions, with fewer systemic AEs. The phase III PROSPECT trial compared patients treated with PROSTVAC +GMC-CSF, PROSTVAC alone, and placebo to further examine the effects of treatment (31). Contrary to the positive results of the phase II clinical trial, neither of the treatment groups effectively improved OS in the interim analysis, and the alive without events

(AWE) rate was similar in both groups at six months. Events including radiographic progression, pain progression, initiation of chemotherapy for prostate cancer, or death, forced early termination of the trial. Regarding the differences in efficacy shown in the PROSPECT trial, an imbalanced allocation of prognostic-related factors in the phase II trial, may have amplified the benefits of OS in the treatment group; also, the smaller number of patients and possible observer bias may have affected the results. Additionally, including patients with multiple prior life-prolonging treatments in the PROSPECT trial may have influenced the positive outcome. Although the results of the phase 3 trial did not meet expectations, the PROSTVAC combination therapy study is still ongoing, and a study (NCT02933255) is exploring the safety and efficacy of PROSTVAC in combination with Nivolumab, and these combination therapies will provide more evidence on the appropriate use of PROSTVAC in the future (32, 33).

DCVAC/PCa is an active immunotherapy based on the activation of antitumor immunity by autologous dendritic cells. Dendritic cells are isolated from mononuclear cells in the peripheral blood of the patient by leukapheresis and brought into contact with dead human prostate adenocarcinoma cell lines, thus enhancing their antitumor activity. A single-arm phase I/II clinical trial in mCRPC patients confirmed that DCVAC/PCa combined with chemotherapy had a good safety profile. No serious adverse events (SAEs) related to DCVAC/PCa were reported in the study, and the median OS was 19 months, which was significantly improved compared with the predicted value of Halabi and MSKCC nomograms (34). Another study demonstrated that DCVAC/PCa produced durable immune responses and significantly prolonged PSA doubling time (PSADT) in prostate cancer patients with low tumor burden (35). The study also reported that the common AEs of DCVAC/PCa were local injection site reactions, fatigue, influenza like-illness, and mild infections, all of which were G1-G2. However, the VIABLE trial (NCT02111577) reported different results (36), with no significant OS benefit in the DCVAC/PCa combination chemotherapy group compared to the placebo group. No difference was observed in either of the primary efficacy endpoints. The VIABLE trial further provided good safety evidence for DCVAC/PCa, with most treatment-related AEs (TRAEs) associated with chemotherapy rather than DCVAC/PCa. The 119 patients who did not develop DCVAC/PCa were included in the efficacy analysis of the VIABLE trial. However, the shorter OS in this group of patients weakened the DCVAC/PCa treatment effect. Study found a dose-dependent treatment effect, with a subgroup of patients receiving more than ten doses of vaccine showing a propensity to benefit OS. Studies evaluating the efficacy of DCVAC/PCa in prostate cancer are still lacking, and more studies are needed to confirm its potential therapeutic value.

pTVG-HP[MVI-816] is a DNA vaccine that encodes the human PAP cDNA. pTVG-HP[MVI-816] has been previously studied for its favorable safety profile in patients with early PSA recurrent prostate cancer, and enhanced vaccine-induced PAP-specific Th1 cell responses have been observed (37). There are no reports on the efficacy and safety of the pTVG-HP[MVI-816] vaccine alone in large trials in patients with mCRPC. In the phase II clinical trial of non-metastatic hormone-sensitive prostate cancer (nmHSPC) with biochemical recurrence (38), there was no significant difference in 2-year metastasis-free survival (MFS) in the pTVG-HP[MVI-816] group (41.8% vs 42.3% P=0.97). Regarding secondary endpoints, no significant differences were observed between the two groups in median MFS and median PSADT; partial immune responses were observed early in treatment but then disappeared. Difficulty in maintaining long-term immune responses may be the main obstacle limiting the antitumor efficacy of pTVG-HP. There is no substantial evidence to support that a single regimen of MVI-816 may make a meaningful difference for patients, and we are counting on whether a combination regimen can enhance its efficacy. A recently published study comparing the effectiveness of MVI-816 in combination with pembrolizumab in patients with mCRPC reported a preliminary exploration of the optimal dosing regimen for combination therapy. The results showed that the combination therapy was superior to PD-1 or PD-L1 monotherapy in PSA declines, tumor volume decreases, and 6-months DCR, while the combination therapy had a good safety profile. G2 or higher TRAEs occurred in 42% of the patients, and common TRAEs were thyroid dysfunction, adrenal insufficiency, colitis, and hepatitis (39). Another DNA vaccine, pTVG-AR [MVI-118], which contains cDNA encoding the ARligand-binding domain (AR-LBD), has been evaluated in a completed multicenter phase I trial (NCT02411786) and showed a favorable safety profile and durable immune responsiveness (40).

In summary, durable immune responses specific to tumor antigens have been observed in studies of multiple prostate cancer therapeutic vaccines. However, there is still a lack of consistent clinical evidence confirming the therapeutic efficacy of vaccines, except for Sipuleucel-T, for which several recent large trials have provided conflicting results. Vaccine combination therapy appears to have gained more attention in recent years, which may provide new approaches for subsequent treatment and provide a rationale guiding and supporting the exploration and use of prostate cancer vaccine therapy.

3 Immune checkpoint therapy

There are antagonistic mechanisms of promotion and suppression of the immune system in the development of tumors: Conversely, when some activating signals stimulate cytotoxic cells with tumor-killing capacity, they will promote functional phenotype transformation and accelerate cell proliferation, which can enhance their ability to kill cancer cells. Conversely, when cytotoxic cells are stimulated by inhibiting signals from the surrounding environment, they will cause their dysfunction and inhibit their proliferation, thus weakening their ability to kill tumor cells. Cells exhibit negative regulatory function *via* a receptor or ligand called an immune checkpoint, and anti-tumor therapy targeting the regulating of immune checkpoints is called Immune Checkpoint Therapy (ICT) (41, 42). Food And Drug Administration (FDA) has approved ICT for the treatment of solid malignancies in multiple organs (43–45). The main

therapeutic targets of ICT in prostate cancer are the cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), programmed cell death protein 1 (PD-1) and its ligand (PD-L1) (46, 47).

Ipilimumab is a humanized monoclonal antibody that blocks CTLA-4 and enhances the immune effect of T cells. It was approved in 2011 for the treatment of melanoma (48). Some early clinical trials that confirmed the anti-tumor activity of Ipilimumab in solid tumors included patients with prostate cancer. However, the results of a phase III trial in patients with asymptomatic or minimally symptomatic mCRPC without visceral metastases who had not previously been treated with chemotherapy (49), did not show a benefit in OS following treatment with ipilimumab compared to placebo. In a separate phase III study (50), the ipilimumab group did not show any significant OS benefit compared with placebo in a population of minimally symptomatic mCRPC patients who had received prior docetaxel chemotherapy and were chemotherapy-sensitive, despite the presence of long-term responders. However, we found that a small group of patients in this study achieved a significant and sustained clinical response with ipilimumab. A follow-up study found that a subgroup of patients with mCRPC with immune characteristics such as higher intratumor infiltrating CD8+ T cells, high IFN-γ response gene signals, and more robust antigen-specific T cell responses was more likely to achieve control of progression with ipilimumab monotherapy and more extended survival benefits, despite the relatively low tumor mutational load in this subset of patients (51). This finding indicates that a more careful selection of appropriate patients is required for ipilimumab treatment.

PD-1 is expressed in activated T cells and binds to PD-L1 and PD-L2 to mediate inhibition of the activity of variable tumor effector cells (52). In patients with metastatic melanoma, objective response rates (ORR) ranging from 20% to 45% were observed after CTLA-4 or PD-1 (53), with response rates up to 60% observed when CTLA-4 was combined with PD-1 blockade (54, 55). However, similar to ipilimumab monotherapy, studies have found that nivolumab and pembrolizumab alone do not achieve the expected treatment outcomes in patients with advanced prostate cancer (56–58). In the KEYNOTE-199 trial (58), the ORR (5% vs 3%) and disease control rate (DCR) (13% vs 18%) in PD-L1-positive patients were similar to the PD-L1-negative patient group; no differences in OS were observed between the two groups, which may be related to the more advanced stage of the disease in the positive PD-L1 group. It is believed that the immunosuppressive tumor microenvironment, tumor mutation burden and immune escape mechanism of prostate cancer are the reasons that hinder the efficacy of immune checkpoint inhibitors (59). At the same time, the shorter duration of treatment in KEYNOTE-199 could diminish the OS benefit. Previous studies have found that ipilimumab treatment significantly increased the number of tumor-infiltrating T cells in prostate cancer patients. However, it induced a compensatory immunosuppressive pathway mediated by PD-1/PD-L1 signaling, negatively affecting antitumor therapy (60). Based on this finding, the subsequent CheckMate650 Phase II trial (NCT02985957) focused on improving treatment outcomes for patients with prostate cancer when ipilimumab was administered in combination with nivolumab (61). We will describe this study in more detail below with regard to the combination therapy strategies. The European Association of Urology (EAU) guidelines suggest that pembrolizumab may be a valuable additional management strategy for mCRPC patients with high microsatellite instability, and with continuous advances in genome sequencing technology, it will be helpful to screen patients who can benefit from immunosuppressive therapy (62, 63).

The difficulty of producing substantial clinical benefits with ICT alone in unselected patients with prostate cancer has been widely recognized. However, earlier studies have reported that a subgroup of prostate cancer patients with defects in the DNA mismatch repair gene and high microsatellite instability characteristics tended to have higher response rates to single ICT (64, 65). The results of the subgroup analysis also suggested that single immunological checkpoint blockade had better efficacy in these patients. However, this idea has been questioned in recent studies: it has been shown that there is no positive correlation between CD8+ T cell numbers and neoantigen load in breast and prostate cancers and that the characteristics of high tumor mutational load is not predictive of the efficacy of ICT in patients with breast and prostate cancer. Therefore, the search for additional biomarkers as predictors of immune checkpoint efficacy may be required in the future (66, 67). This highlights the importance of tumor signature screening for prostate cancer patients and individualized treatment regimens for prostate cancer in terms of immunotherapy strategies.

4 Adoptive cell therapy

Adoptive cell therapy is a rapidly expanding field of medicine in recent years that mediates antitumor, antiviral, or anti-inflammatory effects by isolating, modifying, and expanding autologous or allogeneic tumor-responsive lymphocytes and reinfusing processed lymphocytes back into the patient (68, 69). Of these, cell therapies involving chimeric antigen receptor T (CAR-T) have demonstrated high response rates and durable disease remission in the treatment of hematologic malignancies (70–72), and several companies have received FDA approval for their CAR-T products for the treatment of refractory and complex hematologic diseases in the last 5 years (73).

CAR-T therapy genetically modifies T cells by *in vitro* transfection technology to express engineered chimeric receptors (74). Currently, CAR-T technology has developed to the fourth generation, as the latest generation technology, the structure not only has a co-stimulatory protein intracellular domain but also promotes the release of cytokines such as IL-12, IL15, and IL18 after receptor activation, which can enhance the killing efficiency of T cells (75). When CAR-T cells are cultured and expanded *in vitro* and transfused back to patients, the transmembrane region converts the CAR recognition signal for extracellular targeted tumor antigens into a signal for activation of T cells through the intracellular domain. When CAR-T cells arrive inside the tumor, they cause cytotoxic particles such as cytokines and perforins to be secreted by cytotoxic T cells, leading to the destruction of tumor cells (76). This technique is being applied to treat solid malignancies, including prostate cancer (77, 78). This is a new T cell-mediated antitumor

therapy in which cytotoxic T cells can be activated independently of major histocompatibility complex (MHC), thus eliminating dependence on the traditional T cell receptor-MHC pathway, can be severely compromised in the "cold" tumor microenvironment of prostate cancer (79).

Narayan et al. reported the results of the latest phase I clinical trial of CAR-T therapy in patients with mCRPC (80): In terms of safety, a cytokine release syndrome (CRS) was the most common drug-related SAEs, predominantly G1-3, and most patients exhibiting a CRS resolved spontaneously or with symptomatic treatment, confirming its good safety profile. In terms of tumor responsiveness, PSA levels decreased by at least 30% in 4 of the 13 patients; one patient had a >98% decrease in PSA levels accompanied by significant proliferation of CAR-T cells *in vivo*, and 38.5% of patients maintained stable disease status at three months posttreatment assessed by imaging. The study showed a median OS of 15.9 months and a median progression free survival (PFS) of 4.4 months in patients receiving CAR-T therapy. Although a general immune response was observed in the study, it does not seem to translate into a survival benefit for mCRPC patients. The study also observed that patients had a dose-dependent decrease in peripheral blood CAR-T cell proliferation, inflammatory cytokine expression, clinical CRS, and PSA, which could help guide the appropriate dose selection for future CAR-T therapies in clinical applications (81). This result is generally consistent with the results of the earlier P-PSMA-101-001 trial (NCT04249947). Currently, there is no consensus on whether to receive lymphatic clearance prior to CAR-T therapy, and studies have shown that lymphatic clearance enhances T cell proliferation and viability, thus improving efficacy but also increasing hematologic and systemic toxicity. Thus, more research is needed to select suitable patients to receive lymphatic clearance to achieve maximum therapeutic benefit.

Although CAR-T therapy has shown good therapeutic potential in the treatment of advanced prostate cancer, several barriers remain to be addressed to enhance CAR-T therapy efficacy in solid tumors: 1) physical interference of CAR-T cells by the stroma surrounding solid tumors; 2) abnormal CAR-T function due to the specific suppressive tumor microenvironment of prostate cancer; and 3) CAR-T cell defects: reduced self-replication ability (82, 83). Multiple studies of CAR-T therapies are ongoing (NCT03873805; NCT02744287). A preclinical study has shown that CAR-T combined with docetaxel has synergistic efficacy (84), and this feasibility needs to be supported by evidence in future clinical trials. With the improvement of the structure of CAR-T cells, as well as the determination of therapeutic dose and treatment cycle, CAR-T may become an alternative treatment for prostate cancer patients.

5 Bispecific antibody therapies

Bispecific antibody therapies, especially bispecific T-cell engagers (BiTE) (85), have shown significant therapeutic promise in the treatment of refractory hematologic malignancies. Recently, BiTE therapies have been explored to treat advanced malignant solid tumors. The studies conducted to date have mainly included patients with mCRPC. BiTEs utilize single chain variable fragment (ScFv) technology to recognize specific tumor antigens. Antibodies on one side of the BiTE bind specifically to tumor cell surface tumor antigens, such as PSMA, generating activation signals delivered to the T cell CD3 surface receptors *via* antibodies on the other side (86). Direct engagement of co-stimulatory CD3 receptors bypasses the need for traditional monosynaptic binding and enables MHC non-dependent T cell activation. In recent preclinical studies (87), AMG160 showed promising durable specific antitumor activity and an acceptable nonclinical safety profile in a model of prostate cancer tumor graft. There is a lack of solid evidence supporting the clinical application of BiTE in large trials, with safety and efficacy results of BiTE in patients with prostate cancer reported only in phase I clinical trials (88). In terms of imaging, there is evidence that AMG160 does not interfere with the signal intensity of 68Ga-PSMA-11PET/CT compared to non-PSMA specific BiTE, which has important implications for the post-treatment efficacy assessment (89). BiTE is more readily available for widespread use than CAR-T therapy because it is not a separately produced cellular product. In terms of tumor penetration capacity, BiTE therapy is superior to CAR-T, and in terms of safety, the incidence of BiTE adverse events is lower and relatively controllable. However, there are still many challenges for BiTE therapy, such as loss of target antigen, formation of resistant antibodies, and up-regulation of immune checkpoints (90, 91). The up-regulation of immune checkpoint is a possible resistance mechanism of BiTE, which provides theoretical support for the combination of BiTE therapy and ICI (92). In addition to targeting PSMA, exploring other alternative tumor antigens, such as PSCA, disintegrin and metalloproteinase 17 (ADAM17M) and delta-like ligand 3 (DDL3), may also be a future direction (93–95).

6 Combination therapy

Current evidence shows that treatment with single immunotherapy regimens appear have not achieved the expected therapeutic effects. With the increasing understanding of the regulatory mechanisms of immunotherapy in various preclinical studies, immunotherapy-based combination therapy strategies are gradually becoming and increasing trend. Current combination treatment options include the combination of multiple immunotherapy regimens, immunotherapy combined with hormone therapy, immunotherapy combined with radiation therapy, and immunotherapy combined with chemotherapy.

6.1 Immune dual combination therapy

Uncertainty about the efficacy of immune checkpoint therapy monotherapy regimens facilitated the exploration of combination regimens, and the establishment of the CheckMate650 phase II trial (NCT02985957) (61). The no-chemotherapy cohort and the post-chemotherapy cohort received ipilimumab (3 mg/kg) in combination with nivolumab (1 mg/kg) with a median follow-up of 11.9 months and 13.5 months, respectively, ORR of 25% and 10%

in the two groups, and median OS of 19.0 months and 15.2 months, respectively. Although combination therapy demonstrated significant treatment effects, the study reported a significantly increased incidence of TRAEs, with approximately 40% of patients in both groups requiring the application of high-dose cortisol for immune-mediated AEs. Approximately half of the patients exhibited G3-G4-grade TRAEs and 4 patients experienced TRAE-related deaths. Higher drug-related mortality in patients with mCRPC compared to the same dose regimen previously applied in patients with metastatic melanoma may be associated with advanced age and worse ECOG scores. The significant treatment effect observed in the CheckMate650 trial revealed the therapeutic promise of dual immunosuppressant combinations, and a concomitant increase in drug toxicity needs to be investigated in future studies. We must explore different dosing strategies to find the balance between efficacy and toxicity. The other study investigated the combination of Sipuleucel-T and Ipilimumab in patients with mCRPC, and the combination did not achieve greater efficacy than Ipilimumab monotherapy, which is similar to the results of the previous study (96, 97). It was found that the timing of Ipilimumab administration after Sipuleucel-T vaccination may affect the activation of antigen-specific T cells, but no differences in patient survival benefit and disease progression were observed. Similarly, no additional benefit was shown with Sipuleucel-T plus pTVG-HP as an immune-boosting regimen (98).

6.2 Immunotherapy combined with hormone therapy

Hormone therapy plays an important role in the treatment strategy of prostate cancer patients, and the new generation of AR pathway inhibitors provide more options to treat patients with advanced prostate cancer (99, 100). In recent years, as the mechanisms of androgen activity in prostate cancer have been studied, we have gained a richer understanding of the immune regulatory mechanisms played by androgens and AR in patients with prostate cancer. AR is expressed not only in tumor cells but also in various immune cells *in vivo*, playing an immunomodulatory role (101, 102). Androgens have long been known to inhibit the development and activation of T and B cells through multiple mechanisms (103). In patients with prostate cancer, immunotherapy combined with hormone therapy has emerged as a new combination therapy. However, the efficacy of immunotherapy combined with hormone therapy in patients with prostate cancer has produced uncertain findings. Conflicting treatment outcomes are usually attributed to differences in patient populations. Recent studies have found that AR antagonists interfere with initial T cell activation and may diminish the therapeutic effect of combination therapy (104). However, this immunosuppressive effect can be avoided by judicious selection of the sequential dose timing (105, 106). A recent study published in Nature revealed a potential mechanism by which AR antagonists in combination with anti-PD-1 monoclonal antibodies in clinical trials led to high patient responsiveness (105). The study reported that enzalutamide prevented T cell depletion by inhibiting AR in CD8+ T cells while increasing IFN-γ release and improving responsiveness to targeted PD-1 therapy, which provided a theoretical basis for the administration of hormone therapy in combination with immune checkpoint inhibitors. The IMbassador250 trial (NCT03016312) investigated the impact of co-administration of atezolizumab with enzalutamide compared to enzalutamide alone on the survival benefit of patients with mCRPC (107). Although the incidence of AEs in the combination group was essentially identical to enzalutamide alone, the combination group (median OS: 15.2 months) did not show a survival benefit compared to enzalutamide alone (median OS: 16.6 months) (HR=1.12 95% CI 0.91–1.37), forcing the early termination of the study. In terms of secondary outcomes, the combination group similarly did not show any benefit. It is difficult to provide a plausible explanation for the IMbassador250 trial results. However, previous single-arm studies have found that pembrolizumab combined with enzalutamide produced an 18% response rate in unselected mCRPC. In the latest Nature study, the ADT+enzalutamide+anti-PD-L1 triplet regimen provided a superior OS benefit and the most significant reduction in tumor volume in prostate cancer and sarcoma models compared to the duplex regimen, and it appears that ADT enhanced the synergistic effect of enzalutamide in combination with immunotherapy. A clinical trial of triple combination therapy ADT + enzalutamide + pembrolizumab (NCT04191096) is underway in patients with mHSPC, which will further validate the safety and patient responsiveness of the triple combination regimen. We cannot help but look forward to the therapeutic potential of the triple combination regimen for advanced prostate cancer.

6.3 Immunotherapy combined with radiation therapy

Radiation therapy has been one of the practical tools for the treatment of various malignant tumors, and it can stimulate the production of tumor-specific immune responses by inducing tumor cell death, enhancing the release of tumor-associated antigens, and upregulating the expression of tumor suppressor proteins and cytokines through various pathways and mechanisms (107, 108). In recent years, targeted radiotherapy, represented by Ra-223, has gradually gained clinical popularity as an emerging therapeutic tool for patients with advanced prostate cancer, especially for those with combined bone metastases (109). Ra-223 is a radioactive calcium analogue that selectively binds to areas of increased bone transformation and has a certain degree of “bone targeting”. It produces antitumor effects by releasing alpha particles into surrounding tissues to destroy cellular DNA. Due to the small diameter of the action of the alpha particle (2–10 cell diameters), Ra-233 causes less damage to surrounding normal tissues, giving it a better safety profile (110). In 2013, Ra-223 was approved by the FDA for the treatment of patients with symptomatic mCRPC with bone metastases (111). The survival benefit and the improvement of bone-related events in mCRPC patients have been supported by the results of several large clinical trials, in which Ra-223 significantly prolonged OS and PFS in patients with advanced prostate cancer,

reduced bone pain symptoms considerably, and delayed the onset of bone-related events during treatment (112, 113). Several trials have recently explored the feasibility of a combination immunotherapy regimen with Ra-223. A recently completed trial of Sipuleucel-T in combination with Ra-223 demonstrated superiority to Sipuleucel-T administered alone in patients with mCRPC with bone metastases (114). The study found that Sipuleucel-T in combination with Ra-223 did not increase the incidence or severity of adverse events; however, the combination group did not demonstrate an advantage in secondary outcome indicators related to the immune response. The combination group showed higher PSA responsiveness (33% vs 0%) and longer time to tumor progression in the observation of patient clinical outcomes (median PFS 39w vs 12w; HR=0.32), which was consistent with the findings obtained in previous studies (115), in which Sipuleucel-T combined with Ra-223 produced a more significant benefit in patients with mCRPC without additional toxicity. However, not all combination therapies with Ra-223 produced exciting results; for example, trials exploring the administration of atezolizumab in combination with Ra-223 did not produce any additional therapeutic benefits in the combination group but instead resulted in more significant drug toxicity in the combination group compared to monotherapy, Thus, combination therapy with Ra-223 needs to be further studied (116).

Another radiopharmaceutical with great therapeutic potential, 177Lu-PSMA-617, specifically identifies tumor cells with high expression of PSMA and releases β-particles to destroy tumor cells, was recently evaluated in the just concluded VISION trial (117). Significant benefits in radiology progression-free survival (rPFS) (8.7 vs 3.4 months, HR=0.40) and OS (15.3 vs 11.3 months, HR=0.62) have been reported, and significant improvements were also observed in all secondary endpoints of the study. Because 177Lu-PSMA-617 also has a favorable safety profile, it has been described as a revolutionary precision radiotherapy modality for the treatment of mCRPC. The PSA response rate was superior to that reported for cabazitaxel and docetaxel in other studies (118, 119). The efficacy and safety of 177Lu-PSMA-617 combined with immunotherapy or other drugs have been explored in patients with mCRPC. Considering the low possibility of overlap in toxicity of radiotherapy combined with other therapeutic agents, 177Lu-PSMA-617 combination therapy may provide a relatively safe treatment option for patients with mCRPC.

6.4 Immunotherapy combined with chemotherapy

Chemotherapy, a conventional treatment for cancer patients, is widely used in the treatment of various malignant diseases. Docetaxel and cabazitaxel have been successively approved for the treatment of patients with mCRPC and have been shown to prolong patient survival and control disease progression. Some studies have shown that chemotherapy-induced tumor cell destruction may enhance the development of specific immune responses (120). A study evaluating pembrolizumab in combination with docetaxel for mCRPC reported the efficacy of combination therapy in patients with mCRPC previously treated with enzalutamide or abiraterone. The PSA response rate was 27% and ORR and DCR were 23% and 52%, respectively. The median OS of combination therapy was 20.2 months, which was significantly prolonged compared to the median OS of 9.5 months in the PD-L1 positive cohort in KEYNOTE-199. KEYNOTE-365 showed initial success with PD-L1 antibodies in combination with chemotherapy. However, the patients included in the study were previously chemotherapy naïve patients. All KEYNOTE-199 patients were treated with docetaxel chemotherapy prior to treatment with PD-L1 antibodies. Nonetheless, we cannot define the specific impact of chemotherapy on the benefit of combination therapy. The ongoing KEYNOTE-921 trial includes patients with mCRPC after chemotherapy and will provide further evidence to support this combination strategy (121).

6.5 Immunotherapy combined with PARP inhibitors

PARP inhibitors have recently become one of the most popular drugs in the mCRPC therapeutic area, and is represented by olaparib and rucaparib. PARP plays an important role in DNA damage repair *in vivo*, and PARP1 and PARP2 mediate DNA damage repair through base excision. The restoration of single-stranded DNA (ssDNA) damage can be blocked by PARP inhibitors. Homologous recombination repair proteins can compensate for the above by repairing broken double-stranded DNA. However, under the homologous recombination repair gene defect (HRD), this compensatory pathway is blocked. PARP inhibitors and HRD cause a synthetic lethality of tumor cells, generating tumor neoantigens that increase immunogenicity and improve immune responsiveness in the tumor microenvironment (122). The safety and significant therapeutic effects of olaparib and rucaparib monotherapy in patients with mCRPC have been reported in several studies (123, 124). In the TOPARP-A trial, the ORR to treatment was 32%, with a response rate of 88% in patients with DNA repair gene mutations, and other studies have confirmed that patients with genetic defects such as BRCA1, BRCA2, ATM, FANC, and CHEK2 have higher sensitivity to PARP inhibitors (125). PARP inhibitors have been shown to have synergistic effects with PD-1/PD-L1 or CTLA-4 blockade (126, 127). A recent study of rucaparib in combination with nivolumab for mCRPC reported its results: regardless of previous chemotherapy (128), the CheckMate 9KD study showed significantly improved ORR and PSA response rates with combination therapy in the HRD+ cohort, particularly in patients with BRAC1/2 mutations; in terms of OS and rPFS, the median OS for the A2 cohort without chemotherapy was 20.2 months (95% CI 14.1–22.8 months) and the median rPFS was 8.1 months (95% CI 5.6–10.9 months). Regarding safety, common TRAEs observed following combination therapy were nausea, fatigue, anemia, and loss of appetite, with G3-G4 TRAEs occurring in half of the patients in both cohorts, and neutropenia warranting focus during treatment. In general, rucaparib combined with nivolumab did not appear to show additional benefits in unselected mCRPC patients, which is consistent with the findings of the previous KEYLYNK-010 trial of olaparib combined with

pembrolizumab. It is also encouraging that a significant response to combination therapy was observed in the subgroup of patients with BRAC1 and BRAC2 mutations, but the failure to translate into a survival benefit is difficult to explain and will require further evidence. Many studies have been conducted to screen and identify new, highly effective therapeutic predictive factors for mCRPC patients (129). Continued advances in next-generation exon sequencing technology and liquid biopsy technology will contribute to the precise treatment of mCRPC patients and will provide more significant benefits to patient.

6.6 Immunotherapy combined tyrosine kinase inhibitors (TKIs)

TKIs represented by Cabozantinib and Masitinib belong to the class of small-molecule inhibitors with RARP inhibitors, and both drugs have shown antitumor activity in previous studies. Cabozantinib is a mesenchymal-epithelial transition factor (c-MET) and vascular endothelial factor receptor 2 (VEGFR2) inhibitor that has been approved for the treatment of patients with advanced renal cell carcinoma. In a phase 2 study, cabozantinib significantly prolonged PFS in patients with CRPC (130). However, in the COMET-1 study, mCRPC patients after chemotherapy failed to show an OS benefit (131). Studies have shown that Cabozantinib has immunomodulatory effects and may be synergistic with other immunotherapy combinations (132, 133). The COSMIC 021 trial evaluated the safety and clinical benefit of Cabozantinib in combination with atezolizumab in patients with mCRPC. The study found that the combination regimen had better PSA response rate and DCR than either drug monotherapy. In terms of safety, 95% of patients experienced TRAEs at any grade and 55% of patients experienced G3-G4. The most common G3-G4 AEs were pulmonary embolism, diarrhea, fatigue, and hypertension. The safety profile of cabozantinib combined with atezolizumab was generally consistent with that of the individual agents, but the incidence of pulmonary embolism is higher than that of monotherapy (134). Elderly age and concurrent use of ADT are possible causes of pulmonary embolism. Due to the differences in patient groups included in different studies, it is difficult to compare the specific benefits of combination therapy at present, and future evidence support from other studies is needed.

7 Limitations and future prospects

There are still many obstacles and challenges in prostate cancer immunotherapy, such as the balance between efficacy and toxicity of immunotherapy, the timing of sequential administration, the requirement of individualized dosing regimen for prostate cancer due to tumor heterogeneity, the lack of appropriate biomarkers for efficacy evaluation, and the insufficient understanding of the mechanism of drug resistance. These issues need to be focused on in the future. Due to the extensive differences between the studies of immunotherapy, there is a lack of direct evidence to support the comparison of the treatment effects of different regimens, and more large-scale controlled trials are needed in the future. Immunotherapy of prostate cancer is a promising treatment. With the deepening of research, new tumor-specific antigens have been discovered, which provides more potential targets for immunotherapy. The continuous progress of high-throughput sequencing technology and liquid biopsy technology has promoted the identification of tumor heterogeneity of prostate cancer and promoted the precision treatment of prostate cancer patients. The continuous exploration of drug combination is helpful to the study of drug interaction mechanism. The development of imaging technology represented by PSMA-PET/CT provides a powerful aid in disease diagnosis and efficacy evaluation.

8 Conclusion

Over the past decade, as immunotherapy for solid tumors continues to be explored, our understanding of immunotherapy and immunomodulation of solid tumors, including prostate cancer, has also improved, and a variety of immunotherapeutic agents, including tumor vaccines and immune checkpoint inhibitors, have achieved exciting results in clinical trials for advanced prostate cancer. Although most current trials on immunotherapy for prostate cancer have focused on patients with mCRPC, there is reason to believe that immunotherapy may bring earlier clinical benefits to prostate cancer patients as immunotherapy continues to improve and mature. In this review, we summarize the experience and lessons learnt from recent immunotherapy studies and update the theoretical basis and regulatory mechanisms underlying immunotherapy for prostate cancer, helping to understand the latest progress in immunotherapy for prostate cancer. As more and more clinical trials are conducted, these will provide strong evidence to support and compare the efficacy of immunotherapy for prostate cancer, providing a valuable reference that will allow more patients with prostate cancer to choose their treatment regimen and prolong survival and also improve the quality of patient survival.

Author contributions

HL and YL wrote the first draft of the manuscript. YL, JG, MD, and HL wrote sections of the manuscript. XZ and LH polished the language and searched the literature. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* (2021) 71(3):209–49. doi: 10.3322/caac.21660

2. Foreman KJ, Marquez N, Dolgert A, Fukutaki K, Fullman N, McGaughey M, et al. Forecasting life expectancy, years of life lost, and all-cause and cause-specific mortality for 250 causes of death: reference and alternative scenarios for 2016-40 for 195 countries and territories. *Lancet.* (2018) 392(10159):2052–90. doi: 10.1016/S0140-6736(18)31694-5

3. Mottet N, van den Bergh RCN, Briers E, Van den Broeck T, Cumberbatch MG, De Santis M, et al. EAU-EANM-ESTRO-ESUR-SIOG guidelines on prostate cancer-2020 update. part 1: Screening, diagnosis, and local treatment with curative intent. *Eur Urol* (2021) 79(2):243–62. doi: 10.1016/j.eururo.2020.09.042

4. Schaeffer E, Srinivas S, Antonarakis ES, Armstrong AJ, Bekelman JE, Cheng H, et al. NCCN guidelines insights: Prostate cancer, version 1. *2021. J Natl Compr Canc Netw* (2021) 19(2):134–43. doi: 10.6004/jnccn.2021.0008

5. Eastham JA, Auffenberg GB, Barocas DA, Chou R, Crispino T, Davis JW, et al. Clinically localized prostate cancer: AUA/ASTRO guideline, part II: Principles of active surveillance, principles of surgery, and follow-up. *J Urol.* (2022) 208(1):19–25. doi: 10.1097/JU.0000000000002758

6. Eastham JA, Auffenberg GB, Barocas DA, Chou R, Crispino T, Davis JW, et al. Clinically localized prostate cancer: AUA/ASTRO guideline. part III: Principles of radiation and future directions. *J Urol* (2022) 208(1):26–33. doi: 10.1097/JU.0000000000002759

7. Sandhu S, Moore CM, Chiong E, Beltran H, Bristow RG, Williams SG. Prostate cancer. *Lancet.* (2021) 398(10305):1075–90. doi: 10.1016/S0140-6736(21)00950-8

8. Powers E, Karachaliou GS, Kao C, Harrison MR, Hoimes CJ, George DJ, et al. Novel therapies are changing treatment paradigms in metastatic prostate cancer. *J Hematol Oncol* (2020) 13(1):144. doi: 10.1186/s13045-020-00978-z

9. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. *CA Cancer J Clin* (2021) 71(1):7–33. doi: 10.3322/caac.21654

10. Hofman MS, Lawrentschuk N, Francis RJ, Tang C, Vela I, Thomas P, et al. Prostate-specific membrane antigen PET-CT in patients with high-risk prostate cancer before curative-intent surgery or radiotherapy (proPSMA): a prospective, randomised, multicentre study. *Lancet.* (2020) 395(10231):1208–16. doi: 10.1016/S0140-6736(20)30314-7

11. Wang Y, Galante JR, Haroon A, Wan S, Afaq A, Payne H, et al. The future of PSMA PET and WB MRI as next-generation imaging tools in prostate cancer. *Nat Rev Urol.* (2022) 19(8):475–93. doi: 10.1038/s41585-022-00618-w

12. Shen K, Liu B, Zhou X, Ji Y, Chen L, Wang Q, et al. The evolving role of 18F-FDG PET/CT in diagnosis and prognosis prediction in progressive prostate cancer. *Front Oncol* (2021) 11:683793. doi: 10.3389/fonc.2021.683793

13. Swami U, McFarland TR, Nussenzveig R, Agarwal N. Advanced prostate cancer: Treatment advances and future directions. *Trends Cancer.* (2020) 6(8):702–15. doi: 10.1016/j.trecan.2020.04.010

14. Buck SAJ, Koolen SLW, Mathijssen RHJ, de Wit R, van Soest RJ. Cross-resistance and drug sequence in prostate cancer. *Drug Resist Updat.* (2021) 56:100761. doi: 10.1016/j.drup.2021.100761

15. Cha H-R, Lee JH, Ponnazhagan S. Revisiting immunotherapy: A focus on prostate cancer. *Cancer Res* (2020) 80(8):1615–23. doi: 10.1158/0008-5472.CAN-19-2948

16. Hiam-Galvez KJ, Allen BM, Spitzer MH. Systemic immunity in cancer. *Nat Rev Cancer.* (2021) 21(6):345–59. doi: 10.1038/s41568-021-00347-z

17. Melero I, Castanon E, Alvarez M, Champiat S, Marabelle A. Intratumoural administration and tumour tissue targeting of cancer immunotherapies. *Nat Rev Clin Oncol* (2021) 18(9):558–76. doi: 10.1038/s41571-021-00507-y

18. Gu Z, Thomas G, Yamashiro J, Shintaku IP, Dorey F, Raitano A, et al. Prostate stem cell antigen (PSCA) expression increases with high gleason score, advanced stage and bone metastasis in prostate cancer. *Oncogene.* (2000) 19(10):1288–96. doi: 10.1038/sj.onc.1203426

19. Bostwick DG, Pacelli A, Blute M, Roche P, Murphy GP. Prostate specific membrane antigen expression in prostatic intraepithelial neoplasia and adenocarcinoma: a study of 184 cases. *Cancer.* (1998) 82(11):2256–61. doi: 10.1002/(sici)1097-0142(19980601)82:11<2256::aid-cncr22>3.0.co;2-s

20. Hupe MC, Philippi C, Roth D, Kümpers C, Ribbat-Idel J, Becker F, et al. Expression of prostate-specific membrane antigen (PSMA) on biopsies is an independent risk stratifier of prostate cancer patients at time of initial diagnosis. *Front Oncol* (2018) 8:623. doi: 10.3389/fonc.2018.00623

21. Bou-Dargham MJ, Sha L, Sang Q-XA, Zhang J. Immune landscape of human prostate cancer: immune evasion mechanisms and biomarkers for personalized immunotherapy. *BMC Cancer.* (2020) 20(1):572. doi: 10.1186/s12885-020-07058-y

22. DePeaux K, Delgoffe GM. Metabolic barriers to cancer immunotherapy. *Nat Rev Immunol* (2021) 21(12):785–97. doi: 10.1038/s41577-021-00541-y

23. Briukhovetska D, Dörr J, Endres S, Libby P, Dinarello CA, Kobold S. Interleukins in cancer: from biology to therapy. *Nat Rev Cancer.* (2021) 21(8):481–99. doi: 10.1038/s41568-021-00363-z

24. Higano CS, Small EJ, Schellhammer P, Yasothan U, Gubernick S, Kirkpatrick P, et al. Sipuleucel-T. *Nat Rev Drug Discovery* (2010) 9(7):513–4. doi: 10.1038/nrd3220

25. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N Engl J Med* (2010) 363(5):411–22. doi: 10.1056/NEJMoa1001294

26. Small EJ, Schellhammer PF, Higano CS, Redfern CH, Nemunaitis JJ, Valone FH, et al. Placebo-controlled phase III trial of immunologic therapy with sipuleucel-T (APC8015) in patients with metastatic, asymptomatic hormone refractory prostate cancer. *J Clin Oncol* (2006) 24(19):3089–94. doi: 10.1200/JCO.2005.04.5252

27. Higano CS, Armstrong AJ, Sartor AO, Vogelzang NJ, Kantoff PW, McLeod DG, et al. Real-world outcomes of sipuleucel-T treatment in PROCEED, a prospective registry of men with metastatic castration-resistant prostate cancer. *Cancer.* (2019) 125(23):4172–80. doi: 10.1002/cncr.32445

28. Stein WD, Gulley JL, Schlom J, Madan RA, Dahut W, Figg WD, et al. Tumor regression and growth rates determined in five intramural NCI prostate cancer trials: the growth rate constant as an indicator of therapeutic efficacy. *Clin Cancer Res* (2011) 17(4):907–17. doi: 10.1158/1078-0432.CCR-10-1762

29. Kantoff PW, Schuetz TJ, Blumenstein BA, Glode LM, Bilhartz DL, Wyand M, et al. Overall survival analysis of a phase II randomized controlled trial of a poxviral-based PSA-targeted immunotherapy in metastatic castration-resistant prostate cancer. *J Clin Oncol* (2010) 28(7):1099–105. doi: 10.1200/JCO.2009.25.0597

30. Kantoff PW, Gulley JL, Pico-Navarro C. Revised overall survival analysis of a Phase II, Randomized, double-blind, controlled study of PROSTVAC in men with metastatic castration-resistant prostate cancer. *J Clin Oncol* (2017) 35(1):124–5. doi: 10.1200/JCO.2016.69.7748

31. Gulley JL, Borre M, Vogelzang NJ, Ng S, Agarwal N, Parker CC, et al. Phase III trial of PROSTVAC in asymptomatic or minimally symptomatic metastatic castration-resistant prostate cancer. *J Clin Oncol* (2019) 37(13):1051–61. doi: 10.1200/JCO.18.02031

32. Abdul Sater H, Marté JL, Donahue RN, Walter-Rodriguez B, Heery CR, Steinberg SM, et al. Neoadjuvant PROSTVAC prior to radical prostatectomy enhances T-cell infiltration into the tumor immune microenvironment in men with prostate cancer. *J Immunother Cancer* (2020) 8(1):e000655. doi: 10.1136/jitc-2020-000655

33. Madan RA, Karzai F, Donahue RN, Al-Harthy M, Bilusic M, Rosner II, et al. Clinical and immunologic impact of short-course enzalutamide alone and with immunotherapy in non-metastatic castration sensitive prostate cancer. *J Immunother Cancer* (2021) 9(3):e001556. doi: 10.1136/jitc-2020-001556

34. Podrazil M, Horvath R, Becht E, Rozkova D, Bilkova P, Sochorova K, et al. Phase I/II clinical trial of dendritic-cell based immunotherapy (DCVAC/PCa) combined with chemotherapy in patients with metastatic, castration-resistant prostate cancer. *Oncotarget.* (2015) 6(20):18192–205. doi: 10.18632/oncotarget.4145

35. Fucikova J, Podrazil M, Jarolim L, Bilkova P, Hensler M, Becht E, et al. Phase I/II trial of dendritic cell-based active cellular immunotherapy with DCVAC/PCa in patients with rising PSA after primary prostatectomy or salvage radiotherapy for the treatment of prostate cancer. *Cancer Immunol Immunother* (2018) 67(1):89–100. doi: 10.1007/s00262-017-2068-x

36. Vogelzang NJ, Beer TM, Gerritsen W, Oudard S, Wiechno P, Kukielka-Budny B, et al. Efficacy and safety of autologous dendritic cell-based immunotherapy, docetaxel, and prednisone vs placebo in patients with metastatic castration-resistant prostate cancer: The VIABLE phase 3 randomized clinical trial. *JAMA Oncol* (2022) 8(4):546–52. doi: 10.1001/jamaoncol.2021.7298

37. McNeel DG, Becker JT, Eickhoff JC, Johnson LE, Bradley E, Pohlkamp I, et al. Real-time immune monitoring to guide plasmid DNA vaccination schedule targeting prostatic acid phosphatase in patients with castration-resistant prostate cancer. *Clin Cancer Res* (2014) 20(14):3692–704. doi: 10.1158/1078-0432.CCR-14-0169

38. McNeel DG, Eickhoff JC, Johnson LE, Roth AR, Perk TG, Fong L, et al. Phase II trial of a DNA vaccine encoding prostatic acid phosphatase (pTVG-HP [MVI-816]) in patients with progressive, nonmetastatic, castration-sensitive prostate cancer. *J Clin Oncol* (2019) 37(36):3507–17. doi: 10.1200/JCO.19.01701

39. McNeel DG, Eickhoff JC, Wargowski E, Johnson LE, Kyriakopoulos CE, Emamekhoo H, et al. Phase 2 trial of T-cell activation using MVI-816 and pembrolizumab in patients with metastatic, castration-resistant prostate cancer (mCRPC). *J Immunother Cancer* (2022) 10(3):e004198. doi: 10.1136/jitc-2021-004198

40. Kyriakopoulos CE, Eickhoff JC, Ferrari AC, Schweizer MT, Wargowski E, Olson BM, et al. Multicenter phase I trial of a DNA vaccine encoding the androgen receptor ligand-binding domain (pTVG-AR, MVI-118) in patients with metastatic prostate cancer. *Clin Cancer Res* (2020) 26(19):5162–71. doi: 10.1158/1078-0432.CCR-20-0945

41. Elia AR, Caputo S, Bellone M. Immune checkpoint-mediated interactions between cancer and immune cells in prostate adenocarcinoma and melanoma. *Front Immunol* (2018) 9:1786. doi: 10.3389/fimmu.2018.01786

42. Jiao S, Subudhi SK, Aparicio A, Ge Z, Guan B, Miura Y, et al. Differences in tumor microenvironment dictate T helper lineage polarization and response to immune checkpoint therapy. *Cell* (2019) 179(5):1177–90.e13. doi: 10.1016/j.cell.2019.10.029

43. Fuchs CS, Doi T, Jang RW, Muro K, Satoh T, Machado M, et al. Safety and efficacy of pembrolizumab monotherapy in patients with previously treated advanced gastric and gastroesophageal junction cancer: Phase 2 clinical KEYNOTE-059 trial. *JAMA Oncol* (2018) 4(5):e180013. doi: 10.1001/jamaoncol.2018.0013

44. Hellmann MD, Rizvi NA, Goldman JW, Gettinger SN, Borghaei H, Brahmer JR, et al. Nivolumab plus ipilimumab as first-line treatment for advanced non-small-cell lung cancer (CheckMate 012): results of an open-label, phase 1, multicohort study. *Lancet Oncol* (2017) 18(1):31–41. doi: 10.1016/S1470-2045(16)30624-6

45. Patel MR, Ellerton J, Infante JR, Agrawal M, Gordon M, Aljumaily R, et al. Avelumab in metastatic urothelial carcinoma after platinum failure (JAVELIN solid tumor): pooled results from two expansion cohorts of an open-label, phase 1 trial. *Lancet Oncol* (2018) 19(1):51–64. doi: 10.1016/S1470-2045(17)30900-2

46. Nicholson LT, Fong L. Immune checkpoint inhibition in prostate cancer. *Trends Cancer.* (2020) 6(3):174–7. doi: 10.1016/j.trecan.2020.01.003

47. Claps M, Mennitto A, Guadalupi V, Sepe P, Stellato M, Zattarin E, et al. Immune-checkpoint inhibitors and metastatic prostate cancer therapy: Learning by making mistakes. *Cancer Treat Rev* (2020) 88:102057. doi: 10.1016/j.ctrv.2020.102057

48. Cameron F, Whiteside G, Perry C. Ipilimumab: first global approval. *Drugs.* (2011) 71(8):1093–104. doi: 10.2165/11594010-000000000-00000

49. Beer TM, Kwon ED, Drake CG, Fizazi K, Logothetis C, Gravis G, et al. Randomized, double-blind, phase III trial of ipilimumab versus placebo in asymptomatic or minimally symptomatic patients with metastatic chemotherapy-naive castration-resistant prostate cancer. *J Clin Oncol* (2017) 35(1):40–7. doi: 10.1200/JCO.2016.69.1584

50. Kwon ED, Drake CG, Scher HI, Fizazi K, Bossi A, van den Eertwegh AJM, et al. Ipilimumab versus placebo after radiotherapy in patients with metastatic castration-resistant prostate cancer that had progressed after docetaxel chemotherapy (CA184-043): a multicentre, randomised, double-blind, phase 3 trial. *Lancet Oncol* (2014) 15 (7):700–12. doi: 10.1016/S1470-2045(14)70189-5

51. Subudhi SK, Vence L, Zhao H, Blando J, Yadav SS, Xiong Q, et al. Neoantigen responses, immune correlates, and favorable outcomes after ipilimumab treatment of patients with prostate cancer. *Sci Transl Med* (2020) 12(537):eaaz3577. doi: 10.1126/scitranslmed.aaz3577

52. Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med* (2002) 8(8):793–800. doi: 10.1038/nm730

53. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* (2010) 363(8):711–23. doi: 10.1056/NEJMoa1003466

54. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *N Engl J Med* (2015) 373(1):23–34. doi: 10.1056/NEJMoa1504030

55. Wolchok JD, Chiarion-Sileni V, Gonzalez R, Rutkowski P, Grob J-J, Cowey CL, et al. Overall survival with combined nivolumab and ipilimumab in advanced melanoma. *N Engl J Med* (2017) 377(14):1345–56. doi: 10.1056/NEJMoa1709684

56. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med* (2012) 366(26):2443–54. doi: 10.1056/NEJMoa1200690

57. Hansen AR, Massard C, Ott PA, Haas NB, Lopez JS, Ejadi S, et al. Pembrolizumab for advanced prostate adenocarcinoma: findings of the KEYNOTE-028 study. *Ann Oncol* (2018) 29(8):1807–13. doi: 10.1093/annonc/mdy232

58. Antonarakis ES, Piulats JM, Gross-Goupil M, Goh J, Ojamaa K, Hoimes CJ, et al. Pembrolizumab for treatment-refractory metastatic castration-resistant prostate cancer: Multicohort, open-label phase II KEYNOTE-199 study. *J Clin Oncol* (2020) 38(5):395–405. doi: 10.1200/JCO.19.01638

59. Graf RP, Fisher V, Weberpals J, Gjoerup O, Tierno MB, Huang RSP, et al. Comparative effectiveness of immune checkpoint inhibitors vs chemotherapy by tumor mutational burden in metastatic castration-resistant prostate cancer. *JAMA Netw Open* (2022) 5(3):e225394. doi: 10.1001/jamanetworkopen.2022.5394

60. Gao J, Ward JF, Pettaway CA, Shi LZ, Subudhi SK, Vence LM, et al. VISTA is an inhibitory immune checkpoint that is increased after ipilimumab therapy in patients with prostate cancer. *Nat Med* (2017) 23(5):551–5. doi: 10.1038/nm.4308

61. Sharma P, Pachynski RK, Narayan V, Fléchon A, Gravis G, Galsky MD, et al. Nivolumab plus ipilimumab for metastatic castration-resistant prostate cancer: Preliminary analysis of patients in the CheckMate 650 trial. *Cancer Cell* (2020) 38 (4):489–99.e3. doi: 10.1016/j.ccell.2020.08.007

62. Vestergaard LK, Oliveira DNP, Høgdall CK, Høgdall EV. Next generation sequencing technology in the clinic and its challenges. *Cancers (Basel)* (2021) 13 (8):1751. doi: 10.3390/cancers13081751

63. Yu W, Hurley J, Roberts D, Chakrabortty SK, Enderle D, Noerholm M, et al. Exosome-based liquid biopsies in cancer: opportunities and challenges. *Ann Oncol* (2021) 32(4):466–77. doi: 10.1016/j.annonc.2021.01.074

64. Graff JN, Alumkal JJ, Drake CG, Thomas GV, Redmond WL, Farhad M, et al. Early evidence of anti-PD-1 activity in enzalutamide-resistant prostate cancer. *Oncotarget.* (2016) 7(33):52810–7. doi: 10.18632/oncotarget.10547

65. Schweizer MT, Cheng HH, Tretiakova MS, Vakar-Lopez F, Klemfuss N, Konnick EQ, et al. Mismatch repair deficiency may be common in ductal adenocarcinoma of the prostate. *Oncotarget.* (2016) 7(50):82504–10. doi: 10.18632/oncotarget.12697

66. McGrail DJ, Federico L, Li Y, Dai H, Lu Y, Mills GB, et al. Multi-omics analysis reveals neoantigen-independent immune cell infiltration in copy-number driven cancers. *Nat Commun* (2018) 9(1):1317. doi: 10.1038/s41467-018-03730-x

67. McGrail DJ, Pilié PG, Rashid NU, Voorwerk L, Slagter M, Kok M, et al. High tumor mutation burden fails to predict immune checkpoint blockade response across all cancer types. *Ann Oncol* (2021) 32(5):661–72. doi: 10.1016/j.annonc.2021.02.006

68. Britten CM, Shalabi A, Hoos A. Industrializing engineered autologous T cells as medicines for solid tumours. *Nat Rev Drug Discovery* (2021) 20(6):476–88. doi: 10.1038/s41573-021-00175-8

69. Daher M, Rezvani K. Outlook for new CAR-based therapies with a focus on CAR NK cells: What lies beyond CAR-engineered T cells in the race against cancer. *Cancer Discovery* (2021) 11(1):45–58. doi: 10.1158/2159-8290.CD-20-0556

70. Chong EA, Ruella M, Schuster SJ. Five-year outcomes for refractory b-cell lymphomas with CAR T-cell therapy. *N Engl J Med* (2021) 384(7):673–4. doi: 10.1056/NEJMc2030164

71. Park JH, Rivière I, Gonen M, Wang X, Sénéchal B, Curran KJ, et al. Long-term follow-up of CD19 CAR therapy in acute lymphoblastic leukemia. *N Engl J Med* (2018) 378(5):449–59. doi: 10.1056/NEJMoa1709919

72. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisagenlecleucel in children and young adults with b-cell lymphoblastic leukemia. *N Engl J Med* (2018) 378(5):439–48. doi: 10.1056/NEJMoa1709866

73. Mullard A. FDA Approves fourth CAR-T cell therapy. *Nat Rev Drug Discovery* (2021) 20(3):166. doi: 10.1038/d41573-021-00031-9

74. June CH, Sadelain M. Chimeric antigen receptor therapy. *N Engl J Med* (2018) 379(1):64–73. doi: 10.1056/NEJMra1706169

75. Schepisi G, Cursano MC, Casadei C, Menna C, Altavilla A, Lolli C, et al. CAR-T cell therapy: a potential new strategy against prostate cancer. *J Immunother Cancer.* (2019) 7(1):258. doi: 10.1186/s40425-019-0741-7

76. Fujiwara K, Tsunei A, Kusabuka H, Ogaki E, Tachibana M, Okada N. Hinge and transmembrane domains of chimeric antigen receptor regulate receptor expression and signaling threshold. *Cells* (2020) 9(5):1182. doi: 10.3390/cells9051182

77. Majzner RG, Ramakrishna S, Yeom KW, Patel S, Chinnasamy H, Schultz LM, et al. GD2-CAR T cell therapy for H3K27M-mutated diffuse midline gliomas. *Nature.* (2022) 603(7903):934–41. doi: 10.1038/s41586-022-04489-4

78. Hou AJ, Chen LC, Chen YY. Navigating CAR-T cells through the solid-tumour microenvironment. *Nat Rev Drug Discovery* (2021) 20(7):531–50. doi: 10.1038/s41573-021-00189-2

79. June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CAR T cell immunotherapy for human cancer. *Science.* (2018) 359(6382):1361–5. doi: 10.1126/science.aar6711

80. Narayan V, Barber-Rotenberg JS, Jung I-Y, Lacey SF, Rech AJ, Davis MM, et al. PSMA-targeting TGFβ-insensitive armored CAR t cells in metastatic castration-resistant prostate cancer: a phase 1 trial. *Nat Med* (2022) 28(4):724–34. doi: 10.1038/s41591-022-01726-1

81. Tschernia NP, Norberg SM, Gulley JL. CAR T cells reach clinical milestone in prostate cancer. *Nat Med* (2022) 28(4):635–6. doi: 10.1038/s41591-022-01742-1

82. Dorff TB, Narayan V, Forman SJ, Zang PD, Fraietta JA, June CH, et al. Novel redirected T-cell immunotherapies for advanced prostate cancer. *Clin Cancer Res* (2022) 28(4):576–84. doi: 10.1158/1078-0432.CCR-21-1483

83. Wolf P, Alzubi J, Gratzke C, Cathomen T. The potential of CAR T cell therapy for prostate cancer. *Nat Rev Urol.* (2021) 18(9):556–71. doi: 10.1038/s41585-021-00488-8

84. Zhang X, Sun S, Miao Y, Yuan Y, Zhao W, Li H, et al. Docetaxel enhances the therapeutic efficacy of PSMA-specific CAR-T cells against prostate cancer models by suppressing MDSCs. *J Cancer Res Clin Oncol* (2022) 148(12):3511–20. doi: 10.1007/s00432-022-04248-y

85. Kamat NV, Yu EY, Lee JK. BiTE-ing into prostate cancer with bispecific T-cell engagers. *Clin Cancer Res* (2021) 27(10):2675–7. doi: 10.1158/1078-0432.CCR-21-0355

86. Goebeler M-E, Bargou RC. T Cell-engaging therapies - BiTEs and beyond. *Nat Rev Clin Oncol* (2020) 17(7):418–34. doi: 10.1038/s41571-020-0347-5

87. Deegen P, Thomas O, Nolan-Stevaux O, Li S, Wahl J, Bogner P, et al. The PSMA-targeting half-life extended BiTE therapy AMG 160 has potent antitumor activity in preclinical models of metastatic castration-resistant prostate cancer. *Clin Cancer Res* (2021) 27(10):2928–37. doi: 10.1158/1078-0432.CCR-20-3725

88. Hummel H-D, Kufer P, Grüllich C, Seggewiss-Bernhardt R, Deschler-Baier B, Chatterjee M, et al. Pasotuxizumab, a BiTE® immune therapy for castration-resistant prostate cancer: Phase I, dose-escalation study findings. *Immunotherapy.* (2021) 13 (2):125–41. doi: 10.2217/imt-2020-0256

89. Slaney CY, Wang P, Darcy PK, Kershaw MH. CARs versus BiTEs: A comparison between T cell-redirection strategies for cancer treatment. *Cancer Discovery* (2018) 8 (8):924–34. doi: 10.1158/2159-8290.CD-18-0297

90. Hegde PS, Chen DS. Top 10 challenges in cancer immunotherapy. *Immunity.* (2020) 52(1):17–35. doi: 10.1016/j.immuni.2019.12.011

91. Heitmann JS, Pfluegler M, Jung G, Salih HR. Bispecific antibodies in prostate cancer therapy: Current status and perspectives. *Cancers (Basel)* (2021) 13(3):549. doi: 10.3390/cancers13030549

92. Braig F, Brandt A, Goebeler M, Tony H-P, Kurze A-K, Nollau P, et al. Resistance to anti-CD19/CD3 BiTE in acute lymphoblastic leukemia may be mediated by disrupted CD19 membrane trafficking. *Blood.* (2017) 129(1):100–4. doi: 10.1182/blood-2016-05-718395

93. Giffin MJ, Cooke K, Lobenhofer EK, Estrada J, Zhan J, Deegen P, et al. AMG 757, a half-life extended, DLL3-targeted bispecific T-cell engager, shows high potency and sensitivity in preclinical models of small-cell lung cancer. *Clin Cancer Res* (2021) 27 (5):1526–37. doi: 10.1158/1078-0432.CCR-20-2845

94. Yamamoto K, Trad A, Baumgart A, Hüske L, Lorenzen I, Chalaris A, et al. A novel bispecific single-chain antibody for ADAM17 and CD3 induces T-cell-mediated lysis of prostate cancer cells. *Biochem J* (2012) 445(1):135–44. doi: 10.1042/BJ20120433

95. Einsele H, Borghaei H, Orlowski RZ, Subklewe M, Roboz GJ, Zugmaier G, et al. The BiTE (bispecific T-cell engager) platform: Development and future potential of a targeted immuno-oncology therapy across tumor types. *Cancer.* (2020) 126(14):3192–201. doi: 10.1002/cncr.32909

96. Sinha M, Zhang L, Subudhi S, Chen B, Marquez J, Liu EV, et al. Pre-existing immune status associated with response to combination of sipuleucel-T and ipilimumab in patients with metastatic castration-resistant prostate cancer. *J Immunother Cancer* (2021) 9(5):e002254. doi: 10.1136/jitc-2020-002254

97. Slovin SF, Higano CS, Hamid O, Tejwani S, Harzstark A, Alumkal JJ, et al. Ipilimumab alone or in combination with radiotherapy in metastatic castration-resistant prostate cancer: results from an open-label, multicenter phase I/II study. *Ann Oncol* (2013) 24(7):1813–21. doi: 10.1093/annonc/mdt107

98. Wargowski E, Johnson LE, Eickhoff JC, Delmastro L, Staab MJ, Liu G, et al. Prime-boost vaccination targeting prostatic acid phosphatase (PAP) in patients with metastatic castration-resistant prostate cancer (mCRPC) using sipuleucel-T and a DNA vaccine. *J Immunother Cancer.* (2018) 6(1):21. doi: 10.1186/s40425-018-0333-y

99. Tran C, Ouk S, Clegg NJ, Chen Y, Watson PA, Arora V, et al. Development of a second-generation antiandrogen for treatment of advanced prostate cancer. *Science.* (2009) 324(5928):787–90. doi: 10.1126/science.1168175

100. Fizazi K, Foulon S, Carles J, Roubaud G, McDermott R, Fléchon A, et al. Abiraterone plus prednisone added to androgen deprivation therapy and docetaxel in *de novo* metastatic castration-sensitive prostate cancer (PEACE-1): a multicentre, open-label, randomised, phase 3 study with a 2 × 2 factorial design. *Lancet.* (2022) 399(10336):1695–707. doi: 10.1016/S0140-6736(22)00367-1

101. Ben-Batalla I, Vargas-Delgado ME, von Amsberg G, Janning M, Loges S. Influence of androgens on immunity to self and foreign: Effects on immunity and cancer. *Front Immunol* (2020) 11:1184. doi: 10.3389/fimmu.2020.01184

102. Cioni B, Zaalberg A, van Beijnum JR, Melis MHM, van Burgsteden J, Muraro MJ, et al. Androgen receptor signalling in macrophages promotes TREM-1-mediated prostate cancer cell line migration and invasion. *Nat Commun* (2020) 11(1):4498. doi: 10.1038/s41467-020-18313-y

103. Radojevic K, Arsenovic-Ranin N, Kosec D, Pesic V, Pilipovic I, Perisic M, et al. Neonatal castration affects intrathymic kinetics of T-cell differentiation and the spleen T-cell level. *J Endocrinol* (2007) 192(3):669–82. doi: 10.1677/joe.1.07019

104. Pu Y, Xu M, Liang Y, Yang K, Guo Y, Yang X, et al. Androgen receptor antagonists compromise T cell response against prostate cancer leading to early tumor relapse. *Sci Transl Med* (2016) 8(333):333ra47. doi: 10.1126/scitranslmed.aad5659

105. Guan X, Polesso F, Wang C, Sehrawat A, Hawkins RM, Murray SE, et al. Androgen receptor activity in T cells limits checkpoint blockade efficacy. *Nature.* (2022) 606(7915):791–6. doi: 10.1038/s41586-022-04522-6

106. Madan RA, Gulley JL, Schlom J, Steinberg SM, Liewehr DJ, Dahut WL, et al. Analysis of overall survival in patients with nonmetastatic castration-resistant prostate cancer treated with vaccine, nilutamide, and combination therapy. *Clin Cancer Res* (2008) 14(14):4526–31. doi: 10.1158/1078-0432.CCR-07-5048

107. Powles T, Yuen KC, Gillessen S, Kadel EE, Rathkopf D, Matsubara N, et al. Atezolizumab with enzalutamide versus enzalutamide alone in metastatic castration-resistant prostate cancer: a randomized phase 3 trial. *Nat Med* (2022) 28(1):144–53. doi: 10.1038/s41591-021-01600-6

108. Lin L, Kane N, Kobayashi N, Kono EA, Yamashiro JM, Nickols NG, et al. High-dose per fraction radiotherapy induces both antitumor immunity and immunosuppressive responses in prostate tumors. *Clin Cancer Res* (2021) 27 (5):1505–15. doi: 10.1158/1078-0432.CCR-20-2293

109. Parker C, Lewington V, Shore N, Kratochwil C, Levy M, Lindén O, et al. Targeted alpha therapy, an emerging class of cancer agents: A review. *JAMA Oncol* (2018) 4(12):1765–72. doi: 10.1001/jamaoncol.2018.4044

110. Henriksen G, Breistøl K, Bruland ØS, Fodstad Ø, Larsen RH. Significant antitumor effect from bone-seeking, alpha-particle-emitting (223)Ra demonstrated in an experimental skeletal metastases model. *Cancer Res* (2002) 62(11):3120–5.

111. FDA Approves radiopharmaceutical for metastatic prostate cancer. *Cancer Discovery* (2013) 3(7):OF1. doi: 10.1158/2159-8290.CD-NB2013-088

112. Hoskin P, Sartor O, O'Sullivan JM, Johannessen DC, Helle SI, Logue J, et al. Efficacy and safety of radium-223 dichloride in patients with castration-resistant prostate cancer and symptomatic bone metastases, with or without previous docetaxel use: a prespecified subgroup analysis from the randomised, double-blind, phase 3 ALSYMPCA trial. *Lancet Oncol* (2014) 15(12):1397–406. doi: 10.1016/S1470-2045(14)70474-7

113. Saad F, Carles J, Gillessen S, Heidenreich A, Heinrich D, Gratt J, et al. Radium-223 and concomitant therapies in patients with metastatic castration-resistant prostate cancer: an international, early access, open-label, single-arm phase 3b trial. *Lancet Oncol* (2016) 17(9):1306–16. doi: 10.1016/S1470-2045(16)30173-5

114. Marshall CH, Fu W, Wang H, Park JC, DeWeese TL, Tran PT, et al. Randomized phase II trial of sipuleucel-T with or without radium-223 in men with bone-metastatic castration-resistant prostate cancer. *Clin Cancer Res* (2021) 27 (6):1623–30. doi: 10.1158/1078-0432.CCR-20-4476

115. Twardowski P, Wong JYC, Pal SK, Maughan BL, Frankel PH, Franklin K, et al. Randomized phase II trial of sipuleucel-T immunotherapy preceded by sensitizing radiation therapy and sipuleucel-T alone in patients with metastatic castrate resistant prostate cancer. *Cancer Treat Res Commun* (2019) 19:100116. doi: 10.1016/j.ctarc.2018.100116

116. Fong L, Morris MJ, Sartor O, Higano CS, Pagliaro L, Alva A, et al. A phase ib study of atezolizumab with radium-223 dichloride in men with metastatic castration-resistant prostate cancer. *Clin Cancer Res* (2021) 27(17):4746–56. doi: 10.1158/1078-0432.CCR-21-0063

117. Sartor O, de Bono J, Chi KN, Fizazi K, Herrmann K, Rahbar K, et al. Lutetium-177-PSMA-617 for metastatic castration-resistant prostate cancer. *N Engl J Med* (2021) 385(12):1091–103. doi: 10.1056/NEJMoa2107322

118. Hofman MS, Emmett L, Sandhu S, Iravani A, Joshua AM, Goh JC, et al. [177Lu]Lu-PSMA-617 versus cabazitaxel in patients with metastatic castration-resistant prostate cancer (TheraP): a randomised, open-label, phase 2 trial. *Lancet.* (2021) 397(10276):797–804. doi: 10.1016/S0140-6736(21)00237-3

119. Satapathy S, Mittal BR, Sood A, Das CK, Mavuduru RS, Goyal S, et al. 177Lu-PSMA-617 versus docetaxel in chemotherapy-naïve metastatic castration-resistant prostate cancer: a randomized, controlled, phase 2 non-inferiority trial. *Eur J Nucl Med Mol Imaging.* (2022) 49(5):1754–64. doi: 10.1007/s00259-021-05618-3

120. Heinhuis KM, Ros W, Kok M, Steeghs N, Beijnen JH, Schellens JHM. Enhancing antitumor response by combining immune checkpoint inhibitors with chemotherapy in solid tumors. *Ann Oncol* (2019) 30(2):219–35. doi: 10.1093/annonc/mdy551

121. Petrylak DP, Ratta R, Gafanov R, Facchini G, Piulats JM, Kramer G, et al. KEYNOTE-921: Phase III study of pembrolizumab plus docetaxel for metastatic castration-resistant prostate cancer. *Future Oncol* (2021) 17(25):3291–9. doi: 10.2217/fon-2020-1133

122. Stewart RA, Pilié PG, Yap TA. Development of PARP and immune-checkpoint inhibitor combinations. *Cancer Res* (2018) 78(24):6717–25. doi: 10.1158/0008-5472.CAN-18-2652

123. Mateo J, Carreira S, Sandhu S, Miranda S, Mossop H, Perez-Lopez R, et al. DNA-Repair defects and olaparib in metastatic prostate cancer. *N Engl J Med* (2015) 373(18):1697–708. doi: 10.1056/NEJMoa1506859

124. de Bono J, Mateo J, Fizazi K, Saad F, Shore N, Sandhu S, et al. Olaparib for metastatic castration-resistant prostate cancer. *N Engl J Med* (2020) 382(22):2091–102. doi: 10.1056/NEJMoa1911440

125. Stover EH, Konstantinopoulos PA, Matulonis UA, Swisher EM. Biomarkers of response and resistance to DNA repair targeted therapies. *Clin Cancer Res* (2016) 22 (23):5651–60. doi: 10.1158/1078-0432.CCR-16-0247

126. Shen J, Zhao W, Ju Z, Wang L, Peng Y, Labrie M, et al. PARPi triggers the STING-dependent immune response and enhances the therapeutic efficacy of immune checkpoint blockade independent of BRCAness. *Cancer Res* (2019) 79(2):311–9. doi: 10.1158/0008-5472.CAN-18-1003

127. Higuchi T, Flies DB, Marjon NA, Mantia-Smaldone G, Ronner L, Gimotty PA, et al. CTLA-4 blockade synergizes therapeutically with PARP inhibition in BRCA1-deficient ovarian cancer. *Cancer Immunol Res* (2015) 3(11):1257–68. doi: 10.1158/2326-6066.CIR-15-0044

128. Fizazi K, Retz M, Petrylak DP, Goh JC, Perez-Gracia J, Lacombe L, et al. Nivolumab plus rucaparib for metastatic castration-resistant prostate cancer: results

from the phase 2 CheckMate 9KD trial. *J Immunother Cancer* (2022) 10(8):e004761. doi: 10.1136/jitc-2022-004761

129. De Santis M, Mottet N, Cornford P, Gillessen S. Precision oncology for metastatic prostate cancer: Translation into practice. *Eur Urol.* (2020) 78(6):771–4. doi: 10.1016/j.eururo.2020.08.021

130. Smith DC, Smith MR, Sweeney C, Elfiky AA, Logothetis C, Corn PG, et al. Cabozantinib in patients with advanced prostate cancer: results of a phase II randomized discontinuation trial. *J Clin Oncol* (2013) 31(4):412–9. doi: 10.1200/JCO.2012.45.0494

131. Smith M, De Bono J, Sternberg C, Le Moulec S, Oudard S, De Giorgi U, et al. Phase III study of cabozantinib in previously treated metastatic castration-resistant prostate cancer: COMET-1. *J Clin Oncol* (2016) 34(25):3005–13. doi: 10.1200/JCO.2015.65.5597

132. Patnaik A, Swanson KD, Csizmadia E, Solanki A, Landon-Brace N, Gehring MP, et al. Cabozantinib eradicates advanced murine prostate cancer by activating antitumor innate immunity. *Cancer Discovery* (2017) 7(7):750–65. doi: 10.1158/2159-8290.CD-16-0778

133. Castellano D, Apolo AB, Porta C, Capdevila J, Viteri S, Rodriguez-Antona C, et al. Cabozantinib combination therapy for the treatment of solid tumors: a systematic review. *Ther Adv Med Oncol* (2022) 14:17588359221108691. doi: 10.1177/17588359221108691

134. Petrylak DP, Loriot Y, Shaffer DR, Braiteh F, Powderly J, Harshman LC, et al. Safety and clinical activity of atezolizumab in patients with metastatic castration-resistant prostate cancer: A phase I study. *Clin Cancer Res* (2021) 27(12):3360–9. doi: 10.1158/1078-0432.CCR-20-1981

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 07 March 2023
DOI 10.3389/fonc.2023.1146515

OPEN ACCESS

EDITED BY
Ran Xu,
Second Xiangya Hospital, Central South University, China

REVIEWED BY
Jingchao Wei,
Zhejiang University, China
Yihong Zhou,
The Fifth Affiliated Hospital, Sun Yat-sen University, China

*CORRESPONDENCE
Hengfeng Zhou
zhouxy19990801@163.com
Zhi Long
longzhi522@163.com

SPECIALTY SECTION
This article was submitted to Genitourinary Oncology, a section of the journal Frontiers in Oncology

RECEIVED 17 January 2023
ACCEPTED 13 February 2023
PUBLISHED 07 March 2023

CITATION
Gan Y, He Q, Li C, Alsharafi BLM, Zhou H and Long Z (2023) A bibliometric study of the top 100 most-cited papers in neuroendocrine prostate cancer. *Front. Oncol.* 13:1146515. doi: 10.3389/fonc.2023.1146515

A bibliometric study of the top 100 most-cited papers in neuroendocrine prostate cancer

Yu Gan[1], Qiangrong He[2], Chao Li[2], Bassam Lutf Mohammed Alsharafi[2], Hengfeng Zhou[2*] and Zhi Long[2*]

[1]Department of Urology, Xiangya Hospital, Central South University, Changsha, Hunan, China, [2]Andrology Center, Department of Urology, The Third Xiangya Hospital, Central South University, Changsha, Hunan, China

Background: This study used bibliometrics to define and analyze the characteristics of the first 100 most cited papers on the topic of neuroendocrine prostate cancer (NEPC).

Methods: We explored the Web of Science Core Collection database, and screened the top 100 most frequently cited articles and reviews with the title NEPC or small cell prostate cancer (SCPC). We conducted bibliometrics research on the screening results to identify the most influential journals and authors in the field of NEPC.

Results: The first 100 most cited papers have been cited a total of 14,795 times, from 73 to 833 times (mean ± standard deviation, 147.95 ± 101.68). All top 100 most cited papers were published from 1984 to 2019, and the total number of citations for papers published in 2016 was significantly higher than that for papers published in other years. The journal with the largest number of published papers is "Prostate" (n=8). "Neuroendocrine differentiation" has become the most frequently used author keyword. "Oncology" is the most popular topic in the field of NEPC.

Conclusion: We analyzed the first 100 most cited papers in the NEPC field by collecting detailed information, which provide guiding opinions for finding the most influential journals and authors in NEPC-related fields. We hope to help researchers and readers in this field improve their understanding of NEPC research trends and provide ideas for future research from a unique perspective.

KEYWORDS

bibliometric study, top 100, neuroendocrine prostate cancer, small cell prostate cancer, neuroendocrine differentiation

Introduction

Prostate cancer is one of the most common male malignancies in the world and is the most common malignancy of the genitourinary system (1). Prostatic adenocarcinoma (AD PCa) constitutes 95% of prostate cancers in pathology, and androgen-deprivation therapy (ADT) represents the first-line systemic treatment for metastatic AD PCa (2). Clinical studies have shown that ADT has a good effect on most prostate cancer patients. However, over time, prostate cancer cells eventually adapt to a low level of testosterone secondary to ADT, and develop into castration resistance prostate cancer (CRPC) (3, 4). Once prostate cancer patients enter into the CRPC stage, their median survival period is only 15–30 months. Primary anti-androgen receptor (AR) drugs, such as bicalutamide, have no significant inhibitory effect on CPRC (5). Thus, next-generation AR pathway inhibitors (ARPIs), such as enzalutamide and abiraterone, have been developed. Patients with PCa may nonetheless acquire resistance to ARPIs, whose long-term use can lead to epithelial-mesenchymal transition (EMT) and neuroendocrine differentiation (NED) in some prostate tumor cells (6–10). This type of pathology that has undergone NED is classified as neuroendocrine prostate cancer (NEPC) (11). NEPC is characterized by cancer cells proliferating and growing independently with the AR signaling pathway, and is prone to visceral metastasis and osteolytic metastasis (11–13). The overall survival period is less than one year (12).

At present, there is no effective systematic treatment plan for NEPC. Discovering ways to curb the occurrence of EMT and NED in the initial stage has attracted significant attention (14, 15). While articles on the formation of NEPC have been continuously published in recent years, it can be challenging for researchers to sort through multiple papers, as well as process and summarize huge amounts of information before classifying the papers according to their needs and interests. It is therefore necessary to conduct bibliometrics research on NEPC to facilitate researchers' investigations in this filed.

In 1934, Paul Otlet first introduced bibliometric analysis, which aims to assess the academic influence of publications or countries on a certain topic or field, and explore the development of specific research areas (16). By using stable academic quality standards to statistically analyze published research results, bibliometrics analysis has value in guiding research trends (17, 18). Bibliometrics research has been widely used to explore research trends in various fields, such as microRNA, lncRNA, diabetes, and cancer. Considering the continuous growth of NEPC research results and the lack of published bibliometric analysis research articles in the field of NEPC, it is imperative to use quantitative methods to evaluate and analyze existing research. To a large extent, the frequency of citation can indicate the influence of an article in related disciplines and reflect the recognition of the paper by research peers. Therefore, this research used the number of citations of an article as a condition to screen the top 100 most cited papers in the field of NEPC, then conducted bibliometric research on them. The purpose of this study is to evaluate the relevant factors for the successful citation of research, which can help deepen the understanding of how NEPC-related research develops and expands. Moreover, our study can facilitate researchers' efforts in conducting follow-up studies from different angles.

Methods

Research strategy

We searched the Web of Science (WoS) Core Collection database to gather studies on NEPC on December 20, 2020. The following search strategies were used: TI = (neuroendocrine prostate* OR NEPC OR small cell prostate*).

Inclusion criteria

Editorial materials, letters, revisions, books, biography, news, patent, and unspecified were excluded. Articles and reviews were targeted for screening. The selection results were listed in descending order depending on the total number of citations. We chose the top 100 most-cited papers after an independent review by two experts. The primary selection process is shown in Figure 1.

Data extraction

Two authors (Yu Gan and Hengfeng Zhou) independently collected the data, and a third researcher (Zhi Long) was consulted to deal with discrepancies. The following information were collected: number of citations, journal, first author, corresponding author, country, document type, author keywords, Journal Citation Indicator (JCI) 2021, and 5-year IF. It should be noted that only the first-ranked authors were counted when there are multiple first authors. We calculated the mean and standard deviation (mean ± SD) of the number of citations.

Statistical analysis

We used IBM SPSS Statistics for Windows, version 26.0, software to perform a one-sample t-test and simple linear regression. One-sample t-test was used to compare the difference between specific data and the mean of the population sample. Simple linear regression was used to analyze the linear correlation between the two factors. $p < 0.05$ was considered statistically significant.

Results

Citation

The top 100 most-cited papers are listed in Table S1 in descending order based on the total number of citations. The total number of citations for the 100 papers was 14,795 times. The most cited paper in a single article was cited 833 times, and the

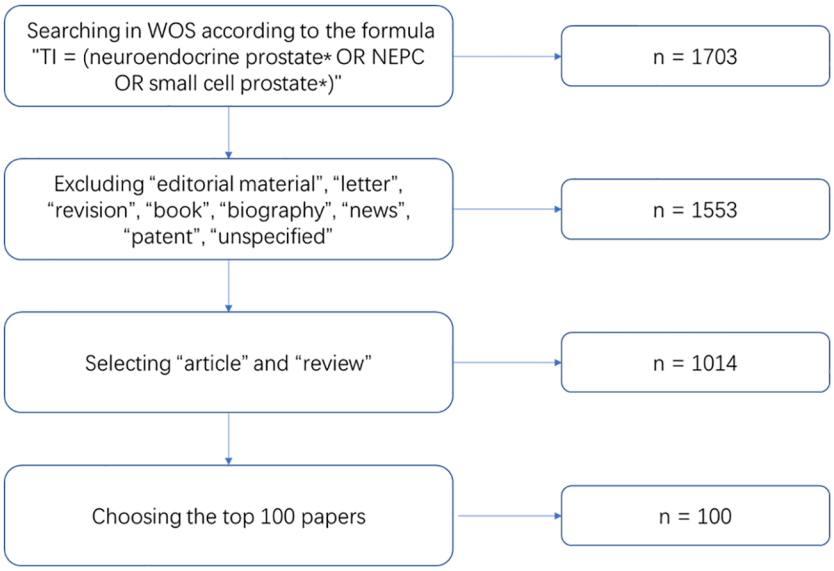


FIGURE 1
The initial search process in Web of Science (WOS).

least cited paper in a single article was cited 73 times, with an average number of citations of 147.95 ± 200.50. The most frequently cited paper was published in the journal "Nature Medicine" in 2016. The paper was written by Himisha Beltran as the first author and corresponding author. Himisha Beltran and Davide Prandi equally contributed to the work. Levi A. Garraway, Mark A., Rubin, and Francesca Demichelis jointly directed this research. This article mainly introduces the role of the evolutionary mechanism of differentiation and cloning in the evolution of NEPC.

Publication year

The top 100 most-cited papers were all published between 1984 and 2019 (Figure 2A). The total number of citations was the highest in 2016, with five articles published (n=1542). Among these five articles published in 2016, "Divergent Clonal Evolution of Castration-Resistant Neuroendocrine Prostate Cancer", which was published by Himisha Beltran, made the greatest contribution (54.021%). In addition, the 5-year IF and JCI 2021 for articles published in 2016 are also the highest at 169.522 and 26.69, respectively (Figures 2C, D). After counting the number of articles published by year (Figure 2B), we can conclude that researchers have continued to publish highly cited articles in the field of NEPC since 1992. It is evident that NEPC has always attracted the attention of researchers.

Journal

The top 100 most-cited papers were published in 48 journals. Eleven journals published three or more articles, and "Prostate" published the most number of articles (n =8). The 5-year IF range of these 48 journals is 2.267 to 68.311, and the JCI 2021 range is 0.31 to 13.00. JCI is a new indicator published by Clarivate to evaluate the impact of literature. JCI controls variables of different subject areas, literature types (e.g., papers, reviews), and year of publication. The resulting value represents the relative citation impact. JCI is the ratio of the influence of a paper to the global baseline; 1.0 represents the world average, and a JCI value of 2.0 means that the influence is twice the average. In order to verify the correspondence between JCI and IF, we produced a combined line chart (Figure 3) for the 5-year IF and JCI 2021 of the 48 journals. The trend of the two lines is roughly the same. Although JCI is a new indicator established in 2021, it can still reflect the influence of journals more objectively. When searching and evaluating journals, JCI can be used as a supplementary mark of IF.

First author

The top 100 most-cited papers were written by 90 different authors, of which eight wrote at least two published articles. Himisha Beltran published the most number of articles (n = 4). We counted the total number of citations published and the total 5-year IF of all papers published by different authors. The top 10 authors with a total 5-year IF and the top 10 authors with the total number of citations are listed in Table 1.

Corresponding author

Seven of the 65 corresponding authors who participated in the publication of the top 100 most-cited papers were involved in the publication of at least two articles. Huang Jiaoti participated in the publication of the most number of articles (n= 5) as a

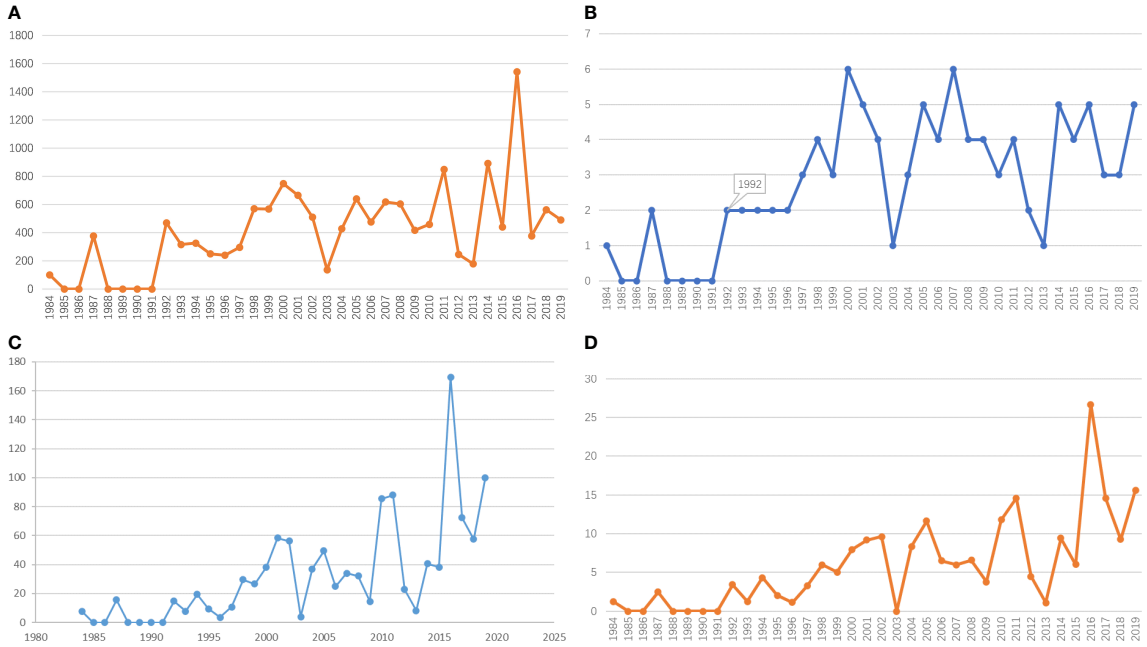


FIGURE 2
Analysis of publication year. **(A)** The relation between publication year and the number of citations. **(B)** The relation between publication year and the number of publications. **(C)** The relation between publication year and the total journal IF. **(D)** The relation between publication year and the total JCI 2021.

corresponding author, followed by Himisha Beltran (n = 4). The top 10 corresponding authors with total IF and the top corresponding authors with the total number of citations are listed in Table 2. We found that although the total number of citations of papers written by Himisha Beltran ranks first, the average number of citations is not outstanding. This is because Beltran participated in the publication of more articles as a corresponding author (n = 4) than other corresponding authors. Among these four articles, "Divergent Clonal Evolution of Castration-Resistant Neuroendocrine Prostate Cancer" has been cited as many as 833 times, far more than the other three.

Country

The top 100 most-cited papers were published by authors from 10 different countries and regions. The United States has the largest number of published articles (n = 60), followed by Canada (n = 15) (Table 3). The number of articles published from the United States and Canada was greater than the average number of articles published (n=11) (p=0.040). This phenomenon is related to the unique distribution characteristics of NEPC. Patients with AD PCa will develop castration resistance after treatment with ADT and ARPIs, and some pathological types of patients will eventually

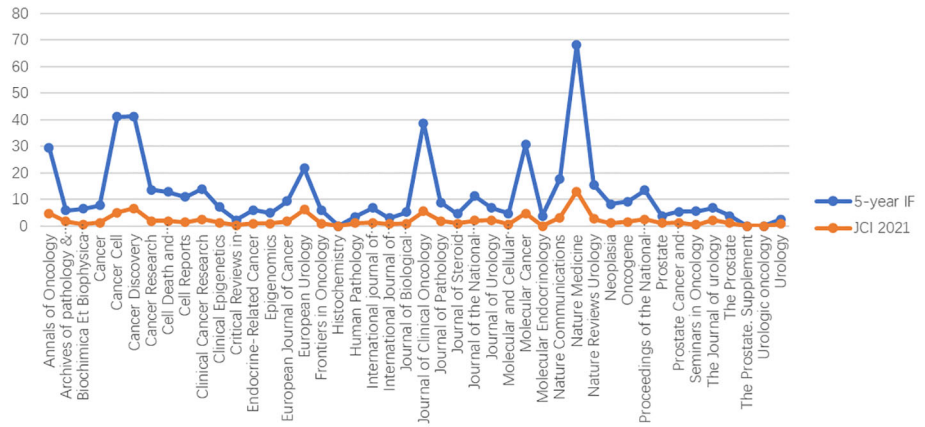


FIGURE 3
The relationship between journal IF and JCI 2021.

TABLE 1 The top 10 authors with total IF and the top 10 authors with the total number of citations.

Author	Total number of citations	Number of papers	Average citation per paper	Total 5-year IF	Average 5-year IF per paper
H. Beltran	1642	4	410.5	137.49	34.37
J. I. Epstein	600	2	300.0	25.16	12.58
P. A. Abrahamsson	503	3	167.8	27.73	9.24
P. A. di Sant'Agnese	403	2	201.5	10.35	5.18
M. E. Cox	309	2	154.5	18.97	9.49
R. Aggarwal	307	2	153.5	38.79	19.49
E. Dardenne	285	1	285.0	41.16	41.16
W. Wang	269	1	269.0	7.58	7.58
H. Bonkhoff	258	2	129.0	29.61	14.81
Y. J. Bang	250	1	250.0	13.45	13.45
J. K. Lee	244	1	244.0	41.16	41.16
C. N. Papandreou	197	1	197.0	38.79	38.79
J. L. Bishop	192	1	192.0	41.23	41.23
J. Qi	182	1	182.0	41.16	41.16

transform into NEPC. Developed countries such as European nations and the United States are the first ones to start using AR pathway-targeted inhibitors. Their proportion of NEPC patients is also higher than that of other countries and regions. Therefore, NEPC has become a hot research topic in these regions.

Document type

The top 100 most-cited papers contain seven different article types. When the same article belongs to different article types, we repeat this article for statistics. The papers were mostly classified as

TABLE 2 The top 10 corresponding authors with total IF and the top corresponding authors with the total number of citations.

·	Total number of citations	Number of papers	Average citation per paper	Total IF	Average IF per paper
Beltran, Himisha	1146	4	286.5	105.69	26.42
Epstein, Jonathan I.	600	1	600	15.16	15.16
Rubin, Mark A.	595	1	595	41.23	41.23
Jiaoti, Huang	478	5	95.6	22.29	4.46
Abrahamsson, PA	416	2	208	27.73	13.87
Aggarwal, Rahul	307	1	307	38.79	38.79
Rickman, David S.	285	1	285	41.16	41.16
Cox, ME	258	2	129	10.09	5.05
Collins, Colin C.	254	2	127	30.60	15.30
Buttyan, R	246	2	123	20.60	10.30
Witte, Owen N.	244	1	244	41.16	41.16
Papandreou, CN	197	1	197	38.79	38.79
Zoubeidi, Amina	192	1	192	41.23	41.23
Ronai, Ze'ev A.	182	1	182	41.16	41.16
Uysal-Onganer, Pinar	115	1	115	30.61	30.61
Diaz-Meco, Maria T.	86	1	86	41.16	41.16

TABLE 3 Countries that published the top 100 most-cited papers.

Countries	Number of publications	Total citations	Average citations per paper
USA	60	9372	156.2
Canada	15	2538	507.6
Italy	8	2441	305.125
France	6	1120	186.667
China	5	506	101.2
Japan	5	651	130.2
Germany	4	1169	292.25
Sweden	3	496	165.333
England	2	195	97.5
Spain	2	201	100.5

an "Article" (n=64), followed by "Journal Article" (n=22), "Review" (n=21), "Research Support" (n=9), "Proceeding Paper" (n=3), "Case Report" (n=1), and "Comparative Study" (n=1). The statistics for each article type can be found in Table 4.

Web of Science categories

According to their respective research topics, the top 100 most-cited papers are divided into 15 WoS categories. Among them, "Oncology" contains the most number of articles (n =56), followed by "Urology & Nephrology" (n=37), "Endocrinology & Metabolism" (n =28), "Biochemistry & Molecular Biology" (n=26), and "Cell Biology" (n =20); the details are listed in Table 5. In addition, we produced Figure 4 to show the number of papers contained in each classification field by year. As can be seen from the figure, "Neurosciences & Neurology," "Pathology," "Reproductive Biology," "Reproductive Biology," "Reproductive Biology," "Reproductive Biology," and "Pharmacology & Pharmacy" did not produce any new papers after 2000. Although there are many articles in "Endocrinology & Metabolism," no frequently cited articles have been produced after 2009.

Author keywords

Since only 38 of the top 100 most-cited papers gave author keywords in WoS, we only included the keywords of these 38 articles in the statistics in this link. These 38 most cited articles contain 103 keywords, with "Neuroendocrine differentiation" showing the most occurrences (n = 12), followed by "prostate cancer" (n = 11). The number of occurrences of the author keywords is listed in Table 6.

Discussion

As the use of ARPIs becomes more widespread, therapy-induced NEPC—as an advanced stage of PCa—is showing a higher prevalence rate and is gradually attracting the attention of many scholars. With the gradual widespread application of pathological biopsy of metastases and immunohistochemistry in clinical diagnosis, the number of patients diagnosed with NEPC is increasing (19, 20). At the same time, as more developed countries begin using secondary generation AR pathway-targeted inhibitors such as abiraterone and enzalutamide, the diagnosis rate of NEPC

TABLE 4 Types of documents in the top 100 most-cited papers.

Type of document	Total number of citations	Number of papers	Average citation per paper
Article	9749	64	152.328
Journal article	3255	22	147.955
Review	2888	21	137.524
Research Support	846	9	94.333
Proceeding Paper	535	3	178.333
Case Report	101	1	101.000
Comparative Study	100	1	100.000

TABLE 5 WOS categories in the top 100 most-cited papers.

WOS categories	Times	Total citation times	Citations per paper	Total IF	Average IF per paper	Total JCI
Oncology	56	8455	150.982	793.627	14.172	127.18
Urology & Nephrology	37	5049	136.459	270.548	7.312	65.14
Endocrinology & Metabolism	28	3793	135.464	150.277	5.367	32.03
Biochemistry & Molecular Biology	26	4123	158.577	255.195	9.815	46.15
Cell Biology	20	3844	192.200	338.805	16.940	53.25
Neurosciences & Neurology	12	1649	137.417	73.875	6.156	16.15
Pathology	9	1513	168.111	57.202	6.356	16.26
Microscopy	8	1051	131.375	44.482	5.560	9.38
Surgery	7	1217	173.857	47.587	6.798	13.57
Reproductive Biology	7	1110	158.571	35.420	5.060	6.91
Geriatrics & Gerontology	7	988	141.143	47.827	6.832	10.42
Genetics & Heredity	7	723	103.286	56.430	8.061	11.09
Respiratory System	6	851	141.833	35.383	5.897	7.37
Immunology	6	788	131.333	40.674	6.779	7.39
Pharmacology & Pharmacy	3	393	131.000	20.332	6.777	4.80

in Europe, America, and other developed nations are also higher than that in developing countries. In the early stage, there have been many debates about the status and significance of NEPC and NED. With the help of technical methods such as gene sequencing and genetically engineered mice, people have gradually realized the uniqueness of NEPC in, for example, gene expression profiles and biological characteristics. Many scholars have therefore chosen to conduct research on NEPC as an independent disease.

In this paper, we selected the top 100 most-cited papers in the NEPC field from the WoS Core English Database. By classifying and counting the top 100 most-cited papers, we discuss the development history and possible future research hotspots of the NEPC.

We chose to conduct our search in the WoS Core English Database as the WoS is the world's largest comprehensive academic information resource covering the broadest scope of disciplines. It contains the core academic journals of various university disciplines, making the data and statistical results comparable to bibliometrics research in other fields. There are some articles originating from journals that are currently out of print. The articles published in these journals can still be searched and cited normally, their inclusion in the statistics has no impact on the statistical results.

We used the number of citations as the main condition for screening articles in this research. Compared with the 5-year IF and JCI 2021, the number of citations can more truly reflect the degree to

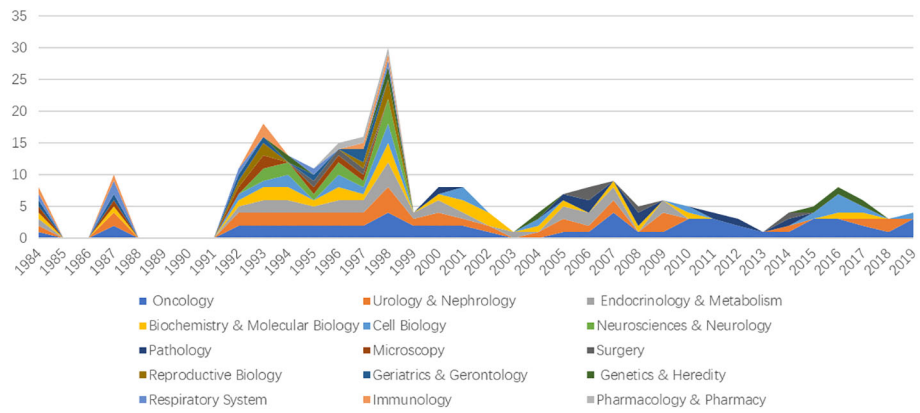


FIGURE 4
The relation between publication year and the number of publications of different WOS categories.

TABLE 6 The author keywords that appear at least three papers in the top 100 most-cited papers.

Keywords	Numbers
Neuroendocrine differentiation	12
prostate cancer	11
neuroendocrine	6
small cell carcinoma	6
neuroendocrine cell	5
androgen receptor	4
immunohistochemistry	3
prognosis	3
hormone refractory	3
neuron-specific enolase	3

which the article is recognized by peers (21, 22). In our statistics, the number of citations for a paper ranges from 73 to 833, and the total number of citations is 14,795. The most cited article is "Divergent Clonal Evolution of Castration-Resistant Neuroendocrine Prostate Cancer." This article proposes a differentiated cloning model of CRPC establishment. In AD PCa tissue, adenocarcinoma cells differentiate into different types of sub-clonal cells. Under the pressure of targeted inhibitors of the AR pathway, sub-clonal cells that can adapt to the environment become the main cells inside the tumor and finally complete the transformation of tumor pathological types. Experimental results and clinical phenomena show that most of the results of adaptation are transformed into NEPC. In addition, this article further proposes that the epigenetic genome and cell plasticity play an important regulatory role in the neuroendocrine differentiation process (23).

The top 100 most-cited papers were published between 1984 and 2019. Since 1994, there has been an output of highly cited articles every year. In particular, the total number of papers published throughout the year was the highest in 2016, followed by 2014 and 2011. However, the statistical results do not include articles published from 2020 to 2022, many of which have a higher 5-year IF. This may be due to the publication time being too close to the statistical time. Even for important results, it is difficult to obtain a high number of citations in a short period of time.

The 5-year IF is the other indicator we pay attention to. The IF reflects the influence of the entire journal in the field in recent years; however, the contribution of each paper in the journal is different. Among the 48 journals counted, except for three journals that do not have a 5-year IF record, the minimum IF is 2.548, which reflects how papers with more citations may be published in journals with lower IF, such as "Proposed Morphologic Classification of Prostate Cancer with Neuroendocrine Differentiation" which was published in the American Journal of Surgical Pathology in 2014. This article reclassifies the clinicopathological types of NEPC. This result will lead to more accurate targeted therapy for patients with different pathological types of prostate cancer (19). Therefore, when assessing the value of a paper, it may be more accurate to focus on the number of citations of the article. However, there is still a large correlation between the journal's 5-year IF value and the number of citations in the paper (r=0.959, p=0.000). When searching for papers about NEPC, we recommend using 5-year IF as an auxiliary search standard.

Our statistical results suggest that the top 100 most-cited papers were written by 90 first authors and published by 65 corresponding authors. Among them, the most noteworthy is Himisha Beltran, who is the director of Clinical and Translational Research at the Englander Institute for Precision Medicine. She has published many high-quality articles on prostate cancer and was involved in the publication of papers that ranked first in both the total number of citations and the total 5-year IF statistics. Himisha Beltran participated in the publication of a total of five articles, whose number of citations is 99 to 833, and all paper types are classified as an "Article." The most cited article is "Divergent Clonal Evolution of Castration-Resistant Neuroendocrine Prostate Cancer" published in "Nature Medicine" in 2016. Scholars who are studying prostate cancer should pay attention to papers published by Himisha Beltran.

Among the 100 most-cited papers, 64 are considered articles. It can be seen that an article is a widely recognized type of paper. Although proceeding papers showed the highest average number of citations, these three papers are still mainly articles. In addition, the majority of these 100 articles classified as "Article," indicating that NEPC is still constantly making progress. The "Article" generally refers to the researcher's detailed global presentation of research results. The "Review" generally refers to the researcher's summary and review of the results of previous experiments or research results in a particular field of study. Thus, the number of papers classified as a "Review" will gradually increase when there are adequate research results.

The top 100 most-cited papers come from 15 different WoS categories. There are more than 20 articles on "Oncology," "Urology and Nephrology," "Endocrinology and Metabolism," "Biochemistry and Molecular Biology," and "Cell Biology." The total number of citations of papers in Oncology has obvious advantages. At the same time, over time, the number of highly cited articles in Endocrinology and Metabolism, Neuroscience and Neurology, Reproductive Biology, Geriatrics and Gerontology, Respiratory System, Immunology, Pharmacology and Pharmacy has decreased significantly. This may be due to the deepening of NEPC understanding, gradually clarifying and refining the direction of NEPC research.

Excluding duplicates, 103 authors' keywords were counted, but only 10 appear frequently. Among them, "neuroendocrine differentiation" has become the most frequently used keyword and appeared in articles from 2000 to 2015. Neuroendocrine differentiation has been confirmed to be one of the theories that promote NEPC formation, and it being a frequently cited keyword can explain why it represents an important research direction of NEPC (24, 25). In addition, although "lineage plasticity" does not appear in the statistical results, it plays a key role in the neuroendocrine differentiation model (2, 26). Therefore, we believe that lineage plasticity may attract more attention in future research.

We acknowledge that our research has several inevitable limitations. First, there have been many debates about the uniqueness and significance of NEPC and NED at the early stage.

The small-cell prostate cancer (SCPC) has not been classified in the NEPC classification. Although we added "SCPC" into the select formula, it is still inevitable that some potentially critical articles may have been missed. Second, our research began on October 30, 2022, so recently published important studies that have not been cited enough may have also been missed. This may be the reason why some important articles have not been included in the statistics. The list of the top 100 most-cited papers may change over time, and we will continue to pay attention to the list and the changes in its statistical results. Third, since only 38 articles provide author keywords in the WoS, the statistical results on keywords may be biased. Fourth, our study only counted papers in the WoS. However, in different databases, the papers included and the data of each paper are not exactly consistent, which may also lead to a distortion of the results.

Despite these limitations, our research is still the first bibliometrics research on NEPC. It presents some of the authors who have made significant contributions to the field of NEPC, and summarizes the development process of NEPC research. Our study provides scholars who wish to join the field of NEPC with the direction to retrieve important literature and try to identify possible future research directions of NEPC.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Author contributions

HZ, CL and QH collected the references. HZ, ZL and YG screened the papers. YG and HZ wrote the initial draft. HZ, ZL and YG reviewed and edited the initial draft. HZ and BA wrote the manuscript. YG acquired the financial support for the project leading to this publication.

Funding

This project is supported by the National Natural Science Foundations of China (82273121 and 81902606) and the Natural Science Foundations of Hunan province (2022JJ20096) to YG.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The handling editor RX declared a shared parent affiliation with the authors at the time of review.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1146515/full#supplementary-material

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *Ca-a Cancer J Clin* (2022) 72(1):7–33. doi: 10.3322/caac.21708

2. Davies AH, Beltran H, Zoubeidi A. Cellular plasticity and the neuroendocrine phenotype in prostate cancer. *Nat Rev Urol* (2018) 15(5):271–86. doi: 10.1038/nrurol.2018.22

3. Wade CA, Kyprianou N. Profiling prostate cancer therapeutic resistance. *Int J Mol Sci* (2018) 19(3):904. doi: 10.3390/ijms19030904

4. Lee AR, Gan Y, Tang YX, Dong XS. A novel mechanism of SRRM4 in promoting neuroendocrine prostate cancer development via a pluripotency gene network. *Ebiomedicine* (2018) 35:167–77. doi: 10.1016/j.ebiom.2018.08.011

5. Tran C, Ouk S, Clegg NJ, Chen Y, Watson PA, Arora V, et al. Development of a second-generation antiandrogen for treatment of advanced prostate cancer. *Science* (2009) 324(5928):787–90. doi: 10.1126/science.1168175

6. Beltran H, Romanel A, Conteduca V, Casiraghi N, Sigouros M, Franceschini GM, et al. Circulating tumor DNA profile recognizes transformation to castration-resistant neuroendocrine prostate cancer. *J Clin Invest* (2020) 130(4):1653–68. doi: 10.1172/JCI131041

7. Beltran H, Hruszkewycz A, Scher HI, Hildesheim J, Isaacs J, Yu EY, et al. The role of lineage plasticity in prostate cancer therapy resistance. *Clin Cancer Res* (2019) 25 (23):6916–24. doi: 10.1158/1078-0432.CCR-19-1423

8. Zhou HF, He QR, Li C, Alsharafi BLM, Deng L, Long Z, et al. Focus on the tumor microenvironment: A seedbed for neuroendocrine prostate cancer. *Front Cell Dev Biol* (2022) 10. doi: 10.3389/fcell.2022.955669

9. Lovnicki J, Gan Y, Feng TT, Li YA, Xie N, Ho CH, et al. LIN28B promotes the development of neuroendocrine prostate cancer. *J Clin Invest* (2020) 130(10):5338–48. doi: 10.1172/JCI135373

10. Gan Y, Li YN, Long Z, Lee AR, Xie N, Lovnicki JM, et al. Roles of alternative RNA splicing of the bif-1 gene by SRRM4 during the development of treatment-induced neuroendocrine prostate cancer. *Ebiomedicine* (2018) 31:267–75. doi: 10.1016/j.ebiom.2018.05.002

11. Fine SW. Neuroendocrine tumors of the prostate. *Modern Pathol* (2018) 31: S122–32. doi: 10.1677/erc.0.0060503

12. Conteduca V, Oromendia C, Eng KW, Bareja R, Sigouros M, Molina A, et al. Clinical features of neuroendocrine prostate cancer. *Eur J Cancer* (2019) 121:7–18. doi: 10.1016/j.ejca.2019.08.011

13. Yamada Y, Beltran H. Clinical and biological features of neuroendocrine prostate cancer. *Curr Oncol Rep* (2021) 23(2):15. doi: 10.1007/s11912-020-01003-9

14. Rich JN. Cancer stem cells: understanding tumor hierarchy and heterogeneity. *Medicine* (2016) 95(1S):S2–7. doi: 10.1097/MD.0000000000004764

15. Zheng XB, Yu CN, Xu MQ. Linking tumor microenvironment to plasticity of cancer stem cells: Mechanisms and application in cancer therapy. *Front Oncol* (2021) 11. doi: 10.3389/fonc.2021.678333

16. Rousseau R. Forgotten founder of bibliometrics. *Nature* (2014) 510(7504):218–8. doi: 10.1038/510218e

17. Zupic I, Cater T. Bibliometric methods in management and organization. *Organizational Res Methods* (2015) 18(3):429–72. doi: 10.1177/1094428114562629

18. Durieux V, Gevenois PA. Bibliometric indicators: Quality measurements of scientific publication. *Radiology* (2010) 255(2):342–51. doi: 10.1148/radiol.09090626

19. Epstein JI, Amin MB, Beltran H, Lotan TL, Mosquera JM, Reuter VE, et al. Proposed morphologic classification of prostate cancer with neuroendocrine differentiation. *Am J Surg Pathol* (2014) 38(6):756–67. doi: 10.1097/PAS.0000000000000208

20. Ishida E, Nakamura M, Shimada K, Tasaki M, Konishi N. Immunohistochemical analysis of neuroendocrine differentiation in prostate cancer. *Pathobiology* (2009) 76(1):30–8. doi: 10.1159/000178153

21. Meho LI, Sonnenwald DH. Citation ranking versus peer evaluation of senior faculty research performance: A case study of Kurdish scholarship. *J Am Soc Inf Sci* (2000) 51(2):123–38. doi: 10.1002/(SICI)1097-4571(2000)51:2<123::AID-ASI4>3.0.CO;2-N

22. Buela-Casal G, Zych I. Analysis of the relationship between the number of citations and the quality evaluated by experts in psychology journals. *Psicothema* (2010) 22(2):270–5. Available at: http://wwwpsicothemacom/pmidlookupasp?pmid=20423632.

23. Beltran H, Prandi D, Mosquera JM, Benelli M, Puca L, Cyrta J, et al. Divergent clonal evolution of castration-resistant neuroendocrine prostate cancer. *Nat Med* (2016) 22(3):298–305. doi: 10.1038/nm.4045

24. Hirano D, Okada Y, Minei S, Takimoto Y, Nemoto N. Neuroendocrine differentiation in hormone refractory prostate cancer following androgen deprivation therapy. *Eur Urol* (2004) 45(5):586–92. doi: 10.1016/j.eururo.2003.11.032

25. Vashchenko N, Abrahamsson PA. Neuroendocrine differentiation in prostate cancer: Implications for new treatment modalities. *Eur Urol* (2005) 47(2):147–55. doi: 10.1016/j.eururo.2004.09.007

26. Chen RQ, Dong XS, Gleave M. Molecular model for neuroendocrine prostate cancer progression. *Bju Int* (2018) 122(4):560–70. doi: 10.1111/bju.14207

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 21 March 2023
DOI 10.3389/fonc.2023.1129028

OPEN ACCESS

EDITED BY
Ran Xu,
Second Xiangya Hospital, Central South University, China

REVIEWED BY
André Vis,
VU Medical Center, Netherlands
Kate Bolam,
Swedish School of Sport and Health Sciences, Sweden

*CORRESPONDENCE
Alberto J. Alves
ajalves@umaia.pt

SPECIALTY SECTION
This article was submitted to Genitourinary Oncology, a section of the journal Frontiers in Oncology

RECEIVED 21 December 2022
ACCEPTED 10 March 2023
PUBLISHED 21 March 2023

CITATION
Capela A, Antunes P, Coelho CA, Garcia CL, Custódio S, Amorim R, Costa T, Vilela E, Teixeira M, Amarelo A, Silva J, Joaquim A, Viamonte S, Brito J and Alves AJ (2023) Effects of walking football on adherence, safety, quality of life and physical fitness in patients with prostate cancer: Findings from the PROSTATA_MOVE randomized controlled trial.
Front. Oncol. 13:1129028.
doi: 10.3389/fonc.2023.1129028

Effects of walking football on adherence, safety, quality of life and physical fitness in patients with prostate cancer: Findings from the PROSTATA_MOVE randomized controlled trial

Andreia Capela[1,2], Pedro Antunes[1,3], César André Coelho[1], Catarina Laranjeiro Garcia[1,4], Sandra Custódio[1,2], Rui Amorim[2], Telma Costa[1,2], Eduardo Vilela[2], Madalena Teixeira[2], Anabela Amarelo[1,2], Joana Silva[1,2], Ana Joaquim[1,2], Sofia Viamonte[1,2], João Brito[5] and Alberto J. Alves[1,4]*

[1]ONCOMOVE® – Associação de Investigação de Cuidados de Suporte em Oncologia (AICSO), Vila Nova de Gaia, Portugal, [2]Centro Hospitalar Vila Nova de Gaia – Espinho, Entidade pública empresaríal (EPE), Vila Nova de Gaia, Portugal, [3]Research Center in Sport Sciences, Health and Human Development (CIDESD), Sport Sciences Department, University of Beira Interior, Covilhã, Portugal, [4]Research Center in Sport Sciences, Health and Human Development (CIDESD), Physical Education and Sport Sciences Department, University of Maia, Maia, Portugal, [5]Portugal Football School, Federação Portuguesa de Futebol, Oeiras, Portugal

Aims: To analyze the feasibility and impact of a walking football (WF) program on quality of life (QoL), cardiorespiratory fitness (CRF), muscle strength, and balance program in men with prostate cancer under androgen deprivation therapy (ADT).

Methods: Fifty patients with prostate cancer (stages IIb-IVb) under ADT were randomized to a 16-week WF program plus usual care (n=25) or usual care control group (n=25). The WF program consisted of three 90-minute sessions per week. Recruitment, withdrawal, adherence, enjoyment rate, and safety of the intervention were recorded throughout the study. Cardiorespiratory fitness was assessed before and after the interventions, while handgrip strength, lower limb muscle strength, static balance, and QoL were assessed before, during (week 8), and after (week 16) the interventions. Adverse events during sessions were also recorded.

Results: The WF group showed high levels of adherence (81.6 ± 15.9%) and enjoyment rate (4.5 ± 0.5 out of 5 points). In the intention-to-treat analysis, the WF group showed an improvement in chair sit-to-stand (p=0.035) compared to the control group. Within-group comparisons showed that handgrip strength in the dominant upper limb (p=0.024), maximal isometric muscle strength in the non-dominant lower limb (p=0.006), and balance in the dominant limb (p=0.009) improved over time in the WF group but not in the usual care group. The results obtained from the per-protocol analysis indicate that CRF improved significantly in the WF group as compared to the control group (p=0.035). Within-group analysis revealed that CRF (p=0.036), muscle strength in dominant (p=0.006) and non-dominant (p=0.001) lower limbs, and balance in

the non-dominant lower limb (p=0.023) improved after 16 weeks of WF, but not in the control group. One major traumatic injury (muscle tear) was reported with a complete recovery before the end of the intervention.

Conclusion: This study suggests that WF is feasible, safe, and enjoyable in patients with prostate cancer under hormonal therapy. Furthermore, patients who adhere to the WF program can expect cardiorespiratory fitness, muscle strength, and balance improvements.

Clinical trials registration: clinicaltrials.gov, identifier NCT04062162.

KEYWORDS

walking football, adherence, safety, quality of life, physical fitness, prostate cancer, rct

Introduction

Prostate cancer is the second most frequent cancer and the fifth leading cause of death from cancer in men worldwide (1). With the purpose of delaying disease progression and enhancing survival, ADT is widely used as a stand-alone treatment or in conjunction with radiation therapy or radical prostatectomy (2). However, despite its undeniable clinical importance, the use of ADT is associated with a vast spectrum of potential side effects (namely loss of muscle mass, bone mass, and physical functionality, increases in fat mass, fatigue, worse metabolic, glycemic, and cardiovascular profile) that considerably reduce QoL. Importantly, an increasing number of patients might be on ADT for prolonged periods and might survive several years following the cessation of the treatments (3). Therefore, it is crucial to implement preventive strategies that contribute to mitigating the toxicity of ADT (4).

Exercise has been proposed as a non-pharmacological useful and viable strategy to counteract some adverse effects of androgen deprivation therapy (5). Exercise has been included in the clinical guidelines from the European Society of Medical Oncology (4), European Association of Urology (6), and the American Society of Clinical Oncology (7). To date, most randomized controlled trials evaluating exercise programs in patients under ADT comprised structured supervised or home-based interventions that commonly combined traditional aerobic (such as walking, jogging, or bicycling) and strength training (8). Despite promising results, such programs may be inadequate to engage and maintain men with prostate cancer in long-term interventions (9). Moreover, permanent behavioral changes concerning engagement with regular physical activity might be difficult to implement in a real-world setting. Indeed, recent data suggest that men with prostate cancer prefer to exercise in a structured group environment, which appears to facilitate the uptake of exercise programs and enhance long-term adherence in this patient population (10). Therefore, developing novel interventions that combine patients' needs, characteristics, and preferences is important.

The popularity of football worldwide, especially among men, appeals to its potential as a health-enhancing recreational physical activity. Currently, several studies on patients with prostate cancer provide interesting results about the multiple beneficial effects of recreational football-based interventions on distinct health outcomes (11, 12), and it is well-established that playing recreational football can also promote enjoyment and positive effects on mental and social well-being (13). However, given its intermittent nature, vigorous efforts, and the possible risk of injuries (due to the potential contact between participants, duels, and tackles), clinicians might be cautious about recommending recreational football practice in patients with prostate cancer undergoing ADT. Adverse events associated with recreational football practice have been reported and might constitute a relevant barrier to the implementation of such programs in these patients (14, 15), who are typically characterized by advanced age, low physical activity levels, and poor fitness (16). To try to minimize potential risks, injuries, and side effects, an adapted version of football has emerged over recent years. Walking football (WF) adheres to the general rules of football, but participants are not allowed to run or engage in physical contact with each other (17). Studies showed that WF programs generally presented high levels of adherence and enjoyment (18–21), and the low rate of adverse events described suggests that it is a feasible and safe exercise strategy (22). In the advanced prostate cancer population, bone metastasis (23) and osteoporosis (6, 24) can be a major concern in the implementation of recreational football practices.

The intensity of WF training characterizes it as generally a light-to-vigorous physical activity (22), which led to promising results on body composition, aerobic fitness, and blood pressure in middle-aged and older individuals (21, 25). However, the effectiveness of WF practice has not been tested in men with prostate cancer undergoing ADT. Given this background, the main aim of this study was to analyze the quality of life and feasibility of a WF program in men with prostate cancer undergoing ADT. The secondary aim was to measure the impact of WF practice on CRF, muscle strength, and balance.

Methods

Study design

This study was a prospective randomized clinical trial, with a parallel 2-arm group design. Patients were recruited by physicians of the Oncology and Urology departments of the Vila Nova de Gaia-Espinho Hospital Centre, Portugal. Patients were randomly allocated to a 16-week WF program plus usual care (intervention group) or usual care alone (control group). Primary and secondary outcomes were assessed at baseline, after 8 weeks of intervention, and 2 days after 16 weeks of intervention, except for CRF, which was assessed only at baseline and after the 16-week intervention. All patients provided written informed consent. The study was approved by the hospital ethics committee (50/2019-2) and registered in clinicaltrials.gov (NCT04062162).

Participants

Adult patients with prostate cancer undergoing ADT for at least 6 months were enrolled in the study if they presented the following inclusion criteria (1): patients treated with radical prostatectomy more than one month passed the procedure and with approval from the urologist (2); patients previously treated with prostatic radiotherapy, at least one month after the end of radiotherapy treatment and with approval from the oncologist; (3) adult patients undergoing hormone therapy with a luteinizing hormone-releasing hormone (LHRH) analogue or antagonist as an initial approach or in the setting of biochemical recurrence. Exclusion criteria included osteoporosis (spine or femur T score of -2.5 or lower) and contraindications for exercise training such as acute coronary syndromes, acute endocarditis, myocarditis or pericarditis, decompensated heart failure, severe aortic stenosis, uncontrolled arrhythmia, uncontrolled hypertension, or any physical disability that precludes safe and adequate exercise testing and training according to the attending physician's assessment (26). All participants were evaluated by a rehabilitation medicine specialist before study entry.

Randomization and allocation

Permuted block randomization was generated with balanced groups (1:1), and strata were defined by age (lower and greater than 65 years) using electronic software (www.sealedenvelope.com).

Outcomes

The primary outcomes were QoL and feasibility assessed by the recruitment rate (the number of invited patients divided by the number of those enrolled), acceptability (number of patient withdrawals and dropouts), adherence (number of sessions attended, number of sessions missed and level of enjoyment) and retention (the number of patients who completed all the exercise sessions divided by the number of patients allocated to the exercise group) of the WF program. The level of enjoyment with the WF program was assessed by a Likert scale (1-not at all satisfied to 5-totally satisfied). Secondary outcomes included CRF, muscle strength, balance, and adverse effects during/after the exercise sessions (e.g., falls and injuries).

Procedures

Clinical and demographic data

Socio-demographic and clinic-pathologic data were collected through patient clinical records.

Quality of life

QoL was assessed using the European Organization for Research and Treatment of Cancer (EORTC) quality of life scale – QLQ30, and its specific module for prostate cancer – PR25 (27).

Cardiorespiratory fitness

CRF was assessed at baseline and after 16 weeks of intervention through a symptom-limited treadmill exercise stress test on a treadmill using a Bruce protocol, and metabolic equivalents (METs) were calculated according to the stage of protocol and time reached at peak exercise. The maximum heart rate (HR) achieved was also recorded for the determination of the intensity of exercise sessions.

Muscle Strength

Maximum voluntary handgrip strength was measured using a digital hand dynamometer (Saehan model SH1001, DHD-1, Saehan Corp. South Korea). Each participant performed a total of 6 trials, 3 on each hand, with an alternating bilateral sequence. Before each trial, the position of the limb was adjusted so that each participant placed the elbow flexed at a 90° angle with the wrist as close to 0° as possible. The average of the respective tests on each member was determined for analysis.

Maximum isometric muscle strength of the knee extensors was measured on both limbs with a digital dynamometer (Advanced Force Gauge, 2500N, Mecmesin Limited, Slinfold, West Sussex, United Kingdom). The participant remained seated during the test with the lower limb flexed at 90°. Two repetitions were performed on each limb and the average value was recorded.

The 30-second chair sit-to-stand test was also used to evaluate muscle strength and endurance of the lower limbs (28). Each participant was instructed to stand up and sit as many times as possible on a 40-cm-high chair for 30 seconds, keeping arms crossed close to the chest (28). The result was determined by the number of repetitions.

Balance

The single-leg stance test with eyes open was used to assess static balance in the dominant and non-dominant limbs. Each participant remained with their arms crossed over their chests and supported in one leg for as long as possible. Time recording began when the patient raised the foot from the floor and ended

when the patient either (1): uncrossed his arms, (2) moved the raised foot or touched the floor, (3) moved the weight-bearing foot, and (4) reached the maximum 45-second time (29). An average of 3 trials were recorded for each limb.

Safety

Adverse effects (AEs) during WF practice were recorded and classified according to the consensus defined by Fuller et al. (30), and their severity was graded. Data on location, type, body side, mechanism of injury (traumatic or overuse), recurrence, time of intervention, the context of the injury (e.g., contact with another participant or object), breach of protocol rules, time until reintegration into an exercise routine, number of missed sessions, need for medical evaluation, date, and description of circumstances of occurrence) were recorded.

Study intervention

The exercise intervention consisted of 3 weekly sessions of WF, on non-consecutive days, for a period of 16 weeks (a total of 48 sessions). The exercise sessions took place at an indoor sports hall, and were divided into four sequential phases (1): a warm-up phase that involved joint mobility exercises and balance exercises (15 min); (2) a skill-developing phase where patients developed football-specific technical skills, such as passing, dribbling, and shooting, as well as fundamental motor skills, including aerobic power, muscular endurance and balance (50 min); (3) a structured small sided game (e.g., 7 vs. 7 or 5 vs. 5) of WF (20 min); and (4) a cool-down phase (5 min). The training sessions were designed, planned, and supervised by a certified football coach (UEFA B license) and two exercise physiologists.

Exercise intensity was continuously monitored during sessions with HR monitors (Firstbeat Sports, Firstbeat Sport®, Finland). Maximum HR was recorded during baseline maximal exercise testing to calculate the intensity of exercise sessions. Effort during exercise sessions was controlled by the rating of perceived exertion (RPE) through the Borg 6-20 scale (minimum effort = 6; maximum effort = 20). Participants were encouraged to exercise with moderate-to-vigorous intensity, as recommended for adults and older adults (64-76% to 77-95% of maximum HR, reporting 12-17 ["a little difficult" to "very difficult"] Borg 6-20 scale) (31). The amount of time spent in very light (1-56%), light (57-63%), moderate (64-76%), vigorous (77-95%), and maximum exercise intensity (96-100%) was determined based on maximum HR, obtained during the treadmill exercise stress test, according to the American College of Sports Medicine (ACSM) physical activity recommendations for adults (31). The control group had only usual medical care, which involves routine follow-up appointments with the attending physician, regular assessments of blood count and bone mineral density, as well as general counseling on issues related to physical inactivity and weight gain. In patients with metastatic prostate cancer, usual care additionally encompasses bone scintigraphy and positron emission tomography/computed tomography (PET/CT) assessments. However, there was no provision for physical activity support as part of the usual care. This group was offered the opportunity of joining the WF program after the 16-week study period. However, although patients of the control group were enrolled later in the WF program, their participation had to be cancelled due to the start of the COVID-19 pandemic.

Statistical analysis

Exploratory data analysis and Shapiro-Wilk tests were performed to determine the normality of the data distribution. Continuous variables are expressed as mean (SD) or median (interquartile range), whereas for categorical variables, counts and percentages are presented. Between-group differences at baseline were tested with unpaired student-t tests or chi-square tests. Two-factor mixed ANOVA was used to assess the effect of the intervention over time across groups in variables with normal distribution and paired-sample ANOVA was performed for within-group comparisons from baseline to the end of the study. Friedman and Wilcoxon's tests were used for within-group comparisons in variables with no normal distribution. Furthermore, we performed a per-protocol analysis including only patients with adherence of 70% or greater to the scheduled exercise sessions. All analyses were conducted with SPSS version 24.0 (SPSS Inc., Chicago, IL, USA). The level of significance was set as $P < 0.05$.

Results

Participants

Of the 50 patients who were considered eligible to participate in the study (Figure 1), 3 refused to participate and 10 were excluded due to electrocardiographic changes during exercise testing. In addition, 2 patients in the exercise group discontinued the intervention and 1 was excluded due to a *de novo* gastric cancer diagnosis. Also, 2 patients in the control group missed follow-up assessments and 1 patient had disease progression. In total, 31 patients were included in the analysis, 16 in the WF group and 15 in the control group. The patient's characteristics are shown in Table 1. Patients were mostly older adults (71.8 ± 5.9 years) with excess body weight (Body Mass Index: 28.3 ± 4.1 kg/m^2), with locally advanced or metastatic cancer (stages III-IV). Patients were submitted to chemotherapy (6.5%), radical prostatectomy (25.8%), radiation therapy (67.7%), and hormonotherapy (100%). No differences were found between groups at baseline concerning patient sociodemographic and clinic-pathologic characteristics.

Feasibility

Two patients (8%) out of the 25 patients from the WF group withdrew their informed consent before participation, and 2 (11%) discontinued their participation from the 19 patients who initiated the program. The remaining patients (n=16) in the WF group

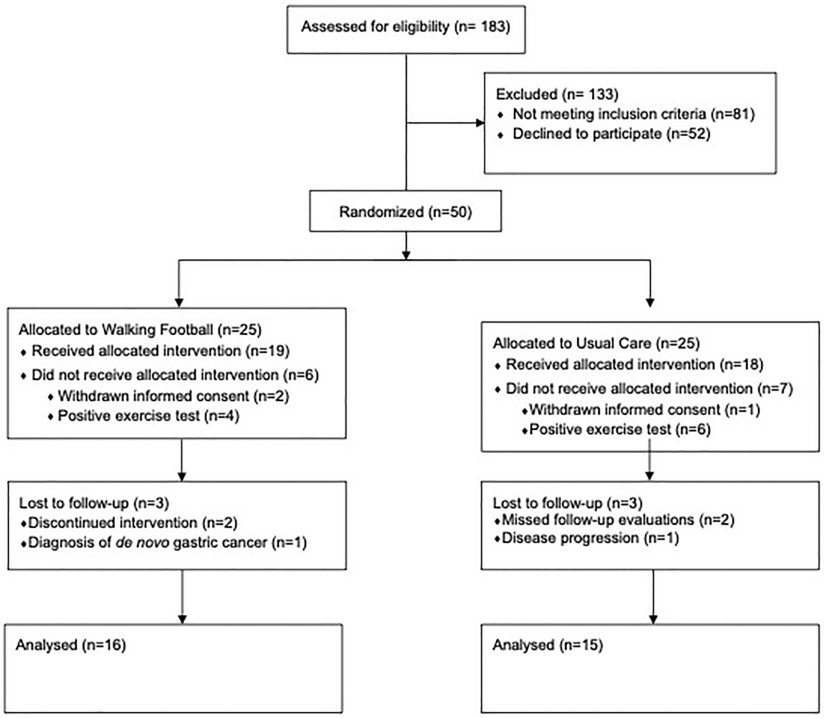


FIGURE 1
Flow diagram depicting the study design.

attended on average 38 ± 8 training sessions. This corresponded to 81.6 ± 15.9% of the total number of training sessions. The median attendance was 90% (minimum 53% and maximum 98%), with none of the patients completing all the training sessions. Three participants attended less than 70% of the sessions. Moreover, a mean of 13 ± 1 patients attended the sessions, and patients' level of enjoyment with the training sessions was very high (4.5 ± 0.5 points on the Likert scale).

Characteristics of the training sessions

Patients showed a mean of 101.9 ± 13.1 bpm during training sessions, which corresponded to 72.8 ± 10.7% of maximum HR. Most of the time of the training sessions was spent on moderate (38.1 ± 16.8 minutes, 45.9 ± 19.4%) and vigorous (22.4 ± 21.5 minutes, 26.8 ± 25.1%) exercise intensity, followed by light (14.7 ± 13.1 minutes, 17.9 ± 15.8%), very light (5.6 ± 10.3 minutes, 6.8 ± 12.2%) and maximum exercise intensities (2.1 ± 6.4 minutes, 2.7 ± 8.4%). The mean perceived exercise effort during the sessions was 13.5 ± 2.6 points on Borg the Scale.

Quality of life

No differences at baseline were observed in the overall score of health-related QoL (EORTC-QLQ-C30) between the intervention and control groups (p=0.883). Moreover, no changes over time were observed in the overall quality of life score in the WF group (median, IQR: 83.3, 66.7-100.0 vs. 83.3, 68.8-100.0 vs. 83.3, 54.2-100.0, p=0.462) or the control group (median, IQR: 83.3, 58.3-91.7 vs. 83.3, 66.7-100.0 vs. 83.3, 45.8-100.0, p=0.462). No differences were also found for any scale subitem except for diarrhea (Table 2). Per-protocol analysis showed no differences in QoL over time when only adherent patients were considered.

Cardiorespiratory fitness

No differences across treatment groups were observed in CRF at baseline (8.1 ± 1.7 vs. 8.0 ± 1.5 METs p=0.865) and between groups over time (-0.1 ± 0.5 vs. 0.3 ± 0.8 METs, p=0.147). However, a per-protocol analysis revealed that, when patients who attended less than 70% of the sessions were excluded from the analysis (n=3), there was a significant difference between groups (p=0.035), with CRF improving in the WF group from baseline to 16 weeks (8.2 ± 1.6 vs. 8.6 ± 1.5 METs, p=0.036) but not in the control group (8.1 ± 1.7 vs. 8.1 ± 1.7 METs, p=0.597).

Muscle strength

No differences were found between groups in handgrip strength and isometric maximal strength in both lower limbs, both at

TABLE 1 Patient baseline characteristics.

	Usual Care (n=15)	Walking Football (n=16)	*P* value
Age (years)	70.7 ± 6.9	72.8 ± 4.9	0.342
Weight (kg)	81.8 ± 16.3	78.8 ± 10.2	0.539
Height (cm)	168.9 ± 5.8	168.8 ± 6.5	0.977
Body mass index (kg/m^2)	28.6 ± 5.0	27.9 ± 3.0	0.646
Cancer history			
Disease Stage			0.361
• II	1 (6.7%)	3 (18.8%)	
• III	7 (46.7%)	9 (56.3%)	
• IV	7 (46.7%)	4 (25.0%)	
PSA (ng/mL)	0.6 ± 1.5	1.7 ± 5.9	0.490
Prostatectomy (n, %)	5 (31.3%)	3 (18.8%)	0.354
Orchiectomy (n, %)	0 (0%)	0 (0%)	–
Hormonotherapy (n, %)	15 (100%)	16 (100%)	–
Chemotherapy (n, %)	0 (0%)	2 (12.5%)	0.157
Radiotherapy (n, %)	10 (66.7%)	11 (68.8%)	0.901
Current ADT (n, %)			0.163
• LHRH agonist	12 (80.0%)	14 (87.5%)	
• LHRH agonist and Bicalutamide	2 (13.3%)	0 (0%)	
• LHRH agonist and Enzalutamide	0 (0%)	2 (12.5%)	
• LHRH agonist and Abiraterone	1 (6.7%)	0 (0%)	
ADT time (weeks)	36.1 ± 43.5	23.8 ± 12.2	0.291
Bone Metastases	5 (33.3%)	3 (18.8%)	0.354
Comorbidities			
Diabetes (n, %)	3 (18.8%)	5 (31.3%)	0.414
Hypertension (n, %)	8 (50.0%)	12 (75.0%)	0.144
Hypercholesterolemia (n, %)	8 (50.0%)	8 (50.0%)	1.000
COPD (n, %)	1 (6.7%)	0 (0%)	0.310
Prior CVD (n, %)	1 (6.3%)	2 (12.5%)	0.612
Depression (n, %)	1 (20.0%)	1 (14.3%)	0.906
Smoking (n, %)	1 (6,7%)	1 (12,5%)	1.000

PSA, Prostate Specific Antigen; PCa, Prostate Cancer; ADT, Androgen Deprivation Therapy; LHRH, luteinising hormone-releasing hormone; COPD, Chronic obstructive pulmonary disease; CVD, Cardiovascular Disease.

baseline and in the changes over time (Table 3). Nonetheless, within-group comparisons showed that handgrip strength and maximal isometric muscle strength in the non-dominant lower limb improved after 8 weeks of WF practice, while no changes over time were observed in the control group.

The per-protocol analysis showed no differences between groups in terms of changes over time in both the dominant (p=0.94) and non-dominant handgrip strength (p=0.37), as well as the dominant (p=0.15) and non-dominant leg strength (p=0.09). However, the WF group improved maximal isometric leg strength in dominant (24.5 ± 5.1 vs. 28.0 ± 5.6 vs. 27.7 ± 4.9 kgf, p=0.006) and non-dominant limbs (24.1 ± 7.2 vs. 27.7 ± 8.3 vs. 28.8 ± 9.5 kgf, p=0.001), but not the control group (23.4 ± 6.9 vs. 22.9 ± 6.4 vs. 24.5 ± 8.1 kgf, p=0.510; 23.6 ± 6.8 vs. 22.9 ± 6.3 vs. 24.6 ± 8.3 kgf, p=0.517).

TABLE 2 Changes over time in health-related quality of life in walking football and usual care groups.

	Usual Care (*N*=15)				Walking Football (*N*=16)			
	Baseline	8 weeks	16 weeks	*p*-value	Baseline	8 weeks	16 weeks	*p*-value
EORTC QLQ-C30								
Quality of Life Global Health Status	83.3 (58.3-91.7)	83.3 (66.7-100.0)	83.3 (45.8-100.0)	0.462	83.3 (66.7-100.0)	83.3 (68.8-100.0)	83.3 (54.2-100.0)	0.674
Functional scales								
Physical functioning	93.3 (73.3-93.3)	86.7 (73.3-93.3)	93.3 (73.3-100.0)	0.167	90.0 (86.7-93.3)	93.3 (86.7-100.0)	93.3 (86.7-100.0)	0.250
Role functioning	100.0 (100.0-100.0)	100.0 (100.0-100.0)	100.0 (100.0-100.0)	0.197	100.0 (100.0-100.0)	100.0 (100.0-100.0)	100.0 (100.0-100.0)	0.336
Emotional functioning	91.7 (75.0-91.7)	91.7 (75.0-100.0)	91.7 (75.0-100.0)	0.384	95.8 (75.0-100.0)	91.7 (75.7-100.0)	95.8 (68.8-100.0)	0.537
Cognitive functioning	83.3 (66.7-100.0)	83.3 (83.3-100.0)	83.3 (83.3-100.0)	0.886	83.3 (83.3-100.0)	100.0 (10.8-100.0)	91.7 (83.3-100.0)	0.478
Social functioning	100.0 (100.0-100.0)	100.0 (100.0-100.0)	100.0 (100.0-100.0)	0.705	100.0 (83.3-100.0)	100.0 (10.8-100.0)	100.0 (100.0-100.0)	0.098
Symptom scales								
Fatigue	22.2 (0.0-33.3)	11.1 (0.0-22.2)	0.0 (0.0-11.1)	0.207	16.7 (0.0-30.6)	11.1 0.0-22.2)	0.0 (0.0-16.7)	0.085
Nausea and Vomiting	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.317	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	1.000
Pain	16.7 (0.0-16.7)	16.7 (0.0-16.7)	16.7 (0.0-16.7)	0.317	0.0 (0.0-16.7)	0.0 (0.0-16.7)	16.7 (0.0-16.7)	0.132
Dyspnea	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.447	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	1.000
Insomnia	0.0 (0.0-33.3)	0.0 (0.0-33.3)	0.0 (0.0-33.3)	0.414	0.0 (0.0-33.3)	0.0 (0.0-33.3)	0.0 (0.0-33.3)	0.705
Appetite loss	0.0 (0.0-0.0)	0.0 (0.0-33.3)	0.0 (0.0-33.3)	1.000	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.317
Constipation	0.0 (0.0-33.3)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.317	0.0 (0.0-25.0)	0.0 (0.0-33.3)	0.0 (0.0-0.0)	0,257
Diarrhea	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.564	0.0 (0.0-0.0)	0.0 (0.0-33.3)	0.0 (0.0-0.0)	0.046
Financial difficulties	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.807	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	1.000
EORTC PR25								
Symptoms scales								
Urinary symptoms	87.5 (69.8-95.8)	83.3 (79.2-91.7)	91.7 (75.0-95.8)	0.127	91.7 (87.5-95.8)	89.6 (84.4-95.8)	91.7 (88.5-100.0)	0.927
Incontinence aid	100.0 (100.0-100.0)	100.0 (100.0-100.0)	100.0 (91.7-100.0)	0.317	100.0 (100.0-100.0)	100.0 (100.0-100.0)	100.0 (100.0-100.0)	0.317
Bowel symptoms	100.0 (91.7-100.0)	100.0 (91.7-100.0)	100.0 (91.7-100.0)	0.565	100.0 (91.7-100.0)	100.0 (91.7-100.0)	100.0 (100.0-100.0)	0.042
Hormonal treatment-related	83.3 (72.2-94.4)	83.3 (77.8-94.4)	94.4 (83.3-94.4)	0.132	94.4 (77.8-100.0)	86.1 (79.1-98.6)	91.7 (83.3-100.0)	0.667
Functional scales								
Sexual activity	16.7 (0.0-33.3)	16.7 (0.0-33.3)	66.7 (50.0-100.0)*†	0.000	16.7 (0.0-33.3)	16.7 (0.0-33.3)	83.3 (66.7-100.0) *†	<0.001
Sexual functioning	50.0 (29.1-58.3)	58.3 (25.0-75.0)	70.8 (31.3-87.5)	0.127	58.3 (41.7-75.0)	50.0 (41.7-66.7)	33.3 (25.0-72.9)	0.497

Data is presented as median (25^{th}-75^{th} quartiles); *P<0.01 (vs. 8 weeks); † P<0.01 (vs. baseline).

Moreover, there were significant differences between groups in the number of repetitions completed during the 30-sec chair sit-to-stand test over time (p=0.035). While the control group's performance remained unchanged, the WF group showed improved performance in the 30-sec chair sit-to-stand test (p<0.001). Results did not change with per-protocol analysis.

Balance

Changes in balance over time among WF and control groups are depicted in Table 4. There was no significant difference in balance at baseline between the groups. Within-group comparisons showed that balance in the dominant leg improved after 8 weeks

TABLE 3 Changes over time in muscle strength in walking football and usual care groups.

	Usual Care (*N*=15)				Walking Football (*N*=16)				
	Baseline	8 weeks	16 weeks	Time *p*-value	Baseline	8 weeks	16 weeks	Time *p*-value	Time*Group *p*-value
Muscle Strength									
Handgrip strength, dominant limb (kgf)	29.1 ± 6.4	30.8 ± 4.3	30.9 ± 5.4	0.217	30.7 ± 4.8	36.0 ± 10.5*	32.2 ± 4.6	0.024	0.880
Handgrip strength, non-dominant limb (kgf)	28.0 ± 6.4	29.8 ± 5.2	30.1 ± 5.2	0.146	29.4 ± 4.8	29.6 ± 5.1	30.2 ± 5.8	0.593	0.467
Lower body strength, dominant limb (kgf)	23.4 ± 6.9	22.9 ± 6.4	24.5 ± 8.1	0.581	24.3 ± 4.7	27.2 ± 6.3	26.2 ± 5.7	0.080	0.221
Lower body strength, non-dominant limb (kgf)	23.6 ± 6.8	22.9 ± 6.3	24.6 ± 8.3	0.517	23.9 ± 6.6	26.7 ± 8.0*	27.3 ± 9.1	0.006	0.173
Chair sit-to-stand (number of repetitions)	11.0 ± 2.0	11.9 ± 2.2	11.7 ± 2.9	0.412	13.8 ± 2.9	16.4 ± 3.6**	17.4 ± 4.7**	<0.001	0.035

*Significantly higher than baseline; $p<0.05$; **Significantly higher than baseline; $p<0.01$.

and 16 weeks of WF practice ($p=0.009$) but remained unchanged in the control group. No differences were found in the non-dominant leg in both groups. After excluding non-exercise adherent patients (per-protocol analysis) from the walking football group, balance improved significantly after 8 weeks and 16 weeks of intervention in the non-dominant leg (median, IQR: 8.1, 3.3-21.3 vs 17.8, 6.6-38.7 vs 19.2, 7.3-33.5, $p=0.023$) and dominant leg, although with borderline significance (median, IQR: 6.9, 3.2-21.8 vs 16.2, 10.4-33.0 vs 20.1, 10.3-27.0, $p=0.058$).

Safety

During the WF sessions, 11 patients had a total of 32 AEs. The maximum number of AEs during a single session per patient was 2. Most of the exercise-related events (n=28, 87.5%) occurred during the formal small-sided game setup (7 vs. 7 or 5 vs. 5 games), whereas the remaining 4 events (12.5%) happened during small-sided exercise drills. The majority was related to falls (n=24), which occurred in 10 patients. In most of the falls (n=21, 87.5%), there was no need for the training session interruption; in a small number of falls (n=3,12.5%) there was a momentaneous exercise interruption, but patients resumed the training session thereafter. Moreover, 1 patient reported fatigue on 3 different occasions (9.4%), and 1 patient reported joint pain (n=4, 12.5%), both of which interrupted temporarily the exercise sessions, and resumed after a recovery break. One traumatic injury was registered (hamstrings muscle tear); despite a complete recovery before the end of the intervention, the patient decided to discontinue exercise intervention. Nonetheless, this patient completed all the following assessments and was therefore included in the intention-to-treat analysis.

Discussion

This study showed that a 16-week program of WF was feasible, safe, and enjoyable. WF practice also significantly improved CRF, muscle strength, and balance in patients with prostate cancer under ADT who adhered to at least 70% of the scheduled exercise sessions. In addition, the results showed that this exercise program allows patients to meet or even overcome the minimal recommendations of physical activity to achieve health benefits (32).

TABLE 4 Changes over time in balance in walking football and control groups.

	Usual Care (*N*=15)				Walking Football (*N*=16)			
	Baseline	8 weeks	16 weeks	*p*-value	Baseline	8 weeks	16 weeks	*p*-value
Balance								
Dominant limb (sec)	14.2 (5.4-25.0)	14.4 (5.5-22.7)	16.2 (11.6-32.5)	0.262	8.9 (3.2-18.3)	16.3 (10.0-31.7)*	20.3 (10.1-27.6)*	0.009
Non-dominant limb (sec)	23.4 (2.7-32.7)	13.5 (7.5-25.4)	20.0 (3.8-35.2)	0.819	7.9 (3.5-22.4)	17.6 (4.9-34.7)	19.9 (7.0-34.3)	0.099

*Significantly higher than baseline; $p<0.01$.

A previous large multicenter study conducted in Denmark also showed that community-based football was a feasible exercise strategy in patients with prostate cancer, by achieving an elevated acceptance rate and retention for 12 weeks and 6 months of the program (33). The current WF program also demonstrated elevated retention. Two patients quit prematurely the program (11%), but compliance was high, as patients attended on average more than 80% of the sessions during 16 weeks. These results are consistent with the elevated level of satisfaction reported. In addition, WF practice was revealed to be safe for patients with prostate cancer, since most of the adverse events related to the exercise program were associated with falls; the great majority of adverse events did not motivate an interruption of the session, and when occurring patients resumed the training session. Only one major traumatic injury (muscle tear) was reported, motivating a permanent interruption of the intervention.

We also observed significant improvements in CRF, muscle strength, and balance in patients who were enrolled in the WF program and complied with at least 70% of the WF sessions. These results are especially relevant because cancer treatments, particularly ADT, can present an overall important burden, eliciting a negative impact on muscle mass and strength, CRF, functional decline, and fatigue (34, 35). It has been shown that prolonged ADT exposure is associated with reduced CRF and increased cardiovascular mortality in patients with prostate cancer (36). Also, muscle loss during hormone treatment is independently associated with increased non-cancer mortality (37). These data reinforce the potential relevance of improvements in physical fitness in prostate cancer patients under ADT. There is evidence showing that aerobic and resistance training can promote significant improvements in fat mass, lean mass, muscle strength, functional capacity, and CRF in patients with prostate cancer during and after treatment (38, 39). Our results add to the current evidence by suggesting that a WF program is an effective exercise strategy to increase physical fitness in patients with prostate cancer. It also shows that WF practice may promote improvements in balance. Notably, current and past patients under ADT are more than twice as likely to have fallen, whilst also presenting more recurrent falling and fall-related injuries compared to men who were never exposed; they are also more likely to be classified as pre-fail than non-users of ADT (40). A recent meta-analysis also concluded that the use of androgen receptor inhibitors is associated with an increased risk of falls and fractures in patients with prostate cancer (41). Even though this was not measured directly, the improvements observed in balance in the WF group suggest that WF practice may be an effective approach to prevent falls and fractures, particularly as most of our patients were older adults.

Previous meta-analyses including randomized clinical trials have shown that exercise training improves QoL in patients with prostate cancer under ADT (42, 43). A recent meta-analysis of 18 randomized controlled trials, including 1477 patients with prostate cancer undergoing androgen deprivation therapy, reported that supervised exercise therapy has a moderately positive effect on disease-specific quality of life compared to no exercise therapy (44). On the other hand, another recent meta-analysis comprising 17 randomized controlled trials, involving 1361 patients with prostate cancer who had received cancer treatment, concluded that exercise had a small effect on cancer-specific QoL, and no differences were observed between exercise modalities (45). In addition, like a previous report (33), we did not observe changes in health-related QoL in patients with prostate cancer that participated in WF practice. Differences in age, assessment methods, treatment regimens, and training programs may explain, at least in part, the discrepancies in results. Of mention, in the current study, patients reported relatively high values of overall QoL at baseline compared to the reference values (46), which may have potentially decreased the margin of improvement in wellbeing with the exercise training.

Limitations

The main limitation of this study is the greater-than-expected loss of patients (38%) after randomization. Although a few patients withdrew (n=3, 6%) their informed consent after being allocated to one of the two groups, and 6 (12%) patients were lost to follow-up, most patients (n=10, 20%) were not enrolled in the trial due to positive exercise tests. Despite this might have resulted in some loss of power, this well-controlled feasibility study highlights the importance of the baseline clinical assessment to determine the safety of exercise training programs in cancer patients, especially in older patients with prostate cancer under androgen deprivation therapy and with multiple cardiovascular comorbidities and cardiovascular risk factors. Osteoporosis is a possible consequence of hormonal therapy. However, our findings in terms of safety cannot be generalized to patients with osteoporosis as they were excluded from this study. Exercise training targeting the musculoskeletal system, involving impact loading exercises plus resistance training, has been shown to attenuate the decline in the spine and femoral neck bone mineral density in patients with prostate cancer (47). Walking football may also be an effective strategy to mitigate the adverse effects of hormonal therapy on bone health, but future studies must address the balance between the risks and benefits of this mode of exercise in this specific population.

Conclusions

This study suggests that WF is a safe, enjoyable, and feasible strategy to meet physical activity recommendations in patients with prostate cancer under hormonal therapy. In addition, cardiorespiratory fitness, muscle strength, and balance are likely to improve in patients who show good adherence to WF.

Data availability statement

The datasets presented in this article are not readily available because Dataset will be available for researchers who provide a methodologically sound proposal. Requests to access the datasets should be directed to AJA, ajalves@umaia.pt.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics committee of Vila Nova de Gaia-Espinho Hospital Centre. The patients/participants provided their written informed consent to participate in this study.

Author contributions

AC, PA, EV, AJ, SV, JB, and AJA conceptualized the trial and were responsible for designing the study and the plan for analysis. AC, SC, PA, EV, AJ, SV, JB, and AJA led the implementation of the study design. AC, SC, RA, AA, JS, AJ, and SV were responsible for patient recruitment and clinical evaluation. AC, PA, CC, CG, SC, RA, TC, EV, MT, AA, JS, AJ, SV, JB, and AJA were responsible for collecting data, monitoring participants, and supervising the exercise intervention. The manuscript was written, read, and edited by all authors. All authors contributed to the article and approved the submitted version.

Funding

Research Center in Sports Sciences, Health and Human Development (CIDESD) is supported by the Portuguese Foundation for Science and Technology (Ref No. UID/DTP/04045/2020).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Rawla P. Epidemiology of prostate cancer. *World J Oncol* (2019) 10(2):63. doi: 10.14740/wjon1191

2. D'Amico AV, Chen MH, Renshaw AA, Loffredo M, Kantoff PW. Androgen suppression and radiation vs radiation alone for prostate cancer: A randomized trial. *JAMA* (2008) 299(3):289–95. doi: 10.1001/jama.299.3.289

3. Nguyen PL, Alibhai SM, Basaria S, D'Amico AV, Kantoff PW, Keating NL, et al. Adverse effects of androgen deprivation therapy and strategies to mitigate them. *Eur Urol* (2015) 67(5):825–36. doi: 10.1016/j.eururo.2014.07.010

4. Parker C, Castro E, Fizazi K, Heidenreich A, Ost P, Procopio G, et al. Prostate cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol* (2020) 31(9):1119–34. doi: 10.1016/j.annonc.2020.06.011

5. Edmunds K, Tuffaha H, Scuffham P, Galvão DA, Newton RU. The role of exercise in the management of adverse effects of androgen deprivation therapy for prostate cancer: A rapid review. *Supportive Care Cancer* (2020) 28(12):5661–71. doi: 10.1007/s00520-020-05637-0

6. Mottet N, van den Bergh RC, Briers E, Van den Broeck T, Cumberbatch MG, De Santis M, et al. EAU-EANM-ESTRO-ESUR-SIOG guidelines on prostate cancer–2020 update. part 1: Screening, diagnosis, and local treatment with curative intent. *Eur Urol* (2021) 79(2):243–62. doi: 10.1016/j.eururo.2020.09.042

7. Ligibel JA, Bohlke K, May AM, Clinton SK, Demark-Wahnefried W, Gilchrist SC, et al. Exercise, diet, and weight management during cancer treatment: ASCO guideline. *J Clin Oncol* (2022) 2491–2507. doi: 10.1200/OP.22.00277

8. Bourke L, Smith D, Steed L, Hooper R, Carter A, Catto J, et al. Exercise for men with prostate cancer: A systematic review and meta-analysis. *Eur Urol* (2016) 69 (4):693–703. doi: 10.1016/j.eururo.2015.10.047

9. Toohey K, Hunter M, Paterson C, Mortazavi R, Singh B. Exercise adherence in men with prostate cancer undergoing androgen deprivation therapy: A systematic review and meta-analysis. *Cancers* (2022) 14(10):2452. doi: 10.3390/cancers14102452

10. Sattar S, Haase K, Bradley C, Papadopoulos E, Kuster S, Santa Mina D, et al. Barriers and facilitators related to undertaking physical activities among men with prostate cancer: A scoping review. *Prostate Cancer prostatic Dis* (2021) 24(4):1007–27. doi: 10.1038/s41391-021-00399-0

11. Brito J, Williams CA, Randers MB. Executive summary: Recreational football training as medicine. *Scand J Med Sci Sports* (2018) 28 Suppl 1:74–6. doi: 10.1111/sms.13246

12. Bjerre ED, Weller S, Poulsen MH, Madsen SS, Bjerre RD, Østergren PB, et al. Safety and effects of football in skeletal metastatic prostate cancer: A subgroup analysis of the FC prostate community randomised controlled trial. *Sports medicine-open* (2021) 7(1):1–7. doi: 10.1186/s40798-021-00318-6

13. Krustrup P, Krustrup BR. Football is medicine: it is time for patients to play! *Br J Sports Med* (2018) 52(22):1412–14. doi: 10.1136/bjsports-2018-099377

14. Uth J, Hornstrup T, Christensen JF, Christensen KB, Jorgensen NR, Schmidt JF, et al. Efficacy of recreational football on bone health, body composition, and physical functioning in men with prostate cancer undergoing androgen deprivation therapy: 32-week follow-up of the FC prostate randomised controlled trial. *Osteoporos Int* (2016) 27 (4):1507–18. doi: 10.1007/s00198-015-3399-0

15. Uth J, Hornstrup T, Schmidt JF, Christensen JF, Frandsen C, Christensen KB, et al. Football training improves lean body mass in men with prostate cancer undergoing androgen deprivation therapy. *Scand J Med Sci Sports* (2014) 24 Suppl 1:105–12. doi: 10.1111/sms.12260

16. Hart NH, Galvao DA, Newton RU. Exercise medicine for advanced prostate cancer. *Curr Opin supportive palliative Care* (2017) 11(3):247–57. doi: 10.1097/SPC.0000000000000276

17. Association WF. *WFA laws of the game*. Association WF (2022) Available at: https://thewfa.co.uk/wp-content/uploads/2022/03/WFA-Rule-Change-V2-210322-PDF.pdf

18. Harper LD, Field A, Corr LD, Naughton RJ. The physiological, physical, and biomechanical demands of walking football: Implications for exercise prescription and future research in older adults. *J Aging Phys activity* (2019) 28(3):478–88. doi: 10.1123/japa.2019-0330

19. Madsen M, Krustrup P, Larsen MN. Exercise intensity during walking football for men and women aged 60+ in comparison to traditional small-sided football–a pilot study. *Managing Sport Leisure* (2021) 26(4):259–67. doi: 10.1080/23750472.2020.1762508

20. McEwan G, Buchan D, Cowan D, Arthur R, Sanderson M, Macrae E. Recruiting older men to walking football: A pilot feasibility study. *Explore* (2019) 15(3):206–14. doi: 10.1016/j.explore.2018.12.001

21. Reddy P, Dias I, Holland C, Campbell N, Nagar I, Connolly L, et al. Walking football as sustainable exercise for older adults–a pilot investigation. *Eur J sport Sci* (2017) 17(5):638–45. doi: 10.1080/17461391.2017.1298671

22. Barbosa A, Brito J, Costa J, Figueiredo P, Seabra A, Mendes R. Feasibility and safety of a walking football program in middle-aged and older men with type 2 diabetes. *Prog Cardiovasc Dis* (2020) 63(6):786–91. doi: 10.1016/j.pcad.2020.06.014

23. Trabulsi EJ, Rumble RB, Jadvar H, Hope T, Pomper M, Turkbey B, et al. Optimum imaging strategies for advanced prostate cancer: ASCO guideline. *J Clin Oncol* (2020) 38(17):1963. doi: 10.1200/JCO.19. 02757

24. Cornford P, van den Bergh RC, Briers E, Van den Broeck T, Cumberbatch MG, De Santis M, 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(2):263–82. doi: 10.1016/j.eururo.2020.09.046

25. Arnold JT, Bruce-Low S, Sammut L. The impact of 12 weeks walking football on health and fitness in males over 50 years of age. *BMJ Open sport Exercise Med* (2015) 1 (1):1–5. doi: 10.1136/bmjsem-2015-000048

26. Fletcher GF, Ades PA, Kligfield P, Arena R, Balady GJ, Bittner VA, et al. Exercise standards for testing and training: a scientific statement from the American heart association. *Circulation* (2013) 128(8):873–934. doi: 10.1161/CIR.0b013e31829b5b44

27. Van Andel G, Bottomley A, Fosså SD, Efficace F, Coens C, Guerif S, et al. An international field study of the EORTC QLQ-PR25: a questionnaire for assessing the health-related quality of life of patients with prostate cancer. *Eur J Cancer* (2008) 44 (16):2418–24. doi: 10.1016/j.ejca.2008.07.030

28. Jones CJ, Rikli RE, Beam WC. A 30-s chair-stand test as a measure of lower body strength in community-residing older adults. *Res Q Exercise sport* (1999) 70(2):113–19. doi: 10.1080/02701367.1999.10608028

29. Springer BA, Marin R, Cyhan T, Roberts H, Gill NW. Normative values for the unipedal stance test with eyes open and closed. *J geriatric Phys Ther* (2007) 30(1):8–15. doi: 10.1519/00139143-200704000-00003

30. Fuller CW, Ekstrand J, Junge A, Andersen TE, Bahr R, Dvorak J, et al. Consensus statement on injury definitions and data collection procedures in studies of football (soccer) injuries. *Br J Sports Med* (2006) 40(3):193–201. doi: 10.1136/bjsm.2005.025270

31. Garber CE, Blissmer B, Deschenes MR, Franklin BA, Lamonte MJ, Lee IM, et al. American College of sports medicine position stand. quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. *Med Sci Sports Exerc* (2011) 43(7):1334–59. doi: 10.1249/MSS.0b013e318213fefb

32. Campbell KL, Winters-Stone K, Wiskemann J, May AM, Schwartz AL, Courneya KS, et al. Exercise guidelines for cancer survivors: consensus statement from international multidisciplinary roundtable. *Med Sci sports Exercise* (2019) 51 (11):2375. doi: 10.1249/MSS.0000000000002116

33. Bjerre ED, Brasso K, Jorgensen AB, Petersen TH, Eriksen AR, Tolver A, et al. Football compared with usual care in men with prostate cancer (FC prostate community trial): A pragmatic multicentre randomized controlled trial. *Sports Med* (2019) 49(1):145–58. doi: 10.1007/s40279-018-1031-0

34. Gonzalez BD, Jim HSL, Small BJ, Sutton SK, Fishman MN, Zachariah B, et al. Changes in physical functioning and muscle strength in men receiving androgen deprivation therapy for prostate cancer: a controlled comparison. *Support Care Cancer* (2016) 24(5):2201–07. doi: 10.1007/s00520-015-3016-y

35. Alibhai SM, Breunis H, Timilshina N, Johnston C, Tomlinson G, Tannock I, et al. Impact of androgen-deprivation therapy on physical function and quality of life in men with nonmetastatic prostate cancer. *J Clin Oncol* (2010) 28(34):5038–45. doi: 10.1200/JCO.2010.29.8091

36. Gong J, Payne D, Caron J, Bay CP, McGregor BA, Hainer J, et al. Reduced cardiorespiratory fitness and increased cardiovascular mortality after prolonged androgen deprivation therapy for prostate cancer. *Cardio Oncol* (2020) 2(4):553–63. doi: 10.1016/j.jaccao.2020.08.011

37. Chiang P-K, Tsai W-K, Chiu W-HA, Lin J-B, Yang F-Y, Lee J. Muscle loss during androgen deprivation therapy is associated with higher risk of non-cancer mortality in high-risk prostate cancer. *Front Oncol* (2021) 11: 722652. doi: 10.3389/fonc.2021.722652

38. Lopez P, Taaffe DR, Newton RU, Galvao DA. Resistance exercise dosage in men with prostate cancer: Systematic review, meta-analysis, and meta-regression. *Med Sci Sports Exerc* (2021) 53(3):459–69. doi: 10.1249/MSS.0000000000002503

39. Bigaran A, Zopf E, Gardner J, La Gerche A, Murphy DG, Howden EJ, et al. The effect of exercise training on cardiometabolic health in men with prostate cancer receiving androgen deprivation therapy: a systematic review and meta-analysis. *Prostate Cancer Prostatic Dis* (2021) 24(1):35–48. doi: 10.1038/s41391-020-00273-5

40. Winters-Stone KM, Moe E, Graff JN, Dieckmann NF, Stoyles S, Borsch C, et al. Falls and frailty in prostate cancer survivors: Current, past, and never users of androgen deprivation therapy. *J Am Geriatr Soc* (2017) 65(7):1414–19. doi: 10.1111/jgs.14795

41. Myint ZW, Momo HD, Otto DE, Yan D, Wang P, Kolesar JM. Evaluation of fall and fracture risk among men with prostate cancer treated with androgen receptor inhibitors: A systematic review and meta-analysis. *JAMA Netw Open* (2020) 3(11): e2025826. doi: 10.1001/jamanetworkopen.2020.25826

42. Vashistha V, Singh B, Kaur S, Prokop LJ, Kaushik D. The effects of exercise on fatigue, quality of life, and psychological function for men with prostate cancer: Systematic review and meta-analyses. *Eur Urol Focus* (2016) 2(3):284–95. doi: 10.1016/j.euf.2016.02.011

43. Teleni L, Chan RJ, Chan A, Isenring EA, Vela I, Inder WJ, et al. Exercise improves quality of life in androgen deprivation therapy-treated prostate cancer: systematic review of randomised controlled trials. *Endocr Relat Cancer* (2016) 23 (2):101–12. doi: 10.1530/ERC-15-0456

44. Ussing A, Mikkelsen MK, Villumsen BR, Wejlgaard J, Bistrup PE, Birkefoss K, et al. Supervised exercise therapy compared with no exercise therapy to reverse debilitating effects of androgen deprivation therapy in patients with prostate cancer: A systematic review and meta-analysis. *Prostate Cancer Prostatic Dis* (2022) 25(3):491–506. doi: 10.1038/s41391-021-00450-0

45. Andersen MF, Midtgaard J, Bjerre ED. Do patients with prostate cancer benefit from exercise interventions? a systematic review and meta-analysis. *Int J Environ Res Public Health* (2022) 19(2):972. doi: 10.3390/ijerph19020972

46. Scott NW, Fayers P, Aaronson NK, Bottomley A, de Graeff A, Groenvold M, et al. *EORTC QLQ-C30 reference values manual*. (2008) Available at: https://www.eortc.org/app/uploads/sites/2/2018/02reference_values_manual2008.pdf.

47. Newton RU, Galvao DA, Spry N, Joseph D, Chambers SK, Gardiner RA, et al. Exercise mode specificity for preserving spine and hip bone mineral density in prostate cancer patients. *Med Sci Sports Exerc* (2019) 51(4):607–14. doi: 10.1249/MSS.0000000000001831

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 23 March 2023
DOI 10.3389/fonc.2023.1142022

OPEN ACCESS

EDITED BY
Ran Xu,
Second Xiangya Hospital, Central South University, China

REVIEWED BY
Biagio Barone,
University of Naples Federico II, Italy
Jianbo Li,
Case Western Reserve University, United States

*CORRESPONDENCE
Sung Gu Kang
kkangsung7@korea.ac.kr

SPECIALTY SECTION
This article was submitted to Genitourinary Oncology, a section of the journal Frontiers in Oncology

RECEIVED 11 January 2023
ACCEPTED 10 March 2023
PUBLISHED 23 March 2023

CITATION
Noh TI, Shim JS, Kang SH, Cheon J and Kang SG (2023) Diagnostic performance of transperineal prostate targeted biopsy alone according to the PI-RADS score based on bi-parametric magnetic resonance imaging.
Front. Oncol. 13:1142022.
doi: 10.3389/fonc.2023.1142022

Diagnostic performance of transperineal prostate targeted biopsy alone according to the PI-RADS score based on bi-parametric magnetic resonance imaging

Tae Il Noh, Ji Sung Shim, Seok Ho Kang, Jun Cheon and Sung Gu Kang*

Department of Urology, Anam Hospital, Korea University College of Medicine, Seoul, Republic of Korea

Purpose: To compare the diagnostic performance of transperineal targeted biopsy (TB) or systematic biopsy (SB) alone based on combined TB+SB and radical prostatectomy (RP) specimen for detecting prostate cancer (PCa) according to the prostate imaging reporting and data system (PI-RADS) score.

Materials and methods: This study included 1077 men who underwent transperineal bi-parametric (bp) magnetic resonance imaging (MRI)–ultrasound (US) fusion TB+SB (bpMRI-US FTSB) between April 2019 and March 2022. To compare the performance of each modality (TB, SB, and combined TB+SB) with the RP specimen (as the standard) for detecting PCa and clinically significant PCa (csPCa), receiver operating characteristic (ROC) curves were plotted.

Results: PCa was detected in 581 of 1077 men (53.9%) using bpMRI-US FTSB. CsPCa was detected in 383 of 1077 men (35.6%), 17 of 285 (6.0%) with PI-RADS 0 to 2, 35 of 277 (12.6%) with PI-RADS 3, 134 of 274 (48.9%) with PI-RADS 4, and 197 of 241 (81.7%) with PI-RADS 5, respectively. The additional diagnostic value of TB vs. SB compared to combined TB+SB for diagnosing csPCa were 4.3% vs. 3.2% ($p=0.844$), 20.4% vs 5.1% ($p<0.001$), and 20.3% vs. 0.7% ($p<0.001$) with PI-RADS 3, 4, and 5, respectively. TB alone showed no significant difference in diagnostic performance for csPCa with combined TB+SB based on RP specimens in patients with PI-RADS 5 ($p=0.732$).

Conclusion: A need for addition of SB to TB in patients with PI-RADS 3 and 4 lesions, however, TB alone may be performed without affecting the management of patients with PI-RADS 5.

KEYWORDS

magnetic resonance imaging, transperineal biopsy, prostate cancer, PI-RADS, target biopsy

Introduction

Prostate cancer (PCa) diagnosis relies on prostate-specific antigen (PSA) and prostate biopsy, and transrectal ultrasonography-guided systematic biopsy (TRUSB) has been considered the standard diagnostic pathway in men with a clinical suspicion of PCa (1).

However, TRUSB has led to missed diagnosis in >30% of patients with PCa and has poor discriminative power in diagnosing cancerous tissue (2, 3). In this regard, to improve the discriminative power and diagnostic accuracy of prostate biopsy, visualization of PCa through magnetic resonance imaging (MRI) has been attempted. Accordingly, the prostate imaging reporting and data system (PI-RADS) was developed to maximize the standardized utilization of MRI for detecting PCa, which led to increased usage of MRI as a guide for targeted biopsy (TB) (4). Studies have suggested that MRI-TB can provide additional value in diagnosis of PCa for clinically significant PCa (csPCa) categorized as International Society for Urological Pathology (ISUP) grade ≥2 (5). Additionally, MRI-TB based on PI-RADS significantly outperforms systematic biopsy (SB) for detection of csPCa with the probability of sparing the potential redundancy of SB (6–8).

However, MRI was missing PCa in 20% of index tumor and 79% of non-index tumor (9). Therefore, the performance of MRI-TB alone may be not good enough to omit systematic biopsy (SB) in every man with a clinical suspicion for PCa (10). TB is the standard pathway in most cancers, nevertheless the current guidelines for detecting PCa have recommended SB and additional TB with a suspicious lesion in MRI (11). However, SB may be associated with over-diagnose the clinically insignificant PCa and result in overtreatment and impose the risk of adverse events, complications, and comes with consequence of medical burden (12, 13). Notably, in PI-RADS 5, MRI-TB have shown good performance with high predictive rates for csPCa that suggests TB alone might also be valuable in diagnosing csPCa (14–16).

The purpose of this study was to compare the diagnostic performance of TB or SB alone according to the PI-RADS scores with combined TB+SB based on the standard transperineal bi-parametric magnetic resolution imaging-ultrasound fusion TB+SB (bpMRI-US FTSB) and radical prostatectomy (RP) specimen.

Abbreviations: ADC, apparent diffusion coefficient; AUC, area under the ROC curve; bpMRI, bi-parametric MRI; bpMRI–US FTSB, bi-parametric MRI-US fusion (transperineal) targeted and systematic biopsy; csPCa, clinically significant prostate cancer; DCE, dynamic contrast enhanced; DWI, diffusion weighted images; FTSB, (transperineal) fusion targeted and systematic biopsy; GA, general anesthesia; MRI, magnetic resonance imaging; mpMRI, multi-parametric MRI; US, ultrasound; PCa, prostate cancer; PI-RADS, prostate Imaging Reporting and Data Systems; ROC, receiver-operating characteristic; ROI, regions of interest; SB, (template) systematic biopsy; TRUS, transrectal ultrasound; TRUSB, transrectal ultrasound guided systematic biopsy; T2WI, T2-weighted images; TB, targeted biopsy; US, ultrasound.

Materials and methods

Study design

We analyzed the medical records of 1077 men, between April 2019 and March 2022, who were clinically suspected for PCa with an elevated prostate-specific antigen (PSA) level (≥ 4.0 ng/mL), and/or abnormal findings on digital rectal examination (DRE). All enrolled patients underwent bi-parametric MRI (bpMRI) prior to the prostate biopsy, and regions of interest (ROIs) on MRI were established according to the PI-RADS version 2.0. Subsequent transperineal bpMRI-US FTSB and RP were performed (Figure 1).

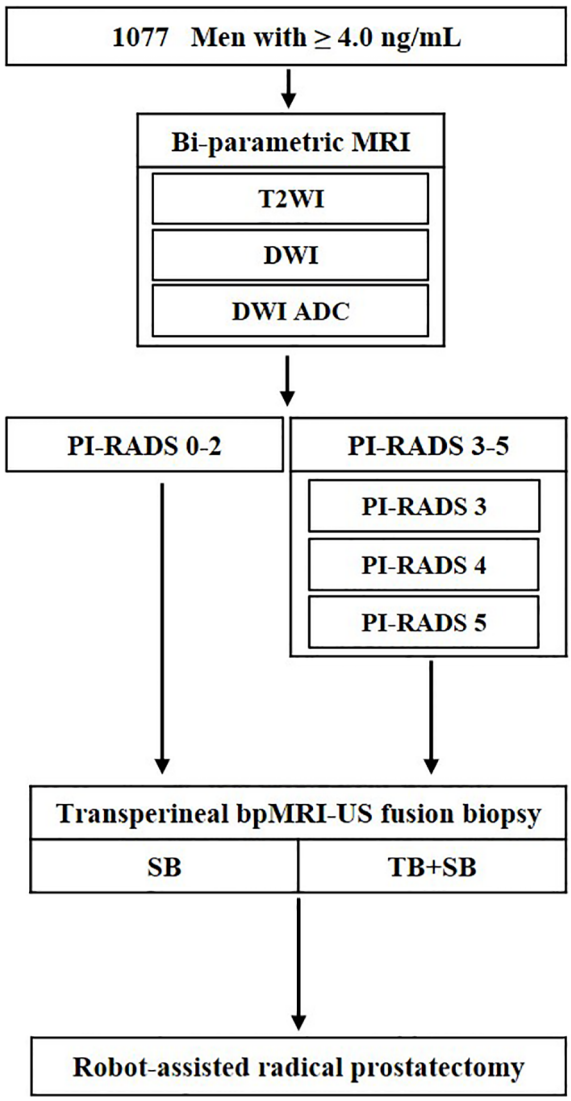


FIGURE 1
Flowchart of study design. ADC, apparent diffusion coefficient; T2WI, T2-weighted images; DWI, diffusion weighted images; TB, targeted biopsy; SB, systematic biopsy; US, ultrasound.

MRI acquisition protocol

The bpMRI, contrast-free protocol, was performed using a 3.0-T scanner (Magnetom Skyra and Prisma, Siemens Healthineers, Erlangen, Germany or Achieva, Philips Healthcare, Best, Netherlands) with a multichannel phased-array external surface coil. T2-weighted images (T2WI) and diffusion-weighted images (DWI) were obtained, whereas dynamic contrast-enhanced (DCE) images were omitted. ROIs on the bpMRI were marked by three dedicated uroradiologists based on PI-RADS version 2.0 (Figure 2A).

Prostate biopsy protocol

We have previously reported a protocol for transperineal bpMRI-US FTSB (16). In brief, the elastic image registration type of the MRI-US fusion technique using a mechanical position encoder and robotic articulated arm system (Biojet, USA) was used and TB and SB were performed by urologists during the same session. Further, we considered suspicious lesions as ROI (PI-RADS ≥3) for TB, and 3-4 cores of TB and sequential 22-cores of SB were performed using a prostate mapping template (modified Barzell-template). The ROI for the TB was not intentionally avoided. Each core was labelled separately and subjected to histopathology. The number of biopsy cores was decided depending on the prostate size. The prostate biopsy results were reported by three uropathologists based on the International Society of Urological Pathology (ISUP) grade groups (GG). Clinically insignificant PCa was defined as an ISUP GG1. Clinically significant PCa was defined as > ISUP GG2 (Figure 2B).

RP and histopathologic examination protocol

Localized PCa with PI-RADS 3-5, sequentially underwent robot-assisted RP (RARP) using da Vinci Si, Xi, or SP system (Intuitive Surgical, Sunnyvale, CA, USA) by two surgeons. For histopathological examination, whole-mount histopathology slides were used, and each prostate was sectioned in the axial plane from the basal to the apex at approximately 4-5 mm intervals (Figure 2C).

Study end points

The endpoint was to compare the impact of TB or SB alone according to PI-RADS scores, referring to the standard of combined TB+SB and RP specimens.

Statistical analysis

To quantify and compare the performance of each modality (TB, SB, and combined TB+SB) in detecting PCa and csPCa,

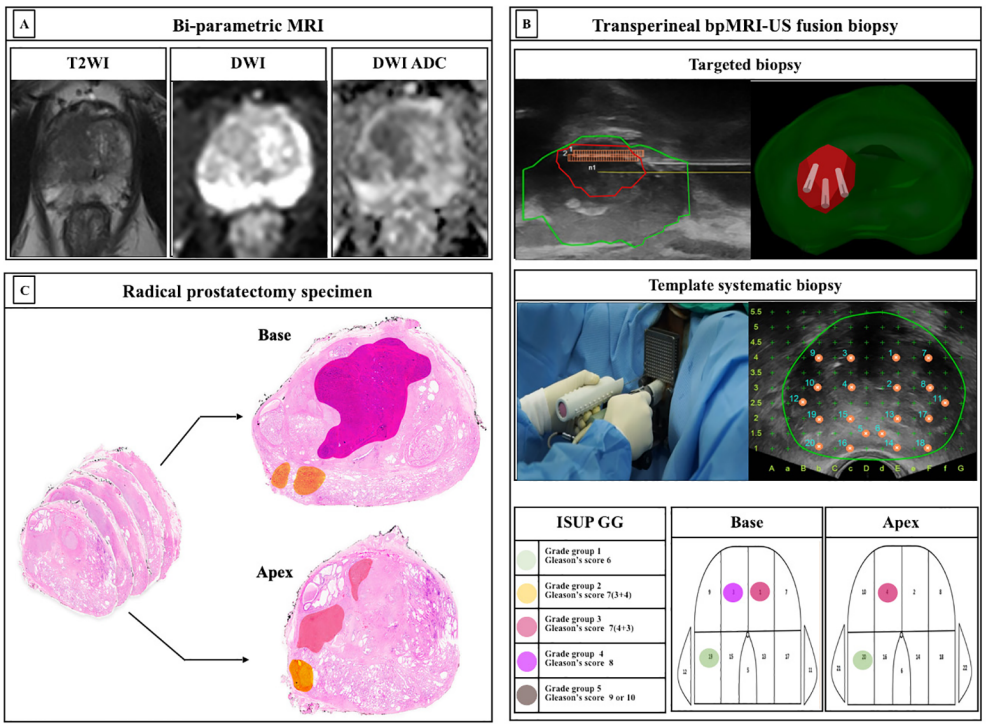


FIGURE 2
Protocols of study. **(A)** Bi-parametric magnetic resonance imaging (bpMRI) **(B)** Transperineal bpMRI-Ultrasound fusion targeted and systematic biopsy **(C)** Whole-mount radical prostatectomy specimen ISUP, International Society for Urological Pathology; GG, grade group; MRI, Magnetic resonance imaging; TB, targeted biopsy; SB, systematic biopsy.

receiver operating characteristic (ROC) curve analyses were performed considering combined TB+SB and RP specimens as standards. Accordingly, the results were summarized using Delong's test as the areas under the ROC curves (AUCs) and 95% CI. All statistical analyses were performed using IBM SPSS version 26.0 (IBM Corp., Armonk, NY, USA). The level of statistical significance was considered P<0.05.

Ethics statement

This study was conducted in accordance with the Declaration of Helsinki and current ethical guidelines. The study was reviewed and approved by the Ethics Committee and Institutional Review Board of Korea University Anam Hospital (IRB No. 2018AN0339).

Result

Patient demographics

In total, 1077 men were included in the analysis. The median (interquartile range (IQR)) age was 69.0 (62.0-75.0) years. The median (IQR) PSA and PSA density (PSAD) were 6.66 (4.57-11.57) ng/mL and 0.18 (0.11-0.35) ng/mL^2. The demographics of the study population are reported in Table 1.

Diagnostic performance of bpMRI-US FTSB

PCa (GG1) was detected in 581 of 1077 men (53.9%) by bpMRI-US FTSB. Accordingly, it was detected in 58 of 285 cases (35.6%) with PI-RADS 0-2, in 91 of 277 cases (32.9%) with PI-RADS 3, in 209 of 274 cases (76.3%) with PI-RADS 4, and in 220 of 241 cases (91.3%) with PI-RADS 5 (Figure 3A). Further, csPCa (≥ GG2) was detected in 383 of 1077 men (35.6%). Accordingly, it was detected in 17 of 285 men (6.0%) with PI-RADS 0-2, in 35 of 277 men (12.6%) with PI-RADS 3, in 134 of 274 men (48.9%) with PI-RADS 4, and in 197 of 241 men (81.7%) with PI-RADS 5 (Figure 3B). The distribution of ISUP grade groups is shown in Table 2. Patients with csPCa (GG2≥2) had higher median PSA, PSAD, and lower prostate volume than those with GG1 pathology; PSA(IQR) [66.0 vs. 72.0, p= 0.038], PSAD (0.14 vs. 0.35, p=0.011), and lower prostate volume (41.2 vs. 30.3, p=0.047) than those with GG1 pathology (Supplementary Table 1).

Comparison of the diagnostic performance of TB or SB alone with the standard of combined TB+SB

In patients with PI-RADS 3 to 5, TB, SB, and Combined TB+SB were able to detect PCa in 61.0%, 54.0%, and 66.0% of cases, respectively. Accordingly, the diagnosis rate of TB, SB, and combined TB+SB for diagnosing PCa were 24.9%, 26.4%, and 32.9% in patients with PI-RADS 3, 70.8%, 63.5%, and 76.3% in patients with PI-RADS 4, and 91.3%, 75.1%, and 92.5% in patients with PI-RADS 5, respectively (Figure 3A). The additional diagnostic value for PCa detection of TB vs. SB compared to combined TB+SB were 12.0% vs. 5.0% (p<0.001) in patients with PI-RADS 3-5; PI-RADS 3: 6.5% vs. 8.0% (p=0.535), PI-RADS 4: 12.8% vs. 5.5% (p<0.001), and PI-RADS 5: 17.4% vs. 1.2% (p<0.0001), respectively (Table 3).

Combined TB+SB showed superior diagnostic performance for TB or SB alone in patients with PI-RADS 3 and 4 (p <0.001). However, TB alone showed no significant difference in diagnostic performance for csPCa with combined TB+SB in patients with PI-RADS 5; PI-RADS 3: area under the curve (AUC) [95% confidence interval (CI)], 0.882 [0.838–0.918], p<0.001; PI-RADS 4: AUC, 0.964 [0.935–0.983], p<0.001; PI-RADS 5: AUC, 0.986 [0.961–0.997], p=0.078 (Table 3).

In patients with PI-RADS 3 to 5, csPCa (ISUP ≥GG2) was detected in 43.1%, 31.4%, and 46.2% cases *via* TB, SB, and combined TB+SB, respectively. Accordingly, the diagnosis rate of TB, SB, and combined TB+SB for diagnosing csPCa were 9.4%, 8.3%, and 12.6% in patients with PI-RADS 3, 43.8%, 28.5%, and 48.9% in patients with PI-RADS 4, and 81.0%, 61.4%, and 81.7% in patients with PI-RADS 5, respectively (Figure 3B). The additional diagnostic value for csPCa detection of TB vs. SB alone compared to combined TB+SB was 14.8% vs. 3.1%(p<0.001) in patients with PI-RADS 3-5; PI-RADS 3: 4.3% vs. 3.2% (p=0.844), PI-RADS 4: 20.4% vs. 5.1% (p<0.001), and PI-RADS 5: 20.3% vs. 0.7% (p<0.001), respectively (Table 3). Further, TB alone showed no significant difference in diagnostic performance for csPCa to combined TB+SB in patients with PI-RADS 5; PI-RADS 3: area under the curve

TABLE 1 Demographics of men according to PI-RADS distribution.

	All	PI-RADS 0-2	PI-RADS 3	PI-RADS 4	PI-RADS 5
Distribution of PI-RADS, n (%)	1077	285 (26.5)	277 (25.7)	274 (25.4)	241 (22.4)
Median Age (IQR)	69.0 (62.0-75.0)	61.0 (56.0-68.0)	66.0 (61.0-72.0)	72.0 (64.8-77.0)	72.0 (68.0-78.0)
Median PSA, ng/mL (IQR)	6.66 (4.57-11.57)	5.27 (4.14-6.73)	5.65 (4.28-8.64)	6.88 (4.89-10.87)	13.3 (7.03-34.3)
Median prostate volume, cm^3 (IQR)	36.3 (26.4-50.1)	38.9 (27.7-54.0)	39.4 (30.2-51.1)	34.9 (25.3-46.4)	32.1 (24.2-44.4)
Median PSA density (IQR)	0.18 (0.11-0.35)	0.13 (0.08-0.20)	0.15 (0.10-0.22)	0.19 (0.13-0.35)	0.45 (0.22-1.03)
Median free/total PSA ratio (IQR)	0.15 (0.10-0.21)	0.17 (0.12-0.24)	0.17 (0.12-0.23)	0.13 (0.10-0.19)	0.12 (0.08-0.17)
DRE nodule, n (%)	122 (11.3)	15 (5.3)	27 (9.7)	42 (15.3)	38 (15.8)

PI-RADS, prostate imaging-reporting and data systems; IQR, interquartile range; PSA, prostate-specific antigen; DRE, digital rectal exam.

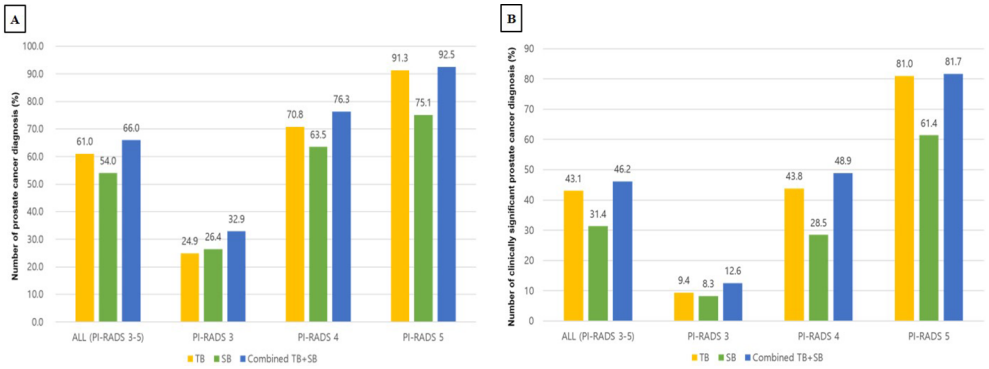


FIGURE 3
Diagnostic performance of TB, SB, TB+SB in patients with PI-RADS 3 to 5 **(A)** Detection rate for prostate cancer **(B)** Detection rate for clinically significant prostate cancer PI-RADS, prostate imaging-reporting and data systems; TB, targeted biopsy; SB, systematic biopsy.

(AUC) [95% confidence interval (CI)], 0.893 [0.851–0.927], p=0.0088; PI-RADS 4: AUC, 0.961 [0.931–0.981], p=0.002; PI-RADS 5: AUC, 0.989 [0.971–0.998], p=0.093 (Table 3).

Comparison of diagnostic performances referring to RP specimen

The RARP was performed in 289 of 483 diagnosed with PCa with PI-RADS 3-5; 59 of 91 (64.8%) with PI-RADS 3, 122 of 209 (58.4%) with PI-RADS 4, and 108 of 220 (49.1%) with PI-RADS 5, respectively (Table 4).

Accordingly, TB alone and combined TB+SB showed 45.7% and 33.2% of any upgrading in RP specimens with PI-RADS 3-5; 76.2% and 37.3% with PI-RADS 3, 50.4% and 39.3% with PI-RADS 4, 28.7% and 24.1% with PI-RADS 5, respectively; and upgrading of GG1 to GG ≥ 2 occurred in 59 of 265 (22.3%) and 59 of 289 (20.4%) cases with PI-RADS 3-5; 17 of 42 (40.5%) and 21of 59 (35.6%) with PI-RADS 3, 38 of 115 (33.0%) and 35 of 122 (28.7%) with PI-RADS 4, and 4 of 108 (3.7%) and 3 of 108 (2.8%) with PI-RADS 5, respectively. Further, downgrading of GG ≥ 2 to GG1 occurred in only one in 289 (0.3%) (Table 4).

The combined TB+SB showed superior diagnostic performance compared to TB alone for diagnosing csPCa when compared to the standard of RP specimen; TB alone vs TB+SB, AUC (95% CI); PI-RADS 3-5: 0.824 (0.777-0.864) vs. 0.860 (0.809-0.911), p=0.034; PI-RADS 3: 0.663 (0.524-0.802) vs. 0.722 (0.593-0.852), p=0.016; PI-RADS 4: 0.817 (0.730-0.904) vs. 0.844 (0.766-0.921), p=0.049. TB alone showed no significant difference in diagnostic performance for csPCa to combined TB+SB in patients with PI-RADS 5; TB alone vs. combined TB+SB, AUC (95% CI), 0.951(0.909-0.994) vs. 0.961(0.924-0.998), p=0.732 (Table 4).

Discussion

In recent years with significant improvements in the accuracy of MRI after implementation of the PI-RADS, the use of prebiopsy

TABLE 2 Diagnostic performance of transperineal MRI-US fusion TB and SB.

	All	PI-RADS 0-2	PI-RADS 3			PI-RADS 4			PI-RADS 5		
	1077	285	277			274			241		
		SB	TB	SB	TB+SB	TB	SB	TB+SB	TB	SB	TB+SB
PCa, n (%)	581 (53.9)	58 (20.4)	69 (24.9)	73 (26.4)	91 (32.9)	194 (70.8)	174 (63.5)	209 (76.3)	220 (91.3)	181 (75.1)	223 (92.5)
csPCa, n (%)	383 (35.6)	17 (6.0)	26 (9.4)	23 (8.3)	35 (12.6)	120 (43.8)	78 (28.5)	134 (48.9)	195 (81.0)	148 (61.4)	197 (81.7)
ISUP*, n (%)											
1	198 (34.1)	41 (14.4)	43 (15.5)	50 (18.1)	56 (20.2)	74 (27.0)	96 (35.0)	75 (27.4)	25 (10.4)	33 (13.7)	26 (10.8)
2	119 (20.5)	10 (3.5)	15 (5.4)	17 (6.1)	22 (7.9)	43 (15.7)	25 (9.1)	44 (16.1)	46 (19.1)	42 (17.4)	43 (17.8)
3	37 (6.4)	5 (1.8)	3 (1.1)	1 (0.4)	2 (0.7)	11 (4.0)	7 (2.6)	15 (5.5)	28 (11.6)	12 (5.0)	15 (6.2)
4	180 (30.9)	1 (0.4)	8 (2.9)	5 (1.8)	11 (4.0)	60 (21.9)	41 (15.0)	66 (24.1)	91 (37.8)	66 (27.4)	102 (42.3)
5	47 (8.1)	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	6 (2.2)	5 (1.8)	9 (3.3)	30 (12.4)	28 (11.6)	37 (15.4)

* ISUP grade groups (GG):1 = Gleason 6 (or less), 2 = Gleason 7(3 + 4), 3 = Gleason 7(4 + 3), 4 = Gleason 8(4 + 4 or 3 + 5 or 5 + 3), and 5 = Gleason 9 or 10. csPCa: ≥ ISUP GG2.
MRI-US, magnetic resonance imaging-ultrasonography; ISUP, International Society for Urological Pathology; TB, targeted biopsy; SB, systematic biopsy; PCa, prostate cancer; PI-RADS, prostate imaging reporting and data system.

TABLE 3 Diagnostic performance of TB or SB alone according to PI-RADS compared to combined TB and SB.

	All (PI-RADS 3-5)			PI-RADS 3			PI-RADS 4			PI-RADS 5		
	792			277			274			241		
	TB	SB	TB +SB	TB	SB	TB +SB	TB	SB	TB +SB	TB	SB	TB +SB
PCa, n (%)	483 (61.0)	428 (54.0)	523 (66.0)	69 (24.9)	73 (26.4)	91 (32.9)	194 (70.8)	174 (63.5)	209 (76.3)	220 (91.3)	181 (75.1)	223 (92.5)
Additional value of TB	12.0%			6.5 %			12.8 %			17.4 %		
Additional value of SB	5.0%			8.0 %			5.5 %			1.2 %		
AUC (CI 95%) Reference to TB+SB	0.932 (0.915-0.947)	0.914 (0.895-0.931)		0.882 (0.838-0.918)	0.904 (0.863-0.937)		0.964 (0.935-0.983)	0.916 (0.877-0.946)		0.986 (0.961-0.997)	0.906 (0.862-0.940)	
p value (vs. TB+SB)	<0.001	<0.001		<0.001	<0.001		<0.001	<0.001		0.078	<0.001	
csPCa (≥ GG2) *, n (%)	341 (43.1)	249 (31.4)	366 (46.2)	26 (9.4)	23 (8.3)	35 (12.6)	120 (43.8)	78 (28.5)	134 (48.9)	195 (81.0)	148 (61.4)	197 (81.7)
Additional value of TB	14.8%			4.3%			20.4%			20.3%		
Additional value of SB	3.1%			3.2%			5.1%			0.7%		
AUC (CI 95%) Reference to TB+SB	0.957 (0.942-0.968)	0.881 (0.895-0.901)		0.893 (0.851-0.927)	0.883 (0.839-0.918)		0.961 (0.931-0.981)	0.841 (0.792-0.882)		0.989 (0.971-0.998)	0.867 (0.817-0.907)	
p value (vs. TB+SB)	<0.001	<0.001		0.009	0.004		0.0021	<0.001		0.093	<0.001	

* ISUP grade groups (GG):1 = Gleason 6 (or less), 2 = Gleason 7(3+4), 3 = Gleason 7(4+3), 4 = Gleason 8(4+4 or 3+5 or 5+3), and 5 = Gleason 9 or 10. csPCa: ≥ ISUP GG2
AUC, area under the curve; CI, confidence interval; ISUP, International Society for Urological Pathology; TB, targeted biopsy; SB, systematic biopsy; PCa, prostate cancer; csPCa, clinically significant prostate cancer; PI-RADS, prostate imaging reporting and data system.

MRI for PCa diagnosis has increased (4, 6, 17). Furthermore, numerous studies have demonstrated that MRI-TB could offer improved diagnostic value for csPCa with pooled sensitivity and specificity of 0.80 (95%CI: 0.69-0.87) and 0.94 (95%CI: 0.90-0.97) (5). However, addition of TB to SB increases the number of csPCa (≥ ISUP GG2) by 6.7-7.6%, while added value of SB to TB is 4.3-5.2% for csPCa (5, 14, 18). Further, MRI was missing PCa in 20% of index tumor and 79% of non-index tumor (9). Therefore, due to the additional diagnostic value of SB and the risk of missing csPCa with TB alone, combined TB + SB has been suggested for dignosing PCa (5, 10, 11).

However, it should be noted that obtaining more prostate cores accompanies with a greater risk of complications, such as prostatitis, sepsis events, visits to the emergency room, rectal bleeding, hematuria, and pain (7, 19, 20). MRI-TB alone with fewer core biopsies per patient might lead to fewer complications. The net benefit of adding SB to TB for prostate biopsy optimization according to PI-RADS score should be weighed against accuracy for csPCa detection and additional burden such as overdiagnosis of indolent PCa, resulting in overtreatment and complications from increased numbers of biopsies. For predicting csPCa, several predictors and their combination such as clinical parameters including PSAD and PI-RADS score have been suggested (21). In addition, for risk assessment to determine the need for biopsy, risk calculators (RCs) have been suggested, thereby may be reducing the number of unnecessary biopsies (22).

Notably, MRI-TB showed good performance and was highly predictive for diagnosing csPCa in cases with PI-RADS 5 (77-85%) (7, 14, 16). In a study comparing the concordances between PI-RADS and histologic reports of the RP specimen, the PI-RADS≥3 was further associated with csPCa in 92.4% of cases, with 100% association in cases with a PI-RADS 5 score (23). High performance of MRI-TB and low additional diagnostic value (2-4%) of SB for detection of csPCa in patients with PI-RADS 5 that suggests the probability of sparing the potential redundancy of SB in PI-RADS 5 (12, 24, 25).

For MRI-TB, mpMRI have shown a high sensitivity and negative predictive value (NPV) of 93.0% and 89.0% for csPCa (6). However, it is time-consuming (~ 40 min) to acquire T2-weighted imaging (T2WI) and diffusion-weighted imaging (DWI), and dynamic contrast-enhanced (DCE) imaging requires intravenous administration of contrast media.

Several studies have demonstrated comparable diagnostic performance of bpMRI (contrast-free protocol) to mpMRI (26). In a systematic review and meta-analysis of the diagnostic accuracy of bpMRI and mpMRI for PCa detection, pooled sensitivity and specificity did not show significant difference and the AUCs were similar; 0.87 and 0.90 for mpMRI and

TABLE 4 Concordance of prostate cancer grade group on targeted, systematic, and combined targeted and systematic biopsy according to radical prostatectomy specimen by PI-RADS scores.

	Radical prostatectomy, n (%)											
	All (PI-RADS 3-5)			PI-RADS 3			PI-RADS 4			PI-RADS 5		
	289			59			122			108		
	TB	SB	TB+SB	TB	SB	TB+SB	TB	SB	TB+SB	TB	SB	TB+SB
PCa	265 (91.7)	239 (82.7)	289 (100.0)	42 (71.2)	47 (79.7)	59 (100.0)	115 (94.2)	104 (85.2)	122 (100.0)	108 (100.0)	84 (77.8)	108 (100.0)
Any upgrading of GG*	121 (45.7)	168 (70.3)	96 (33.2)	32 (76.2)	35 (74.5)	22 (37.3)	58 (50.4)	79 (76.0)	48 (39.3)	31 (28.7)	54 (64.3)	26 (24.1)
Any downgrading of GG*	73 (27.5)	55 (23.0)	93 (32.2)	6 (14.3)	3 (6.4)	9 (15.3)	30 (26.1)	24 (23.1)	37 (30.3)	37 (34.3)	28 (33.3)	47 (43.5)
GG1*	79 (29.8)	113 (47.3)	89 (30.8)	26 (61.9)	33 (70.2)	36 (61.0)	44 (38.3)	62 (59.6)	45 (36.9)	9 (8.3)	23 (27.9)	8 (7.4)
Upgrading GG1 to GG ≥ 2	59 (22.3)	94 (39.3)	59 (20.4)	17 (40.5)	21 (44.7)	21 (35.6)	38 (33.0)	54 (51.9)	35 (28.7)	4 (3.7)	19 (22.6)	3 (2.8)
GG ≥ 2*	186 (70.2)	126 (52.7)	200 (69.2)	16 (38.1)	14 (29.8)	23 (39.0)	71 (61.7)	42 (40.4)	77 (63.1)	99 (91.7)	66 (78.6)	100 (92.6)
Downgrading GG ≥ 2 to GG 1	1 (0.4)	0 (0.0)	1 (0.3)	1 (2.3)	0 (0.0)	1 (1.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
AUC (CI 95%) Reference to RP specimen	0.824 (0.777-0.864)	0.719 (0.665-0.768)	0.860 (0.809-0.911)	0.663 (0.524-0.802)	0.667 (0.531-0.802)	0.722 (0.593-0.852)	0.817 (0.730-0.904)	0.688 (0.559-0.816)	0.844 (0.766-0.921)	0.951 (0.909-0.994)	0.820 (0.711-0.929)	0.961 (0.924-0.998)
p value (vs. TB+SB)	0.034	<0.001		0.016	0.021		0.049	<0.001		0.732	<0.001	

* ISUP grade groups (GG):1 = Gleason 6 (or less), 2 = Gleason 7(3 + 4), 3 = Gleason 7(4 + 3), 4 = Gleason 8(4 + 4 or 3 + 5 or 5 + 3), and 5 = Gleason 9 or 10. csPCa: ≥ ISUP GG2.

AUC, area under the curve; CI, confidence interval; ISUP, International Society for Urological Pathology; TB, targeted biopsy; SB, systematic biopsy; PCa, prostate cancer; csPCa, clinically significant prostate cancer; PI-RADS, Prostate Imaging Reporting and Data System.

10.3389/fonc.2023.1142022

bpMRI (27). In this regard, bpMRI is more rapid (~15 min) due to exclusion of DCE, and safer from potential side effects of contrast media than mpMRI while retaining a sufficient diagnostic value (16).

In the current study, we compared the impact of TB, SB, and combined TB+SB according to the PI-RADS score. Accordingly, the SB had only additional diagnostic values of 1.2% and 0.7% for detection of PCa and csPCa in patients with PI-RADS 5. Further, TB alone showed no significant difference of diagnostic performance with combined TB+SB for csPCa. Similarly, in a study conducted on 112 patients with PI-RADS 5 on MRI and subsequentially 78 of RP, TB alone could diagnose PCa with very high probability (97%) in patients with PSAD >0.15ng/ml^2 (12). Accordingly, if SB was omitted, none of the PCa cases and only 4% of csPCa cases would be missed. Thus, the authors suggest that SB might be omitted for cases with PI-RADS 5 and PSAD >0.15ng/ml^2.

Since the upgrading grade group of RP specimens from prostate biopsy has been reported, the omission of SB may lead to misclassification of PCa; TB (30.9%) and TB+SB (14.4%) of the upgraded grade group (10). These inconsistencies between biopsy and specimen of prostate, upgrading and misclassification of PCa, are the inherent limitations of prostate needle biopsy (28). Nevertheless, in this study, upgrading from GG1 to ≥ GG2, which has a potential risk of changing subsequent clinical management, showed difference in only one patient; TB alone vs. combined TB +SB, 4 of 108 (3.7%) vs. 3 of 108 (2.8%). Similarly, in another study, MRI-TB alone in PI-RADS 5 cases had meager upgrade rate (3.4%) (29). Further, addition of SB to TB in PI-RADS 5 cases altered only 3.1% of the highest grade group of PCa patients, all of whom had already been categorized as GG≥2 based on TB, and SB did not change subsequent clinical management (24). Current study supports the need for SB in patients with PI-RADS 3 and 4 lesions. However, minimal additional diagnostic values of SB and comparable diagnostic performance of MRI-TB suggest that SB potentially can be omitted in patients with PI-RADS 5.

The limitations of this study are its retrospective nature and accompanying bias. The other limitation is that this study was performed in a single tertiary center with transperineal prostate biopsy and bpMRI, and transrectal prostate biopsy with mpMRI, which is the common practice, was not considered. This may raise concerns toward extrapolating a general trend from our results. Nevertheless, this study can support that performing TB alone in patients with PI-RADS 5 lesions, might mitigate the medical burden by SB omission.

Conclusion

The current study suggests a need for addition of SB to TB in patients with PI-RADS 3 and 4 lesions, and TB alone may be performed for diagnosing csPCa in patients with PI-RADS 5, without changing the subsequent clinical management.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

This study was approved by the Ethics Committee and the Institutional Review Board of KUMC (IRB No. 2018AN0339).

Author contributions

TIN: protocol/project development, data acquisition, data analysis and interpretation, drafting of the manuscript. JSS: Protocol/project development, supervision. SHK: Protocol/project development, Data acquisition, supervision. JC: Protocol/project development, supervision. SGK: Protocol/project development, Data acquisition, supervision. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1142022/full#supplementary-material

References

1. Mottet N, Bellmunt J, Bolla M, Briers E, Cumberbatch MG, De Santis M, et al. EAU-ESTRO-SIOG guidelines on prostate cancer. part 1: Screening, diagnosis, and local treatment with curative intent. *Eur Urol* (2017) 71:618. doi: 10.1016/j.eururo.2016.08.003

2. Das CJ, Razik A, Sharma S, Verma S. Prostate biopsy: when and how to perform. *Clin Radiol* (2019) 74:853. doi: 10.1016/j.crad.2019.03.016

3. Epstein JI, Feng Z, Trock BJ, Pierorazio PM. Upgrading and downgrading of prostate cancer from biopsy to radical prostatectomy: incidence and predictive factors using the modified Gleason grading system and factoring in tertiary grades. *Eur Urol* (2012) 61:1019. doi: 10.1016/j.eururo.2012.01.050

4. Weinreb JC, Barentsz JO, Choyke PL, Cornud F, Haider MA, Macura KJ, et al. PI-RADS prostate imaging - reporting and data system: 2015, version 2. *Eur Urol* (2016) 69:16. doi: 10.1016/j.eururo.2015.08.052

5. Drost FH, Osses DF, Nieboer D, Steyerberg EW, Bangma CH, Roobol MJ, et al. Prostate MRI, with or without MRI-targeted biopsy, and systematic biopsy for detecting prostate cancer. *Cochrane Database Syst Rev* (2019) 4:Cd012663. doi: 10.1002/14651858.CD012663.pub2

6. Ahmed HU, El-Shater Bosaily A, Brown LC, Gabe R, Kaplan R, Parmar MK, et al. Diagnostic accuracy of multi-parametric MRI and TRUS biopsy in prostate cancer (PROMIS): a paired validating confirmatory study. *Lancet* (2017) 389:815. doi: 10.1016/S0140-6736(16)32401-1

7. Kasivisvanathan V, Rannikko AS, Borghi M, Panebianco V, Mynderse LA, Vaarala MH, et al. MRI-Targeted or standard biopsy for prostate-cancer diagnosis. *N Engl J Med* (2018) 378:1767. doi: 10.1056/NEJMoa1801993

8. Siddiqui MM, Rais-Bahrami S, Turkbey B, George AK, Rothwax J, Shakir N, et al. Comparison of MR/ultrasound fusion-guided biopsy with ultrasound-guided biopsy for the diagnosis of prostate cancer. *JAMA* (2015) 313:390. doi: 10.1001/jama.2014.17942

9. Le JD, Tan N, Shkolyar E, Lu DY, Kwan L, Marks LS, et al. Multifocality and prostate cancer detection by multiparametric magnetic resonance imaging: correlation with whole-mount histopathology. *Eur Urol* (2015) 67:569. doi: 10.1016/j.eururo.2014.08.079

10. Ahdoot M, Wilbur AR, Reese SE, Lebastchi AH, Mehralivand S, Gomella PT, et al. MRI-Targeted, systematic, and combined biopsy for prostate cancer diagnosis. *N Engl J Med* (2020) 382:917. doi: 10.1056/NEJMoa1910038

11. Mottet N, van den Bergh RCN, Briers E, Van den Broeck T, Cumberbatch MG, De Santis M, et al. EAU-EANM-ESTRO-ESUR-SIOG guidelines on prostate cancer-2020 update. part 1: Screening, diagnosis, and local treatment with curative intent. *Eur Urol* (2021) 79:243. doi: 10.1016/j.eururo.2020.09.042

12. Tafuri A, Iwata A, Shakir A, Iwata T, Gupta C, Sali A, et al. Systematic biopsy of the prostate can be omitted in men with PI-RADS™ 5 and prostate specific antigen density greater than 15. *J Urol* (2021) 206:289. doi: 10.1097/JU.0000000000001766

13. Weiner AB, Manjunath A, Kirsh GM, Scott JA, Concepcion RD, Verniero J, et al. The cost of prostate biopsies and their complications: a summary of data on all medicare fee-for-service patients over 2 years. *Urol Pract* (2020) 7:145. doi: 10.1097/UPJ.0000000000000072

14. Rouvière O, Puech P, Renard-Penna R, Claudon M, Roy C, Mège-Lechevallier F, et al. Use of prostate systematic and targeted biopsy on the basis of multiparametric MRI in biopsy-naive patients (MRI-FIRST): a prospective, multicentre, paired diagnostic study. *Lancet Oncol* (2019) 20:100. doi: 10.1016/S1470-2045(18)30569-2

15. Filson CP, Natarajan S, Margolis DJ, Huang J, Lieu P, Dorey FJ, et al. Prostate cancer detection with magnetic resonance-ultrasound fusion biopsy: The role of systematic and targeted biopsies. *Cancer* (2016) 122:884. doi: 10.1002/cncr.29874

16. Noh TI, Tae JH, Kim HK, Shim JS, Kang SG, Sung DJ, et al. Diagnostic accuracy and value of magnetic resonance imaging-ultrasound fusion transperineal targeted and template systematic prostate biopsy based on bi-parametric magnetic resonance imaging. *Cancer Res Treat* (2020) 52:714. doi: 10.4143/crt.2019.716

17. Oberlin DT, Casalino DD, Miller FH, Meeks JJ. Dramatic increase in the utilization of multiparametric magnetic resonance imaging for detection and management of prostate cancer. *Abdom Radiol (NY)* (2017) 42:1255. doi: 10.1007/s00261-016-0975-5

18. van der Leest M, Cornel E, Israël B, Hendriks R, Padhani AR, Hoogenboom M, et al. Head-to-head comparison of transrectal ultrasound-guided prostate biopsy versus multiparametric prostate resonance imaging with subsequent magnetic resonance-guided biopsy in biopsy-naïve men with elevated prostate-specific antigen: A Large prospective multicenter clinical study. *Eur Urol* (2019) 75:570. doi: 10.1016/j.eururo.2018.11.023

19. Klotz L, Chin J, Black PC, Finelli A, Anidjar M, Bladou F, et al. Comparison of multiparametric magnetic resonance imaging-targeted biopsy with systematic transrectal ultrasonography biopsy for biopsy-naive men at risk for prostate cancer: A phase 3 randomized clinical trial. *JAMA Oncol* (2021) 7:534. doi: 10.1001/jamaoncol.2020.7589

20. Ghani KR, Dundas D, Patel U. Bleeding after transrectal ultrasonography-guided prostate biopsy: a study of 7-day morbidity after a six-, eight-and 12-core biopsy protocol. *BJU Int* (2004) 94:1014. doi: 10.1111/j.1464-410X.2004.05096.x

21. Massanova M, Vere R, Robertson S, Crocetto F, Barone B, Dutto L, et al. Clinical and prostate multiparametric magnetic resonance imaging findings as predictors of general and clinically significant prostate cancer risk: A retrospective single-center study. *Curr Urol* (2023). doi: 10.1097/cu9.0000000000000173

22. Alberts AR, Roobol MJ, Verbeek JFM, Schoots IG, Chiu PK, Osses DF, et al. Prediction of high-grade prostate cancer following multiparametric magnetic resonance imaging: Improving the Rotterdam European randomized study of screening for prostate cancer risk calculators. *Eur Urol* (2019) 75:310–8. doi: 10.1016/j.eururo.2018.07.031

23. Rapisarda S, Bada M, Crocetto F, Barone B, Arcaniolo D, Polara A, et al. The role of multiparametric resonance and biopsy in prostate cancer detection: comparison with definitive histological report after laparoscopic/robotic radical prostatectomy. *Abdom Radiol (NY)* (2020) 45:4178–84. doi: 10.1007/s00261-020-02798-8

24. Drobish JN, Bevill MD, Tracy CR, Sexton SM, Rajput M, Metz CM, et al. Do patients with a PI-RADS 5 lesion identified on magnetic resonance imaging require systematic biopsy in addition to targeted biopsy? *Urol Oncol* (2021) 39:235 e1. doi: 10.1016/j.urolonc.2020.12.015

25. Kilic M, Vural M, Coskun B, Acar Ö, Saglican Y, Akpek , et al. Accuracy of sampling PI-RADS 4-5 index lesions alone by MRI-guided in-bore biopsy in biopsy-naive patients undergoing radical prostatectomy. *Eur Urol Focus* (2020) 6:249. doi: 10.1016/j.euf.2019.04.010

26. Bass EJ, Pantovic A, Connor M, Gabe R, Padhani AR, Rockall A, et al. A systematic review and meta-analysis of the diagnostic accuracy of biparametric prostate MRI for prostate cancer in men at risk. *Prostate Cancer Prostatic Dis* (2021) 24:596. doi: 10.1038/s41391-020-00298-w

27. Alabousi M, Salameh JP, Gusenbauer K, Samoilov L, Jafri A, Yu H, et al. Biparametric vs multiparametric prostate magnetic resonance imaging for the detection of prostate cancer in treatment-naïve patients: a diagnostic test accuracy systematic review and meta-analysis. *BJU Int* (2019) 124:209. doi: 10.1111/bju.14759

28. Noh TI, Shim JS, Kang SG, Cheon J, Lee JG, Lee JH, et al. Concordance between biparametric MRI, transperineal targeted plus systematic MRI-ultrasound fusion prostate biopsy, and radical prostatectomy pathology. *Sci Rep* (2022) 12:6964. doi: 10.1038/s41598-022-10672-4

29. Arabi A, Deebajah M, Yaguchi G, Pantelic M, Williamson S, Gupta N, et al. Systematic biopsy does not contribute to disease upgrading in patients undergoing targeted biopsy for PI-RADS 5 lesions identified on magnetic resonance imaging in the course of active surveillance for prostate cancer. *Urology* (2019) 134:168. doi: 10.1016/j.urology.2019.08.035

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 03 April 2023
DOI 10.3389/fonc.2023.1108937

OPEN ACCESS

EDITED BY
Sifeng Qu,
Shandong University, China

REVIEWED BY
Jeanny B. Aragon-Ching,
Inova Schar Cancer Institute, United States
Martin Schoen,
Saint Louis University, United States

*CORRESPONDENCE
Dawid Sigorski
dawid.sigorski@uwm.edu.pl

SPECIALTY SECTION
This article was submitted to Genitourinary Oncology, a section of the journal Frontiers in Oncology

RECEIVED 26 November 2022
ACCEPTED 27 February 2023
PUBLISHED 03 April 2023

CITATION
Sigorski D, Wilk M, Gawlik-Urban A, Sałek-Zań A, Kiszka J, Malik M, Czerko K, Kuć K, Szczylik C, Kubiatowski T, Cybulska-Stopa B, Filipczyk-Cisarż E, Bodnar L and Skoneczna I (2023) Real-life data of abiraterone acetate and enzalutamide treatment in post-chemotherapy metastatic castration-resistant prostate cancer in Poland. *Front. Oncol.* 13:1108937. doi: 10.3389/fonc.2023.1108937

Real-life data of abiraterone acetate and enzalutamide treatment in post-chemotherapy metastatic castration-resistant prostate cancer in Poland

Dawid Sigorski[1,2*], Michał Wilk[3], Angelika Gawlik-Urban[4,5], Agata Sałek-Zań[4], Joanna Kiszka[6], Mateusz Malik[7], Katarzyna Czerko[8], Kamil Kuć[9], Cezary Szczylik[3], Tomasz Kubiatowski[1,2], Bożena Cybulska-Stopa[4], Emilia Filipczyk-Cisarż[7], Lubomir Bodnar[8,10] and Iwona Skoneczna[3,11,12]

[1]Department of Oncology, Collegium Medicum, University of Warmia and Mazury, Olsztyn, Poland, [2]Department of Oncology and Immuno-Oncology, Warmian-Masurian Cancer Center of the Ministry of the Interior and Administration Hospital, Olsztyn, Poland, [3]Department of Oncology, Centre of Postgraduate Medical Education, European Health Centre, Otwock, Poland, [4]Department of Clinical Oncology, Maria Sklodowska-Curie National Research Institute of Oncology, Kraków, Poland, [5]Faculty of Health Sciences, University of Applied Sciences in Tarnów, Tarnów, Poland, [6]Department of Clinical Oncology, Subcarpathian Cancer Center, Brzozów, Poland, [7]Department of Clinical Oncology, Lower Silesian Oncology, Pulmonology and Hematology Centre, Wroclaw, Poland, [8]Department of Clinical Oncology and Radiotherapy, St. John Paul II Mazovia Regional Hospital in Siedlce, Siedlce, Poland, [9]Department of Oncology, St. Pio's Provincial Hospital, Przemyśl, Poland, [10]Faculty of Medical and Health Sciences, University of Natural Sciences and Humanities, Siedlce, Poland, [11]Department of Oncology, Grochowski Hospital, Warsaw, Poland, [12]Cancer & Cardio-Oncology Diagnostics, Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Poland

Background: Abiraterone acetate (ABI) and Enzalutamide (ENZA) are second-generation hormone drugs that show breakthrough activity in post-chemotherapy, metastatic castration-resistant prostate cancer (mCRPC). The leading oncological and urological guidelines indicate both drugs with the same strong recommendation. There is a lack of randomized trials which compare the efficacy of ABI and ENZA. The current study aimed to compare the effectiveness of the drugs with an analysis of prognostic factors related to those drugs.

Patients and methods: The study included 420 patients with docetaxel (DXL) pretreated mCRPC from seven Polish cancer centers. Patients were treated according to inclusion and exclusion criteria in the Polish national drug program (1000 mg ABI and 10 mg prednisone, *n*=76.2%; ENZA, 160 mg; *n*=23.8%). The study retrospectively analyzed the overall survival (OS), time to treatment failure (TTF), PSA 50% decline rate (PSA 50%) and selected clinic-pathological data.

Results: In the study group, the median OS was 17 months (95% CI: 15.6-18.3). The median OS (26.1 vs. 15.7 mo.; $p<0.001$), TTF (14.2 vs. 7.6 mo.; $p<0.001$) and PSA 50% (87.5 vs. 56%; $p<0.001$) were higher in ENZA than in ABI treatment. Multivariate analysis shows that ENZA treatment and PSA nadir <17.35 ng/mL

during or after DXL treatment were related to longer TTF. ENZA treatment, DXL dose ≥750 mg, PSA nadir <17.35 ng/mL during or after DXL treatment was related to longer OS.

Conclusions: ENZA treatment may be related to more favorable oncological outcomes than ABI treatment in the studied Polish population of patients. A 50% decline in PSA is an indicator of longer TTF and OS. Due to the non-randomized and retrospective nature of the analysis, the current results require prospective validation.

KEYWORDS

real-word study, abiraterone acetate, enzalutamide, metastatic prostate cancer, targeted therapy

1 Introduction

Prostate cancer (PCa) is one of the most common cancers worldwide. According to the Surveillance Epidemiology and End Results Program, it is estimated that in 2022 as many as 34,500 people will die of PCa (1, 2). Androgen deprivation therapy (ADT) remains the key systemic therapy for patients with metastatic prostate cancer. Despite the initial sensitivity to ADT, PCa transforms into the uncurable castration-resistant stage of the disease (3). Currently registered treatment options for PCa patients consist of chemotherapy (docetaxel (DXL), cabazitaxel) and second-generation antiandrogens (abiraterone acetate (ABI), enzalutamide (ENZA), apalutamide, darolutamide), radiopharmaceutical therapy (Radium-223), immunotherapy (sipuleucel-T, pembrolizumab) and PARP inhibitors (olaparib, rucaparib) (4, 5). The drug selection depends on the castration status, tumor stage and genetic mutation status. According to National Comprehensive Cancer Network, oncological guidelines ABI and ENZA are the first-choice treatment options for patients who progressed after prior DXL chemotherapy and were not treated with novel hormone therapy (category 1) (6).

Advances in cancer pathobiology and understanding the mechanisms contributing to cancer progression allow for designing antiandrogen-targeted therapy. ABI and ENZA changed the treatment landscape for patients with PCa (7, 8). Several differences between these drugs include mechanisms of action, dosing method and pattern of side effects. ABI inhibits 17α-hydroxylase/C17.20-lyase (CYP17), an enzyme involved in the biosynthesis of androgens (8). ENZA inhibits the androgen receptor signaling pathway affecting androgen binding to androgen receptors, translocation of androgen receptors to the nucleus and interaction with DNA (7). Both drugs are used once daily, ABI needs additional steroid supplementation. ABI and ENZA are registered treatment options for chemotherapy-naive and chemotherapy-pretreated PCa patients with metastases. COU-AA-301 and AFFIRM were the phase III randomized clinical trials which showed that ABI and ENZA increase overall survival (OS) in PCa patients previously treated with chemotherapy (7, 8). There is a lack of established predictive factors and head-to-head comparative studies, which may facilitate clinicians' choice between these two drugs. The current study aimed to compare the oncological outcome measures and evaluate prognostic factors affecting survival in real-life populations of patients treated with ABI and ENZA in Poland.

2 Patients and methods

This retrospective analysis was approved by the local bioethics committee in Olsztyn (5/21/VII.) and was conducted in accordance with the Declaration of Helsinki. The analyzed data were obtained from the Polish National Health Fund Drug Program database. The study population consisted of patients with metastatic castration-resistant prostate cancer (mCRPC) receiving 1,000 mg ABI with 10 mg prednisone or 160 mg ENZA once a day who progressed after chemotherapy with DXL and were qualified for this treatment in seven comprehensive cancer centers in Poland (Olsztyn, Otwock, Cracow, Brzozów, Wrocław, Siedlce, Przemysl) between 2014-2021 (ABI) and 2018-2021 (ENZA). The inclusion and exclusion criteria were based on the Polish National Health Fund Department, which reimbursed the treatment. The criteria are mostly equivalent to those from clinical trials.

Inclusion criteria included: age over 18 years, a pathological diagnosis of prostate adenocarcinoma with radiologic evidence of metastases, and a serum testosterone level of 50 ng/dL or less (≤1.7 nmol/L) due to ADT (surgical or pharmacological). All qualified patients received DXL before ABI or ENZA treatment. The disease progression after or during chemotherapy was defined as biochemical progression if a patient had two consecutive increases in the prostate-specific antigen (PSA) concentration (from the lowest PSA level reached during or after DXL) or radiological progression (radiographic evidence of disease progression in bone or soft tissue). All patients had an Eastern

Cooperative Oncology Group (ECOG) performance status score of 0 or 1. Patients could not be qualified if they had significant hepatic dysfunction, unstable or uncontrolled cardiovascular disorders, or a history of prior abiraterone acetate, enzalutamide or ketoconazole therapy. All the patients' characteristics were registered before the initiation of ABI/ENZA treatment.

The primary endpoints of the analysis were:

- The OS, defined as the time between the start of treatment and death from any cause;
- The time to treatment failure (TTF) is described as the time between the initiation of ABI/ENZA to the moment of its termination (due to cancer progression*, unaccepted toxicity, hypersensitivity to the drug or the patient's death);
- PSA 50% rate - patients with PSA decline 50% or more during the treatment.

Disease progression* was defined according to the Polish drug program:

I. The occurrence of at least two of the following three types of progression in total:
   1) Clinical - defined as pain progression (inclusion of a new opioid for more than two weeks) or the occurrence of skeletal-related events or ECOG ≥2 (according to the WHO classification).
   2) Biochemical- defined as PSA progression (three consecutive increases in PSA, measured at least in weekly intervals, with proven increases of at least 50% from ABI/ENZA baseline).
   3) Radiological - the appearance of at least two new metastatic lesions confirmed by bone scan.

II. Response Evaluation Criteria In Solid Tumors ver. 1.1 were met (regardless of other types of progression mentioned above).

The analyzed clinicopathological data included:

- Characteristics of PCa: date of diagnosis, Gleason score (GS), primary stage at PCa diagnosis (non-metastatic (M0) vs. metastatic (M1)), sites of metastases at the beginning of ABI/ENZA (bone, only extra bone, both localizations).
- History of PCa treatment: treatment approach at PCa diagnosis (radical vs. palliative), type of castration (LHRH agonists, LHRH antagonists, surgical), the timing of DXL (for hormone-sensitive PCa vs. mCRPC), total administered dose of DXL, duration of chemotherapy, PSA nadir during or after DXL treatment, subsequent lines after ABI/ENZA treatment, type of progression at ABI/ENZA treatment initiation (biochemical, radiological or both).
- Patient characteristics: age, ECOG and body mass index (BMI).

The statistically significant variables in univariate analysis were chosen for multivariate analysis.

Statistical analyses were performed using Stata® Software ver. 14.1 (StataCorp LLC). Nominal parameters were presented as a percentage frequency. The study used the $\chi 2$ test (categorical variables), the independent t-test (continuous, normally distributed variables) and the Mann-Whitney U (non-normally distributed variables) for comparisons between the groups. The r-Pearson and Spearman correlations were used for assessing the association between continuous variables. Survival curves and Cox proportional hazard model (univariate and multivariate) were used to determine the predictors for longer TTF/OS during ABI/ENZA treatment. A level of $p<0.05$ was recognized as statistically significant.

3 Results

3.1 Characteristics of the study group (overall population)

A summary of the basic characteristics of the patients is presented in Table 1.

The study enrolled 420 patients who met inclusion criteria and were treated with ABI or ENZA. 72.6% of patients were treated with ABI and 23.8% with ENZA. Most of the patients were younger (51.2%) than 70 years. The median GS was 8, ECOG 1 (76.4%). In 59.1% of cases, PCa was diagnosed in the disseminated stage of disease, in 46.4% with the bone-only confined disease. DXL was the most commonly used in the mCRPC stadium of the disease (66.4%). The median PSA was 107 ng/mL at the start of ABI/ENZA treatment. The most common type of ADT was treatment with LHRH agonists (90%). Most enrolled patients were qualified for the program based on the biochemical and radiological progression (70.4%). Median PSA nadir during or after DXL was 17.35 ng/mL (IQR 3.43 – 76.69 ng/mL). The median DXL dose was 750 mg (IQR 510 – 990).

ABI and ENZA populations were statistically different in terms of several clinicopathologic factors, i.e. grading, pattern of metastases, and ECOG. In the ENZA group, PCa was less differentiated (≥8; 72.6 vs. 51.4; $p<0.001$), metastases occurred more frequently in the bone than in the viscera (56.2 vs. 41.8%; $p=0.013$), and patient performance status was better in the ENZA group (ECOG 0 32.3 vs. 16.3; $p=0.001$). A statistical comparison of the ABI and ENZA groups is shown in Table 1.

3.2 Analysis of TTF in the study group

Median TTF for the overall population was 9.2 months (95% CI: 8.0 – 10.1). Median TTF in the ENZA group was statistically longer than in the ABI group (14.2 vs. 7.6 mo.; $p<0.001$; Figure 1). Median TTF was longer in ECOG 0 patients vs. ECOG 1 (11.3 vs. 8.8 mo.; $p=0.021$), BMI ≥25 vs. BMI <25 (9.5 vs. 6.5 mo.; $p=0.009$). Patients

10.3389/fonc.2023.1108937

TABLE 1 Clinicopathological characteristics of the study group.

Drug n (%)	All 420 (100)	ABI 320 (76.2)	ENZA 100 (23.8)	p-value ABI vs ENZA
Age				
n=	420	320	100	
Median (IQR), years	69 (64-75)	69 (64 – 76)	69 (63.5-74)	0.679
< 70 years. n (%)	215 (51.2)	141 (50.3)	54 (54)	0.520
≥ 70 years. n (%)	205 (48.8)	159 (49.7)	46 (46)	
Gleason scale				
n=	381	286	95	
Median (IQR)	8 (7-9)	-	–	<0.001*
≥8. n (%)	216 (51.4)	147 (45.94)	69 (69)	
<8. n (%)	165 (39.3)	139 (43.44)	26 (26)	
Missing data. n (%)	39 (9.3)	34 (10.62)	5 (5)	
ECOG				
n=	402	306	96	
0. n (%)	81 (19.3)	50 (15.63)	31 (31)	0.001*
1. n (%)	321 (76.4)	256 (80.0)	65 (65)	
Missing data. n (%)	18 (4.3)	14 (4.37)	4 (4)	
Treatment approach at PCa diagnosis				
n =	412	312	100	0.672
Radical. n (%)	164 (39)	126 (39.37)	38 (38)	
Palliative. n (%)	248 (59.1)	186 (58.13)	62 (62)	
Missing data. n (%)	8 (1.9)	8 (2.5)	–	
Site of metastases				
n=	413	315	98	
Bone only. n (%)	195 (46.43)	138 (43.13)	57 (57)	0.013
Visceral ± bone. n (%)	218 (51.90)	177 (55.31)	41 (41)	
Missing data. n (%)	7 (1.67)	5 (1.56)	2 (2)	
PSA ng/mL median (IQR)	107 (31.6 – 309.9)	130.1 (38.4 – 362.7)	59.5 (16.9 – 181)	0.002*
Type of ADT				
Bilateral orchidectomy. n (%)	19 (4.5)	13 (4.1)	6 (6.0)	0.984
LHRH agonists. n (%)	378 (90)	291 (90.9)	87 (87.0)	
LHRH antagonists. n (%)	15 (3.6)	10 (3.1)	5 (5.0)	
Missing data. n (%)	8 (1.9)	6 (1.9)	2 (2.0)	
Docetaxel treatment				
n=	404	306	95	
For mHSPC. n (%)	122 (29.1)	94 (29.38)	28 (28)	0.818
For mCRPC. n (%)	279 (66.4)	212 (66.25)	67 (67)	
Missing data. n (%)	19 (4.5)	14 (4.37)	5 (5)	

(Continued)

TABLE 1 Continued

Drug n (%)	All 420 (100)	ABI 320 (76.2)	ENZA 100 (23.8)	p-value ABI vs ENZA
Type of progression at inclusion				
n=	418	318	100	
Biochemical. n (%)	82 (19.52)	66 (20.63)	16 (16)	0.105
Radiological. n (%)	41 (9.76)	23 (7.19)	18 (18)	
Both. n (%)	295 (70.24)	229 (71.56)	66 (66)	
Missing data. n (%)	2 (0.48)	2 (0.62)	–	
Median year of treatment	-	2017	2019	
BMI kg/m^2				
n=	410	310	100	
median (IQR)	28.1 (25.4-31.3)	28.1 (25-31.1)	28.1 (25-31.9)	0.332
≥25. n (%)	314 (74,8)	240 (75)	74 (74)	
<25. n (%)	96 (22.8)	70 (21.87)	26 (26)	
Missing data. n (%)	10 (2.4)	10 (3.13)	–	
≥50% PSA decline				
n=	343	255	88	
Yes. n (%)	220 (52.38)	143 (44.69)	77 (77)	<0.001*
No. n (%)	123 (29.29)	112 (35)	11 (11)	
Missing data. n (%)	77 (18.33)	65 (20.31)	12 (12)	
Total DXL dose				
n=	339	259	80	
≥750 mg. n (%)	176 (41.90)	126 (39.38)	50 (50)	0.030*
< 750 mg. n (%)	163 (38.81)	133 (41.56)	30 (30)	
Missing data. n (%)	81 (19.29)	61 (19.06)	20 (20)	
PSA nadir during or after DXL treatment				
n=	336	248	88	
≥17.35 ng/mL. n (%)	168 (40)	141 (44.06)	27 (27)	<0.001*
<17.35 ng/mL. n (%)	168 (40)	107 (33.44)	61 (61)	
Missing data. n (%)	84 (20)	72 (22.5)	12 (12)	

ABI, Abiraterone acetate; ADT, androgen deprivation therapy; BMI, body mass index; DXL, docetaxel; ECOG, Eastern Cooperative Oncology Group; ENZA, enzalutamide; IQR, interquartile range; mCRPC, metastatic castration-resistant prostate cancer; mHSPC, metastatic hormone-sensitive prostate cancer; PCa, prostate cance; LHRH, a luteinizing-hormone-releasing hormone; PSA, prostate-specific antigen, *- statistically significant.

who were qualified for ABI/ENZA therapy with concurrent biochemical and radiological progression had shorter median TTF than patients who experienced the single type of progression (8.8 vs. 10.8 mo.; p<0.001).

DXL treatment in metastatic hormone-sensitive prostate cancer (mHSPC) vs. mCRPC did not affect TTF (p=0.743). However, patients who received ≥750 mg of DXL in total had longer median TTF (9.6 vs. 7.7 mo.; p=0.015). Patients with PSA nadir <17.35 ng/mL during or after DXL treatment had longer median TTF versus those who did not reach PSA levels below that value (12.2 vs. 6.5 mo., respectively; p<0.001). All analyzed variables are presented in Table 2. In the time-subgroup analysis (treatment between 2018 - 2021), the median TTF for both drugs was 10.6 mo. (95% CI: 9.2 – 12.0). TTF of ABI and ENZA was 7.6 mo (95% CI: 6.2 – 9.5) and 14.2 mo (95% CI: 11.3 -17.1), respectively (p <0.001).

In multivariate analysis, ENZA treatment (p=0.002) and PSA nadir during or after DXL treatment < 17.35 ng/mL (p<0.001) were related to statistically longer TTF (Table 2).

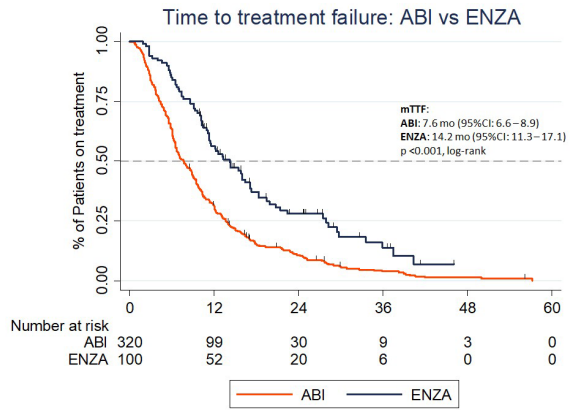


FIGURE 1
The time to treatment failure in the Abiraterone acetate and Enzalutamide group.

3.3 Analysis of OS in the study group

For the whole population, the median OS was 17 months (95% CI:15.6-18.3). Median OS for patients treated with ENZA (26.1 mo.; 95% CI: 17.8-29.4) was significantly longer than for treatment with ABI (15.7, 95% CI 13.5 - 17.4; p<0.001, Figure 2). In univariate analysis, treatment with ENZA (p<0.001), ECOG 0 (p=0.014), BMI ≥25 (p=0.002), eligible for treatment due to one type of progression vs. multiple (p=0.002), treatment with DXL of at least 750 mg in total (p=0.001), ≥50% PSA response (p<0.001) during ABI or ENZA and PSA nadir < 17.35 during or after DXL treatment (p<0.001) were associated with longer median OS (Table 3). There was a positive, non-significant trend in longer median OS in patients who were primarily diagnosed with early-stage cancer (p=0.054). In patients treated between 2018 and 2021, the population's median OS was 19.5 mo (95% CI: 16.7 – 22.8). The mOS in the ABI group was 16.7 mo (95% CI: 13.2 – 20.4). The median OS in the ENZA group was 26.1 mo (95% CI: 17.7 – 24.9). The difference was statistically significant (p = 0.001).

In multivariate analysis, ENZA treatment (p=0.019), total DXL dose >750 mg in total (p=0.027) and PSA nadir <17.35 during or after DXL treatment (p=0.004) were associated with longer OS (Table 3).

3.4 PSA response during ABI/ENZA

The 50% PSA decline was higher in the ENZA group than in the ABI group (87.5% vs. 56%; p<0.001). Patients who experienced ≥ 50% PSA decline during ABI/ENZA had statistically longer TTF in comparison with men who had <50% PSA decline (13.9 vs. 5.6 mo., respectively; p<0.001, log-rank). Median OS was also longer in men with ≥ 50% PSA decline (23.3 vs. 10.5 mo.; p<0.001, log-rank).

3.5 Treatment after progression

After progression on ABI or ENZA, 75/166 patients (45.18%) were treated with a subsequent line of therapy (ABI: 50 patients, 41.3%; ENZA: 25 patients, 55.5%). The patients were treated with DXL rechallenge, cabazitaxel, radium-223 dichloride, ABI/ENZA and clinical trials. The OS for patients treated with at least one subsequent line of therapy was 21.2 months (95% CI: 16.56 - 26.15), and the median of OS for patients who were not treated was 11 months (95% CI 7.57 - 14.61). There were no statistical differences in the number of subsequent therapy lines between ABI and ENZA (p=0.101).

4 Discussion

Real-life studies allow for a better understanding of disease courses in a specific population of patients and facilitate the selection of a drug, which is especially important for practicing oncologists. The choice of the drug by clinicians may be based on patient comorbidities, expected side effects, patient preferences and cost-effectiveness. The multicenter retrospective analysis in the current study is the first study to describe the outcomes for mCRPC patients treated with ABI and ENZA in Poland. The study was conducted on all subsequent patients from these centers who met the criteria for participation in the Polish drug program, which meets the criteria of a retrospective case-control study (evidence level IIIE). There is a lack of randomized, comparative phase III trials in patients treated with ENZA and ABI in mCRPC, which justifies the current real-life clinical data analysis.

COU-AA-301 and AFFIRM were registered trials that determined the role of ABI and ENZA in mCRPC patients pretreated with DXL chemotherapy (7, 8). A total of 800 patients were treated with ENZA, and 300 were treated with a placebo in AFFIRM. The final analysis revealed that the median of OS was 18.4 vs. 13.6 months, respectively (p < 0.001, HR: 0.63; 95% CI: 0.53–0.75). The trial also met the secondary endpoints, including the proportion of patients with a reduction in the PSA level by 50% or more (54% vs. 2%, p<0.001), the time to PSA progression (8.3 vs. 3.0 mo.; p<0.001), radiographic progression-free survival (rPFS) (8.3 vs. 2.9 mo.; p<0.001). The analysis of HR for death showed the superiority of ENZA over placebo in all patient subgroups, including age (65< vs. ≥65 years), baseline ECOG, type of progression at entry study, visceral disease and PSA level at baseline (7). In the COU-AA-301 trial, 797 patients were treated with ABI and prednisone, and 398 received a placebo. The median OS for the study group was 15.8 months (95% CI: 14.8-17) vs. 11.2 months (10.4-13.1; HR 0.74, 95% CI: 0.64-0.86; p<0·0001). The trial also met the secondary endpoints: median time to PSA progression (8.5 vs. 6.6 mo. p<0·0001), median rPFS (5.6 vs. 3.6 mo. p<0.0001), and proportion of patients who had a PSA response (29.5% vs. 5.5%; p<0.0001). The analysis of HR also confirmed the superiority of the study drug over the placebo in subgroups, including age, ECOG and type of progression (8).

In the current study, the median OS was 15.7 months in the ABI group (95% CI: 13.5 - 17.4) and 26.1 months (95% CI: 17.8-29.4) in the ENZA group. The difference between drugs was statistically significant. TTF was also longer in the ENZA group (14.2; 95% CI: 11.3-17.6 mo.) than the ABI group (7.6; 95% CI: 6.64-8.85; p<0.001). The 50% PSA decline was higher in the ENZA group

10.3389/fonc.2023.1108937

TABLE 2 Predictive factors determining the time to treatment failure (univariate and multivariate analysis).

Variable	Median TTF (months)	HR	95% CI	*p*-value
A. Univariate analysis				
Drug type				
ABI (ref.)	7.6	–	–	–
ENZA	14.2	0.51	0.39 – 0.66	<0.001*
ECOG				
0 (ref.)	11.3	–	–	–
1	8.8	1.37	1.05 – 1.79	0.021*
BMI				
<25	6.5	–	–	–
≥25	9.5	0.73	0.57 – 0.92	0.009*
Gleason score				
<8 (ref.)	10.3	–	–	–
≥8	7.9	1.18	0.95 - 1.46	0.128
Age				
≥ 70 (ref.)	9.3	–	–	
<70	9.0	0.96	0.78- 1.17	0.685
Primary stage				
I-III (ref.)	9.4	–	–	–
IV)	9.1	1.10	0.89 - 1.35	0.386
Castration method				
Surgical (ref.)	9.6	–	–	–
Pharmacological	8.9	1.02	0.64- 1.65	0.916
Location of metastases				
Bone only (ref.)	8.9	–	–	–
Visceral ± bone	8.8	0.98	0.80 - 1.20	0.859
≥ 50% PSA decline				
No (ref.)	5.5	–	–	–
Yes	13.8	0.28	0.22 – 0.35	<0.001*
DXL therapy				
mHSPC (ref.)	8.8	–	–	–
mCRPC	9.3	1.04	0.83 - 1.30	0.743
Total DXL dose (mg)				
<750 (ref.)	7.7	–	–	–
≥750	9.6	0.75	0.60 - 0.95	0.015*
PSA nadir during or after DXL (ng/mL)				
<17.35 (ref.)	12.2	–	–	–
≥ 17.35	6.5	1.91	1.55 - 2.46	<0.001*

(Continued)

TABLE 2 Continued

Variable	Median TTF (months)	HR	95% CI	*p*-value
Initial progression type				
Radiological or PSA (ref.)	10.8	–	–	–
Both	8.8	1.57	1.25 – 1.99	< 0.001*
B. Multivaraite analysis				
Drug type				
ABI (ref.)	–	0.59	0.43 – 0.83	0.002*
ENZA				
ECOG				
0 (ref.)	–	1.11	0.81 - 1.53	0.520
1				
BMI				
<25 (ref.)	–	0.87	0.65 - 1.18	0.373
≥25				
Total DXL dose (mg)				
<750 (ref.)	–	0.87	0.66 – 1.14	0.317
≥750				
PSA nadir during or after DXL (ng/mL)				
<17.35 (ref.)	–	1.77	1.33 – 2.34	<0.001*
≥ 17.35				
Initial progression type				
Radiological or PSA (ref.)	–	1.33	0.99 – 1.79	0.057
Both				

ABI, abiraterone acetate; BMI, body mass index; CI, confidence interval; DXL, docetaxel; ECOG, Eastern Cooperative Oncology Group; ENZA, enzalutamide; HR, hazard ratio; mCRPC, metastatic castration-resistant prostate cancer; mHSPC, metastatic hormone-sensitive prostate cancer; PSA, prostate-specific antigen; ref, reference value; TTF, time to treatment failure; *- statistically significant.

than in the ABI group (87.5 vs. 56%; $p<0.001$), which was higher than in the registered trials (56 vs. 29.5%). The 50% PSA decline was observed more frequently in the ENZA group (87.5%) than in the ABI group (56%; $p<0.001$). The result of OS in the current study and the registered trial was comparable to the ABI group (15.7 vs. 15.8 months) but differed from the ENZA group (26.1 vs. 18.4 months). However, the length of treatment was longer in the ENZA group ($p<0.001$), TTF and rPFS are not comparable oncological outcome measures.

The observational study of real-life clinical data was analyzed in many countries, and results vary between the different populations of patients. The main differences between the studies include the number of patients, the line of treatment (pre or post-chemotherapy) and the following lines of treatment after progression.

The most extensive observational study was published by Schoen et al. and presented the results of treatment on 5,822 US veterans. It shows that the OS was longer in patients treated with

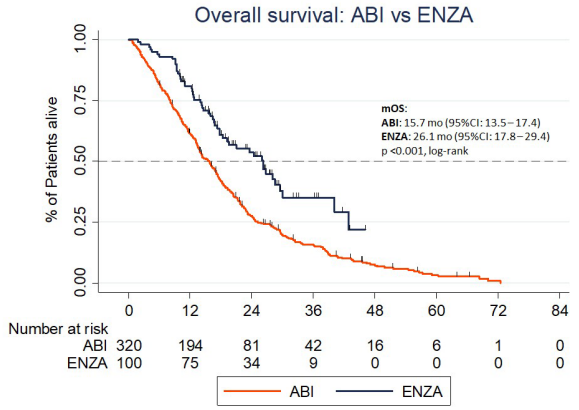


FIGURE 2
The overall survival in the Abiraterone acetate and Enzalutamide group.

10.3389/fonc.2023.1108937

TABLE 3 Prognostic factors determining the overall survival (univariate and multivariate analysis).

Variable	Median OS (months)	HR	95% CI	*p*-value
A. Univariate analysis				
Drug type				
ABI (ref.)	15.7	–	–	–
ENZA	26.1	0.54	0.40 - 0.73	<0.001*
ECOG				
0 (ref.)	22.5	–	–	–
1	16.1	1.45	1.08 - 1.95	.014*
BMI				
<25	12.7	–	–	–
≥25	17.8	0.67	0.52 - .86	0.002*
Gleason score				
<8 (ref.)	18.7	–	–	–
≥8	14.3	1.17	0.93 - 1.47	0.181
Age				
≥ 70 (ref.)	17.7	–	–	–
<70	16.1	1.04	0.84 - 1.29	0.716
Primary stage				
I-III (ref.)	18.7	–	–	–
IV	15.9	1.24	0.99 - 1.55	0.054
Castration method				
Surgical (ref.)	20	–	–	–
Pharmacological	16.8	0.88	0.54 - 1.43	0.597
Location of metastases				
Bone only (ref.)	17.6	–	–	–
Visceral ± bone	16.3	1.00	0.81 - 1.24	0.968
≥ 50% PSA decline				
No (ref.)	10.5	–	–	–
Yes	23.3	0.37	0.29 - 0.47	<0.001*
DXL therapy				
mHSPC (ref.)	16.3	–	–	–
mCRPC	17.6	0.91	0.72 - 1.16	0.459
Total DXL dose (mg)				
<750 (ref.)	15.8	–	–	–
≥750	18.4	0.66	0.52 – 0.84	0.001*
PSA nadir during or after DXL (ng/mL)				
<17.35 (ref.)	22.5	–	–	–
≥ 17.35	14.3	1.89	1.48 – 2.42	<0.001*

(Continued)

TABLE 3 Continued

Variable	Median OS (months)	HR	95% CI	*p*-value
Initial progression type				
Radiological or PSA (ref.)	20.3	–	–	–
Both	16.1	1.46	1.15 - 1.86	0.002*
B. Multivaraite analysis				
Drug type				
ABI (ref.)	–	0.62	0.42 – 0.93	0.019*
ENZA				
ECOG				
0 (ref.)	–	1.22	0.85 - 1.75	0.270
1				
BMI				
<25 (ref.)	–	0.92	0.66 - 1.28	0.610
≥25				
Primary stage				
I-III (ref.)	–	1.17	0.88 – 1.54	0.276
IV				
Total DXL dose (mg)				
<750 (ref.)	–	0.71	0.53 – 0.96	0.027*
≥750				
PSA nadir during or after DXL (ng/mL)				
<17.35 (ref.)	–	1.56	1.15 – 2.16	0.004*
≥ 17.35				
Initial progression type				
Radiological or PSA (ref.)	–	1.16	0.85 - 1.59	0.350
Both				

ABI, abiraterone acetate; BMI, body mass index; CI, confidence interval; DXL, docetaxel; ECOG, Eastern Cooperative Oncology Group; ENZA, enzalutamide; mCRPC, metastatic castration-resistant prostate cancer; mHSPC, metastatic hormone-sensitive prostate cancer; OS, overall survival; PSA, prostate-specific antigen; ref, reference value; *- statistically significant.

ENZA than ABI (24.2 vs 22.1 months) in pre- and post-chemotherapy treated patients. Importantly, patients with cardiovascular diseases also had better survival (9). Also, a direct comparison of drugs in 10,308 chemotherapy-naive patients with CRPC based on the 2014-2018 French population study showed that patients treated with ENZA had a better OS than ABI (34.2 vs. 31.7 mo.) (10). Li et al. also recently published the results of a retrospective cohort population-based study in the unselected Taiwanese population, which showed that treatment with ENZA (n=118) was associated with better OS than treatment with ABI (n=1046), although without differences in TTF (11). In another unselected Australian population of 250 patients (53% of ABI; 38% of ENZA post-chemo), patients treated with ENZA had a greater PSA response (70.3% vs. 39.5%). The OS was longer in the ENZA group, 29 months (95% CI: 21.3-36.7) vs. 7 months (95% CI: 0-18.5%; p=0.002) in the ABI group. The authors did not find any factors significantly associated with OS, including the Gleason scale (12). Interesting data come from Chowdhury et al., who analyzed the oncological outcomes among patients from 16 countries with mCRPC treated in the first line of therapy with ABI, ENZA or chemotherapy. The OS for ABI and ENZA was the same (27.1 months) (13).

Data from selected post-DXL-treated patients show similar results. Contrary to the pre-DXL setting, in post-DXL, the OS in the ENZA (26 ± 7 (12.3-39.7)) was longer than the ABI (13 ± 1.6 (9.8-16.2); 0.021) cohort of patients. Moreover, the rPFS was longer in the ENZA (11± 5.1 (1.1-20.9) than the ABI (pre-and post-DXL) group. PSA ≥50% decline occurred more frequently in the ENZA group than in the ABI group (p=0.02). The authors concluded that the good prognostic factors for rPFS were ENZA treatment, age ≥75 years and PSA ≥50% decline at 12 weeks of treatment. The authors did not find any prognostic factors for OS (14).

Another Taiwanese study that indirectly compared the ENZA (n=13) and ABI (n=63) in post-DXL chemotherapy showed that the

OS from second-line hormone treatment was 30.2 months in the ABI group and 16.2 months in the ENZA group, although statistical significance was not shown. The same study shows no difference between PSA and PFS responses. PSA 50% response was seen in 48.4% in ABI and 69.2% in the ENZA group (p=0.171); the median PFS was 7.3 months (95% CI: 4.79-9.80) in ABI and 9.5 months (95%CI: 5.743-13.257) in ENZA (p of log rank=0.766) (15). In Austrian populations of patients, the OS for ABI was 14 months (mean: 15.8 ± 0.9 months), and for ENZA it was 19 months (mean: 17.2 ± 1.4 months). A randomized phase II cross-over study confirmed that ENZA is associated with better biochemical response, however, without changes in time to PSA progression (16). Hu et al. found a difference in OS between ENZA and ABI (17.9 vs. 15.4 mo.; p=0.8224) (17). Other studies show different OS in post-DXL treated with ABI. In the Marret et al. trial, the OS was 13.4 months, although only 58 patients were analyzed (18).

Finally, Wei et al. performed a meta-analysis of 5,199 patients treated with ABI and ENZA in randomized clinical trials. Contrary to rPFS and time to PSA progression, which were significantly better in the ENZA group, the mOS did not vary significantly between ABI and ENZA (HR=1.03, 95% CI: 0.854-1.242) (19). The same results regarding OS came from Bianchi et al. analysis (20). Another indirect analysis of AFFIRM and COU-AA-301 trials favors ENZA in terms of time to PSA progression, PSA response and radiological PFS, although without difference in OS (21). Chung et al. also studied the effectiveness of both drugs in sequential treatment. The sequence ABI-ENZA is better regarding oncological outcomes than ENZA-ABI (22). In Poland, sequential treatment is not reimbursed.

The current study attempted to identify the prognostic factors associated with ABI and ENZA therapy. Although the univariate analysis revealed that ENZA treatment, ECOG 0, BMI ≥25 kg/m^2, total DXL dose ≥750 mg, ≥50% PSA decline, and PSA nadir during or after DXL treatment <17.35 ng/mL are factors associated with better survival, the multivariate analysis shows that ENZA treatment and total DXL dose ≥750 mg and PSA nadir <17.35 ng/mL during or after DXL treatment were independent prognostic factors for longer OS. Early PSA response is a good independent prognostic factor in next-generation androgen receptor inhibitors (23, 24). The ≥50% drop in PSA from baseline within the three months of treatment correlated with better OS and PFS (23). However, although it was found that DXL dose has an impact on the prognosis of patients with PCa, treatment with an early DXL was not found to be a prognostic factor. Patients with hormone-sensitive PCa were not included in clinical trials, and data in such a population of patients are limited and not well explored. Additionally, it was studied if BMI affects prognosis during antiandrogen treatment. The majority of patients (74.4%) were overweight or obese. In univariate analysis, patients with BMI ≥ 25 kg/m^2 had longer TTF and OS, although this lacked statistical significance in multivariate analysis. Other studies suggest that obesity may play a protective role associated with increased survival (25–27).

In COU-AA-301, the authors of the study found that patients who received DXL for more than three months had better OS than those treated for less than three months (8). Chi et al. published a risk model for predicting OS in chemotherapy-pretreated patients treated with ABI, including ECOG 2, presence of liver metastases and time from ADT to the start of ABI ≤36 months (28). The multivariate analysis of the hazard ratio for death showed that ECOG 0, mean pain score on Brief Pain Inventory-Short Form <4, PSA progression at study entry, and no visceral disease at screening were associated with better survival (7). Patients with age > 75, Charlson comorbidity scores > 2, presence of symptoms, time from prostate cancer diagnosis < 3 years and time from last chemotherapy < 6 months had lower survival (17). Multivariate analysis revealed that PSA response, Gleason score ≥8 and PSA-doubling time <2 months correlated with OS. Patients with visceral metastases had worse oncological outcomes in terms of OS (2.8 vs. 18; p=0.0007) and PFS (2.8 vs. 6.8; p=0.0088) (29). Another study showed that low levels of miR-21 are an unfavorable prognostic factor in PCa patients (30).

In the current study, it was shown that ENZA treatment may be related to more favorable oncological outcomes than ABI treatment in the Polish population of patients. There was no difference in the efficacy of ABI and ENZA on time to treatment failure in docetaxel-naive and docetaxel-pretreated prostate cancer patients. A 50% decline in PSA is an indicator of longer TTF and OS. Due to the non-randomized and retrospective nature of the analysis, the current results require prospective validation. Although many different observational and metanalyses show that treatment with enzalutamide has a better treatment outcome, there is no single explanation for the results. The main difference between drugs includes different action mechanisms because abiraterone inhibits 17α-hydroxylase/C17,20-lyase (CYP17), and enzalutamide has a threefold mechanism of action. First, it is a potent, competitive binder of androgens at the level of the androgen receptor (AR), and it prevents the translocation of the AR from the cytoplasm to the nucleus. Within the nucleus, it inhibits AR binding to chromosomal DNA, which prevents further transcription of tumor genes. Therefore, compared to abiraterone, enzalutamide may act more selectively and comprehensively on the AR signaling pathway in prostate cancer cells (31). The indirect analysis of drugs has several limitations which may also affect the results. One of the major disadvantages of real-life data trials is the heterogenicity of the groups and follow-up time in the study. In the current cohort of patients, the differences included the ECOG scale, GS and location of metastases. The population of patients treated with ENZA was in better performance status (ECOG 0: 32.3 vs. 16.3%), had a higher percent of bone-only limited metastases (58.2 vs. 43.8%), received the higher cumulative dose of DXL (62.5 vs. 48.7%), and more patients had PSA nadir of <17.35 during or after DXL (69.3% vs 43.1%) but had less differentiated tumors (72.6 vs. 51.4%) which may interfere with the results. ABI was introduced earlier than ENZA in Poland, which limits the adjustment of drug selection. Patients included in our study were not treated during the same period (ABI 2014-2021, ENZA 2018-2021); however, the subgroup analysis of patients treated during the same time supports the superiority of ENZA in terms of TTP and OS. The unbalanced population of patients were also an issue in similar studies. The current study limitations include an unbalanced study population, the retrospective nature of the study and the lack of some clinical aspects like pain score or quality of life (similar to other published studies).

The current study confirmed the clinical activity of ABI and ENZA in the Polish population of patients. The presented analysis suggests that treatment with ENZA may be related to more favorable outcomes than treatment with ABI.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by local bioethics committee in Olsztyn (5/21/VII.). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

Conceptualization, DS and MW; methodology, DS and MW; statistical analysis, MW; writing—original draft preparation, DS, data collection: all the authors, supervision: IS. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the University of Warmia and Mazury in Olsztyn, and the University of Applied Sciences in Tarnów.

Conflict of interest

DS and KK received honoraria for lectures from Astellas and Janssen. MW received travel grants from Pfizer, Novartis and Bayer, and honoraria for lectures from Pfizer. KC received travel grants from Janssen. IS received grants/research support, honoraria or consultation fees from Astellas, Janssen.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Miller KD, Nogueira L, Devasia T, Mariotto AB, Yabroff KR, Jemal A, et al. Cancer treatment and survivorship statistics, 2022. *CA Cancer J Clin* (2022) 72:409–436. doi: 10.3322/CAAC.21731

2. *Surveillance, epidemiology, and end results program*. Available at: https://seer.cancer.gov/ (Accessed July 28, 2022).

3. Freedman-Cass D, Berardi R, Shead DA, Schaeffer EM, An Y, Armstrong AJ, et al. NCCN guidelines version 4.2022 prostate cancer. Available at: https://www.nccn.org/home/ (Accessed July 28, 2022).

4. Cornford P, van den Bergh RCN, Briers E, Van den Broeck T, Cumberbatch MG, De Santis M, et al. EAU-EANM-ESTRO-ESUR-SIOG guidelines on prostate cancer. part II–2020 update: Treatment of relapsing and metastatic prostate Cancer[Formula presented]. *Eur Urol* (2021) 79:263–82. doi: 10.1016/J.EURURO.2020.09.046

5. Potocki LP, Potocki PM, Wysocki PJ. Evolution of prostate cancer therapy. part 1. *Oncol Clin Pract* (2022) 18:177–88. doi: 10.5603/OCP.2021.0001

6. Freedman-Cass D, Berardi R, Shead DA, Schaeffer E, Armstrong AJ, Cheng HH, et al. *NCCN guidelines version 1.2022 prostate cancer* (2021). Available at: https://www.nccn.org/home/ (Accessed November 1, 2021).

7. Scher HI, Fizazi K, Saad F, Taplin M-E, Sternberg CN, Miller K, et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. *N Engl J Med* (2012) 367:1187–97. doi: 10.1056/nejmoa1207506

8. de Bono JS, Logothetis CJ, Molina A, Fizazi K, North S, Chu L, et al. Abiraterone and increased survival in metastatic prostate cancer. *N Engl J Med* (2011) 364:1995–2005. doi: 10.1056/nejmoa1014618

9. Schoen MW, Carson KR, Eisen SA, Bennett CL, Luo S, Reimers MA, et al. Survival of veterans treated with enzalutamide and abiraterone for metastatic castrate resistant prostate cancer based on comorbid diseases. *Prostate Cancer Prostatic Dis* (2022) published online. doi: 10.1038/s41391-022-00588-5

10. Scailteux L-M, Campillo-Gimenez B, Kerbrat S, Despas F, Mathieu R, Vincendeau S, et al. Overall survival among chemotherapy-naive patients with castration-resistant prostate cancer under abiraterone versus enzalutamide: A direct comparison based on a 2014–2018 French population study (the SPEAR cohort). *Am J Epidemiol* (2021) 190:413–22. doi: 10.1093/aje/kwaa190

11. Li PY, Lu YH, Chen CY. Comparative effectiveness of abiraterone and enzalutamide in patients with metastatic castration-resistant prostate cancer in Taiwan. *Front Oncol* (2022) 12:822375. doi: 10.3389/FONC.2022.822375

12. Raju R, Sahu A, Klevansky M, Torres J. Real-world data on outcomes in metastatic castrate-resistant prostate cancer patients treated with abiraterone or enzalutamide: A regional experience. *Front Oncol* (2021) 11:656146. doi: 10.3389/FONC.2021.656146

13. Chowdhury S, Bjartell A, Lumen N, Maroto P, Paiss T, Gomez-Veiga F, et al. Real-world outcomes in first-line treatment of metastatic castration-resistant prostate cancer: The prostate cancer registry. *Target Oncol* (2020) 15:301–15. doi: 10.1007/S11523-020-00720-2

14. Demirci A, Bilir C, Gülbağcı B, Hacıbekiroğlu İ, Bayoğlu İV, Bilgetekin İ, et al. Comparison of real-life data of abiraterone acetate and enzalutamide in metastatic castration-resistant prostate cancer. *Sci Rep* (2021) 11:14131. doi: 10.1038/S41598-021-93659-X

15. Chang LW, Hung SC, Wang SS, Li JR, Yang CK, Chen CS, et al. Abiraterone acetate and enzalutamide: Similar efficacy in treating post docetaxel metastatic castration-resistant prostate cancer: Single center experience. *Anticancer Res* (2019) 39:3901–8. doi: 10.21873/ANTICANRES.13541

16. Chi KN, Annala M, Sunderland K, Khalaf D, Finch D, Oja CD, et al. A randomized phase II cross-over study of abiraterone + prednisone (ABI) vs enzalutamide (ENZ) for patients (pts) with metastatic, castration-resistant prostate cancer (mCRPC). *J Clin Oncol* (2017) 35:5002–2. doi: 10.1200/JCO.2017.35.15_SUPPL.5002

17. Uj K, Dasari A, Behera A, Agrawal S, Ra K, Dixit M, et al. Pcn52 abiraterone versus enzalutamide in the post-chemotherapy setting in metastatic castration-resistant prostate cancer: Population-based study. *Value Heal* (2019) 22:S445. doi: 10.1016/J.JVAL.2019.09.249

18. Marret G, Doucet L, Hennequin C, Fizazi K, Culine S. Abiraterone in metastatic castration-resistant prostate cancer: Efficacy and safety in unselected patients. *Cancer Treat Res Commun* (2018) 17:37–42. doi: 10.1016/J.CTARC.2018.10.001

19. Wei ZH, Chen CX, Li BW, Li YY, Gu H. Efficacy and safety of abiraterone acetate and enzalutamide for the treatment of metastatic castration-resistant prostate

cancer: A systematic review and meta-analysis. *Front Oncol* (2021) 11:732599. doi: 10.3389/FONC.2021.732599

20. Bianchi E, Fantini M, Nicoletti SLV, Drudi F, Tamburini E, Cherubini C, et al. Enzalutamide (E) vs abiraterone acetate (AA) in the treatment of metastatic, castration-resistant prostate cancer. indirect comparisons and network meta-analysis for clinical practice. *Ann Oncol* (2017) 28:vi20. doi: 10.1093/ANNONC/MDX423.011

21. Chopra A, Haaland B, Lopes G. Comparative effective analysis between enzalutamide and abiraterone in the treatment of metastatic castration-resistant prostate cancer. *J Cli Onco* (2013) 31:217–7. doi: 10.1200/JCO.2013.31.6_SUPPL.217

22. Chung DY, Kang DH, Kim JW, Kim DK, Lee JY, Hong CH, et al. Comparison of oncologic outcomes between two alternative sequences with abiraterone acetate and enzalutamide in patients with metastatic castration-resistant prostate cancer: A systematic review and meta-analysis. *Cancers (Basel)* (2019) 12:8. doi: 10.3390/CANCERS12010008

23. Fuerea A, Baciarello G, Patrikidou A, Albigès L, Massard C, Di Palma M, et al. Early PSA response is an independent prognostic factor in patients with metastatic castration-resistant prostate cancer treated with next-generation androgen pathway inhibitors. *Eur J Cancer* (2016) 61:44–51. doi: 10.1016/J.EJCA.2016.03.070

24. Facchini G, Caffo O, Ortega C, D'Aniello C, Di Napoli M, Cecere SC, et al. Very early PSA response to abiraterone in mCRPC patients: A novel prognostic factor predicting overall survival. *Front Pharmacol* (2016) 7:123/BIBTEX. doi: 10.3389/FPHAR.2016.00123/BIBTEX

25. Pan J, Wang J, Wei Y, Zhang T, Zhang S, Ye D, et al. Combination of body mass index and albumin predicts the survival in metastatic castration-resistant prostate cancer patients treated with abiraterone: A *post hoc* analysis of two randomized trials. *Cancer Med* (2021) 10:6697. doi: 10.1002/CAM4.4205

26. Antoun S, Bayar A, Ileana E, Laplanche A, Fizazi K, Di Palma M, et al. High subcutaneous adipose tissue predicts the prognosis in metastatic castration-resistant prostate cancer patients in post chemotherapy setting. *Eur J Cancer* (2015) 51:2570–7. doi: 10.1016/J.EJCA.2015.07.042

27. Cheranda N, Luo S, Riekhof F, Govindan S, Sanfilippo KM, Schoen MW. Survival of patients with metastatic prostate cancer and comorbid obesity. *J Cli Onco* (2022) 40:116–6. doi: 10.1200/JCO.2022.40.6_SUPPL.116

28. Chi KN, Kheoh T, Ryan CJ, Molina A, Bellmunt J, Vogelzang NJ, et al. A prognostic index model for predicting overall survival in patients with metastatic castration-resistant prostate cancer treated with abiraterone acetate after docetaxel. *Ann Oncol Off J Eur Soc Med Oncol* (2016) 27:454–60. doi: 10.1093/ANNONC/MDV594

29. Poon DMC, Chan K, Lee SH, Chan TW, Sze H, Lee EKC, et al. Abiraterone acetate in metastatic castration-resistant prostate cancer - the unanticipated real-world clinical experience. *BMC Urol* (2016) 16:12. doi: 10.1186/S12894-016-0132-Z

30. Sharova E, Maruzzo M, Del Bianco P, Cavallari I, Pierantoni F, Basso U, et al. Prognostic stratification of metastatic prostate cancer patients treated with abiraterone and enzalutamide through an integrated analysis of circulating free microRNAs and clinical parameters. *Front Oncol* (2021) 11:626104. doi: 10.3389/FONC.2021.626104

31. He Y, Xu W, Xiao YT, Huang H, Gu D, Ren S. Targeting signaling pathways in prostate cancer: Mechanisms and clinical trials. *Signal Transduct Target Ther* (2022) 7:198. doi: 10.1038/s41392-022-01042-7

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 02 May 2023
DOI 10.3389/fonc.2023.1162653

OPEN ACCESS

EDITED BY
Ran Xu,
Central South University, China

REVIEWED BY
Wensheng Zhang,
Xavier University of Louisiana, United States
Ye An,
Huazhong University of Science and Technology, China

*CORRESPONDENCE
Yan Wang
wy_smmu@163.com
Xu Gao
gaoxu.changhai@smmu.edu.cn

†These authors have contributed equally to this work

RECEIVED 09 February 2023
ACCEPTED 17 April 2023
PUBLISHED 02 May 2023

CITATION
Zhang J, Jiang S, Gu D, Zhang W, Shen X, Qu M, Yang C, Wang Y and Gao X (2023) Identification of novel molecular subtypes and a signature to predict prognosis and therapeutic response based on cuproptosis-related genes in prostate cancer.
Front. Oncol. 13:1162653.
doi: 10.3389/fonc.2023.1162653

Identification of novel molecular subtypes and a signature to predict prognosis and therapeutic response based on cuproptosis-related genes in prostate cancer

Jili Zhang[1†], Shaoqin Jiang[1,2†], Di Gu[1†], Wenhui Zhang[1], Xianqi Shen[1], Min Qu[1], Chenghua Yang[1], Yan Wang[1*] and Xu Gao[1*]

[1]Department of Urology, Changhai Hospital, Second Military Medical University, Shanghai, China, [2]Department of Urology, Fujian Union Hospital, Fujian Medical University, Fuzhou, Fujian, China

Background: Prostate cancer (PCa) is the most common malignant tumor of the male urinary system. Cuproptosis, as a novel regulated cell death, remains unclear in PCa. This study aimed to investigate the role of cuproptosis-related genes (CRGs) in molecular stratification, prognostic prediction, and clinical decision-making in PCa.

Methods: Cuproptosis-related molecular subtypes were identified by consensus clustering analysis. A prognostic signature was constructed with LASSO cox regression analyses with 10-fold cross-validation. It was further validated in the internal validation cohort and eight external validation cohorts. The tumor microenvironment between the two risk groups was compared using the ssGSEA and ESTIMATE algorithms. Finally, qRT-PCR was used to explore the expression and regulation of these model genes at the cellular level. Furthermore, 4D Label-Free LC-MS/MS and RNAseq were used to investigate the changes in CRGs at protein and RNA levels after the knockdown of the key model gene B4GALNT4.

Results: Two cuproptosis-related molecular subtypes with significant differences in prognoses, clinical features, and the immune microenvironment were identified. Immunosuppressive microenvironments were associated with poor prognosis. A prognostic signature comprised of five genes (B4GALNT4, FAM83D, COL1A, CHRM3, and MYBPC1) was constructed. The performance and generalizability of the signature were validated in eight completely independent datasets from multiple centers. Patients in the high-risk group had a poorer prognosis, more immune cell infiltration, more active immune-related functions, higher expression of human leukocyte antigen and immune checkpoint molecules, and higher immune scores. In addition, anti-PDL-1 immunotherapy prediction, somatic mutation, chemotherapy response prediction, and potential drug prediction were also analyzed based on the risk signature. The validation of five model genes' expression and regulation in qPCR was consistent with the

results of bioinformatics analysis. Transcriptomics and proteomics analyses revealed that the key model gene B4GALNT4 might regulate CRGs through protein modification after transcription.

Conclusion: The cuproptosis-related molecular subtypes and the prognostic signature identified in this study could be used to predict the prognosis and contribute to the clinical decision-making of PCa. Furthermore, we identified a potential cuproptosis-related oncogene B4GALNT4 in PCa, which could be used as a target to treat PCa in combination with cuproptosis.

KEYWORDS

prostate cancer, cuproptosis, unsupervised clustering, tumor microenvironment, signature

1 Introduction

Globally, prostate cancer (PCa) accounts for about 1.4 million new cases and 375,000 deaths yearly, making it the second most common cancer and the most common malignant tumor of the male urinary system (1). For patients with localized cancer, radical prostatectomy or radical radiotherapy is the standard treatment (2). Unfortunately, about 20-30% of patients will develop biochemical recurrence after radical treatment, followed by clinical recurrences and metastases (3–5). For advanced PCa, androgen deprivation therapy (ADT) remains the preferred treatment, inhibiting PCa growth by reducing circulating testosterone and inhibiting androgen receptor function (5, 6). However, due to the resistance to ADT, almost all patients progress to castration-resistant PCa (CRPC) after 1 to 2 years of ADT treatment (7). So far, there is no effective treatment for CRPC, and patients usually die within 2-4 years (8, 9). Therefore, it is urgent to explore further the underlying progression mechanisms and new therapeutic targets for advanced PCa.

Although prostate specific antigen level, Gleason score, AJCC TNM staging, and other clinicopathological features have provided important references for monitoring the disease progression and predicting the prognosis of PCa patients (10, 11), the predictive value of these routine features is often limited for patients with an unclear clinical diagnosis or in intermediate grades or stages (12). Furthermore, emerging treatments such as neoadjuvant therapy, chemotherapy, targeted therapy, radionuclide therapy, and immunotherapy have achieved some efficacy in advanced PCa. However, the survival gains from these treatments are unclear for some patients, and these treatments may even lead to severe complications (9). Therefore, due to the heterogeneity of PCa, a reliable prediction tool is required to accurately evaluate the prognosis of patients, which can help clinicians choose the best treatment and determine whether to proceed with more aggressive treatment.

Regulated cell death (RCD), which also refers to programmed cell death (PCD), is a form of cell death that can be regulated by various biological macromolecules (13). In recent years, an increasing number of RCD forms, including apoptosis, necroptosis, autophagy, ferroptosis, and pyroptosis, have been proven to be involved in various pathological and physiological processes, including tumorigenesis (14). Apoptosis, the earliest and most well-studied form of RCD, is the treatment target of almost all tumors (15, 16). However, resistance to apoptosis may be the main reason for the failure of these therapeutic strategies (17). Therefore, it is necessary to discover a new form of RCD and to study its role in tumorigenesis in depth.

Copper, a trace metal, plays a vital role in many biological processes, and maintaining its homeostasis in living organisms is necessary for life (18, 19). On the one hand, copper deficiency in cells can destroy the function of copper-binding enzymes; on the other hand, copper accumulation leads to cell death (20). It has been shown that dysregulation of copper homeostasis contributes to cancer growth, angiogenesis, and metastasis (21). A recent study clarified that excessive copper binds directly to lipoylated components of the tricarboxylic acid (TCA) cycle (22), leading to aggregation of the lipoylated protein and then the loss of iron-sulfur cluster protein, which ultimately kills cells after proteotoxic stress (23). Unlike any other, this novel form of RCD was called "cuproptosis". Recent studies have shown that cuproptosis is closely associated with the tumor microenvironment (TME) and prognosis of various tumors, including bladder cancer, hepatocellular carcinoma, breast cancer and melanoma (24–27). Recently, Yuzhi Xu et al. demonstrated a significant inhibitory effect of a copper nanomaterial on bladder tumor growth in mice with negligible systemic toxicity (28). This suggests that selective killing of cancer cells by modulating the concentration of copper ions in cancer cells is a feasible and promising new direction for cancer therapy. However, as a novel form of RCD, the role of cuproptosis in PCa remains unclear.

In this study, we first visualized the expression, prognostic network, and somatic alteration of CRGs in the TCGA PCa cohort. Two molecular subtypes associated with cuproptosis were identified. Then, prognosis, clinicopathological features, function enrichment, TME, and immunotherapy response were compared

between the two molecular subtypes. Next, on the basis of the differentially expressed genes (DEGs) between the two cuproptosis-related subtypes, we established and tested a prognostic signature consisting of five genes to evaluate prognosis independently for PCa in the TCGA database and validated the performance and generalizability of the signature in eight completely independent datasets. We also established a clinically applicable nomogram and analyzed the function enrichment, TME, somatic mutations, chemotherapy response prediction, and potential drug prediction on the basis of the risk signature. Finally, we validated the expression of model genes in cells, explored the regulation of these genes in the presence of copper ions and copper ionophore Elesclomol to induce cuproptosis, and further investigated the changes of CRGs at RNA and protein levels after knockdown of the key model gene B4GALNT4 by proteomics and transcriptomics analysis.

So far, the study of cuproptosis in PCa is still in its infancy. Our study explores this promising uncharted area in PCa and provides a reference for future research on cuproptosis in PCa.

2 Materials and methods

2.1 Data collection

This study included nine independent PCa cohorts (Table 1, Supplementary Table S1). Transcriptome profiles (Transcripts Per Kilobase Million, TPM) of 497 PCa cases and 52 normal cases were obtained from the Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/). The corresponding clinical and progression-free survival (PFS) information in TCGA were downloaded from the UCSC (University of California, Santa Cruz) Xena public data hub (https://xenabrowser.net/).

Eight completely independent cohorts were included as the external validation sets, including DFKZ (The German Cancer Research Center, Deutsches Krebsforschungszentrum, n=81) (29), MSKCC (The Memorial Sloan Kettering Cancer Center, n = 140) (30), CPGEA (Chinese Prostate Cancer Genome and Epigenome Atlas, n=125) (31), GSE46602(n=36) (32), GSE70768 (n=111) (33), GSE70769 (n=92) (33), GSE70770 (n=203) (33), GSE54460 (n=91) (34). The cases included in the 8 external datasets were all radical surgery PCa cases with complete survival information. All 8 external datasets were used as validation sets only, and none of them were involved in the construction of the prediction model. The RNA sequence data profiles and the corresponding clinical information of DFKZ and MSKCC were obtained from the cBioPortal for Cancer Genomics (https://www.cbioportal.org/). The RNA sequence data of CPGEA were downloaded from (http://www.cpgea.com/download.php). Our team published the CPGEA dataset in Nature in 2020 (31), and we used the latest survival data in this study. The microarray data profiles and corresponding clinical information of GSE46602, GSE70768, GSE70769, GSE70770, and GSE54460 were obtained from Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/).

We downloaded the complete expression data and detailed clinical information of the cohort of metastatic urothelial carcinoma treated with atezolizumab (an anti-PDL-1 agent) in a large phase 2 trial (IMvigor210) from the R package IMvigor210Core Biologies (version 1.0.0) (35). CRGs, including NFE2L2, NLRP3, ATP7B, ATP7A, SLC31A1, FDX1, LIAS, LIPT1, LIPT2, DLD, DLAT, PDHA1, PDHB, MTF1, GLS, CDKN2A, DBT, GCSH, and DLST, were obtained from the literature published in *Science* by Tsvetkov et al. (23).

2.2 Somatic mutation and copy number alteration analysis

We downloaded the somatic mutation data of PCa from the TCGA database and performed gene mutation waterfall plots through the "maftools" R package. Tumor mutation burden (TMB) was calculated for each patient, and differences in TMB were compared between different molecular subtypes and risk groups. Survival analysis was conducted based on the TMB score.

TABLE 1 Detailed information of PCa cohort used in this study.

Datasets	Platform	Number of Input (tumor)	Application
TCGA	Illumina HumanHT-12 V4.0 expression beadchip	497	Construction and Test of the Prognostic Signature
DKFZ	Illumina HumanHT-12 V3.0 expression beadchip	81	Validation of the Prognostic Signature
MSKCC	Affymetrix Human Exon 1.0 ST Array	140	Validation of the Prognostic Signature
CPGEA	Illumina HiSeq X TEN	125	Validation of the Prognostic Signature
GSE46602	GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	36	Validation of the Prognostic Signature
GSE70768	GPL10558 Illumina HumanHT-12 V4.0 expression beadchip	111	Validation of the Prognostic Signature
GSE70769	GPL10558 Illumina HumanHT-12 V4.0 expression beadchip	92	Validation of the Prognostic Signature
GSE70770	GPL10558 Illumina HumanHT-12 V4.0 expression beadchip	203	Validation of the Prognostic Signature
GSE54460	GPL11154 Illumina HiSeq 2000 (Homo sapiens)	91	Validation of the Prognostic Signature

We downloaded the somatic copy number alterations (SCNA) data of PCa from the UCSC Xena public data hub and compared the frequency of CRGs copy number gain and loss. And then, the somatic mutation frequencies of the model genes were exhibited using the cBioPortal database.

2.3 Consensus clustering analysis

Univariate cox regression analysis was conducted to screen out prognostic CRGs for PCa. Based on the expression of the prognostic CRGs, consensus clustering analysis was conducted with the R software package "ConsensusClusterPlus" to identify cuproptosis-related molecular subtypes. The Kaplan-Meier (K-M) analysis was used to compare the prognosis between the two groups. The correlation of clusters with CRGs and clinicopathological features was displayed by a heat map, and the differences in clinicopathological features between subtypes were compared by a chi-square test.

2.4 Gene set variation analysis and gene set enrichment analysis

Utilizing the "GSVA" R package, GSVA was performed to compare the differences in biological pathways between molecular subtypes. The adjusted $p < 0.05$ was used as the criterion for judging statistically significant differences in pathway enrichment among different subgroups by the "limma" package. The R package "clusterProfiler" was used to perform GSEA.

2.5 Immune landscape analysis

Each sample's immune cell infiltration and functional activity were calculated using ssGSEA. Previous studies provided us with the marker genes of different immune cells (Supplementary Table S2) (36, 37). Immune, stromal, and estimate scores were calculated using the ESTIMATE algorithm based on the proportion of immune and stromal cells. We also compared the expression of major histocompatibility complex (MHC) and immune checkpoint molecules between subtypes and between the risk groups (38) and the expression of genes that inhibit the cancer-immunity cycle based on cluster analysis (39). These genes that inhibit the cancer-immunity cycle were downloaded from https://biocc.hrbmu.edu.cn/TIP/index.jsp . Tumor Immune Dysfunction and Exclusion (TIDE) score related to poorer immune checkpoint blockade therapy was calculated through the TIDE database.

2.6 Construction and validation of the prognostic signature

Firstly, we performed DEGs analysis between the two molecular subtypes by limma package in R software. The threshold for differential analysis was "Adjusted $p<0.05$ and $|\log2FoldChange| > 0.585$". Sixty-three prognostic DEGs for PCa were identified through univariate Cox regression analysis. Subsequently, we randomly divided 497 PCa patients from the TCGA cohort into a training group (n=249) and a test group (n=248). To eliminate potential overfitting between the prognostic DEGs, we used the least absolute shrinkage and selection operator (LASSO) algorithm with the penalty parameter (λ) determined by the lowest partial likelihood deviance based on the R package "glmnet" to establish a prognostic signature. The LASSO cox regression analysis with 10-fold cross-validation was conducted in the TCGA training group with the glmnet package in R to further select DEGs with the greatest predictive power. Finally, the forward stepwise selection and the multivariate cox regression model were utilized to develop a prognostic signature according to the candidate DEGs generated by the above screening. Then, the regression coefficients calculated by multivariate cox regression analysis were used to construct the cuproptosis-related risk score (CRRS).

According to the median risk score value of the training cohort, the TCGA cohort (including the training and test cohorts) was divided into high- and low-risk groups. The performance of the model was assessed using K-M analysis and area under the curve (AUC) of the receiver operating characteristic (ROC) curve. Furthermore, the reliability and generalizability of the model were validated by eight completely independent datasets (DFKZ, MSKCC, CPGSA, GSE46602, GSE70768, GSE70769, GSE70770, and GSE54460). Based on the model built from the training set in the TCGA dataset, risk scores for each patient in these external datasets were calculated separately. Then, patients in each external dataset were classified into high- and low-risk groups based on the optimal cutoff of risk scores calculated by the "surv_cutpoint" algorithm of the survminer R package. Finally, the progression-free survival time between the two groups was compared through K-M analysis and AUC of the ROC curve. In addition, we confirmed that CRRS is an independent prognostic factor for PCa using univariate and multivariate cox regression analyses and established a clinically applicable nomogram.

2.7 Chemotherapy response and small-molecule drugs

The response to chemotherapeutic drugs was predicted using the Genomics of Drug Sensitivity in Cancer (GDSC) database (37). The Half Maximal Inhibitory concentration (IC50) was calculated through the "pRRophetic" package (37).

The Connectivity Map (cMap) Database, a database of biological applications combining disease, gene expression, and small-molecule drugs, can predict compounds that may induce or reverse tumor biological processes by comparing up-and down-regulated genes between the two risk groups (37). Enrichment scores range from -100 to 0, indicating that these compounds may be potential candidates for PCa treatment. 3D structural maps of the six most likely candidates were obtained from the PubChem database (37).

2.8 Cell culture and drug therapy *in vitro*

Four PCa cell lines (C4-2, PC3m, PC3, and LNCaP) were used in this study, and these cell lines were purchased from the Cell Bank of the Chinese Academy of Science (Shanghai, China). All these cell lines were cultured in RPMI-1640 with 10% fetal bovine serum and 1% penicillin-streptomycin solution at 37°C in a humid incubator with 5% CO2. We purchased copper ionophore Elesclomol and copper chloride from Selleck and Sangon, respectively. The cells were treated with 2mM copper chloride or 20nM Elesclomol when the cells were adherent and morphologically diffused. After 24h of treatment, cells were collected, and RNA was isolated.

2.9 Real-time quantitative polymerase chain reaction

The total RNA of the above cells was isolated using the Fast Pure Cell Total RNA Isolation Kit (Vazyme, RC101-01). Then, reverse transcription was conducted with the HiScript III RT SuperMix for qPCR (+gDNA wiper) Kit (Vazyme, R323-01). Next, RT-qPCR was performed in triplicate with ChamQ Universal SYBR qPCR Master Mix (Vazyme, Q711). The mRNA expression level of B4GALNT, FAM83D, COL1A1, CHRM3, and MYBPC1 was normalized by β-actin mRNA. All experiments were conducted following the manufacturer's protocol. The primer sequences are listed in Table S3.

2.10 Transfection of C4-2 cells with B4GALNT4-specific shRNA plasmid

The shRNA sequences for B4GALNT4 and the shRNA control were designed through GPP Web Portal (https://portals.broadinstitute.org/gpp/public/gene/search). The sequences are also listed in Table S3. The lentivirus expression system was used to generate targeted virus supernatant for infection of C4-2 cells. After 48h of infection, the target cells were screened with puromycin. Then, western blotting confirmed the expression of B4GALNT4 in these target cells.

2.11 Western blot

The cells were lysed in RIPA (Radio Immunoprecipitation Assay) solution. After separation with 10% SDS-PAGE, the proteins were transferred to PVDF membranes and detected with antibodies. Anti-B4GALNT4 was purchased from Biorbyt (Cambridge, UK). Anti-GAPDH was purchased from ProteinTech (Chicago, USA). GAPDH was used as an internal reference.

2.12 4D label-free LC-MS/MS (liquid chromatography tandem-mass spectrometry) proteomics and data processing

We obtained samples from the C4-2 stable cell lines (shB4GALNT4 vs. shControl) by sonicating them three times on ice in lysis buffer (8 M urea, 1% protease inhibitor cocktail) with a high-intensity sonication processor (Scientz). BCA kits were used to measure the protein concentration of these samples after centrifugation at 12000 g for 10 minutes at 4°C. The following reduction with 5 mM dithiothreitol for 30 minutes at 56°C, the protein solution was alkylated for 15 minutes at room temperature with 11 mM iodoacetamide. Following that, 100 mM TEAB was added to the protein (urea concentration was below 2 M). Finally, the peptide was desalted by the C18 SPE column after digestion with trypsin. A reverse phase assay column (25 cm length, 75/100 mm internal diameter) was loaded directly with the tryptic peptide dissolved in solvent A (0.1% formic acid, 2% acetonitrile/water). For the separation of peptides, a gradient of 6% to 24% solvent B (0.1% formic acid in acetonitrile) was used for no less than 70 minutes, followed by a gradient of 24% to 35% in 14 minutes, 80% in 3 minutes, and 80% for the final 3 minutes. Peptides processed by capillary source were analyzed by timsTOF Pro (Bruker Daltonics) mass spectrometry (MS).

MaxQuant search engine (v.1.6.15.0) was used to process the obtained MS/MS data. The reverse decoy database was linked to the human SwissProt database (20422 entries) when searching tandem MS. Trypsin/P was designated as a lyase, allowing cleavage of up to 2 deletions. A mass tolerance of 20 ppm is set for the first precursor ion, five ppm for the main search, and 0.02 Da for the fragment ion. The false discovery rate (FDR) < 0.01 and Fold Change ≥1.2 were used to determine whether the expression differed significantly.

2.13 The transcriptomics analysis

Samples were obtained from the abovementioned C4-2 stable cell lines (shB4GALNT4 vs. shControl). Wash and dissolve the sample with 1 ml of TRizol reagent. With the help of a NanoPhotometer spectrophotometer (IMPLEN, California, USA), the purity of the RNA was determined. After the measurement of the concentration and integrity of RNA, the sequencing libraries were established with the NEBNext UltraTM RNA library Prep Kit for Illumina (NEB, USA). Then, based on the established libraries, paired-end reads were generated using the Illumina Hiseq 2500 platform. The depth of sequencing coverage was 10-fold, and the sequence read length was 200-250. Prior to data analysis, raw data was processed by eliminating reads with adapters, ploy-N, and low quality. The edgeR package was used to analyze the differential expression of two samples (without biological replicates). The threshold was the FDR < 0.01 and |log 2 (Fold Change) | ≥1.

10.3389/fonc.2023.1162653

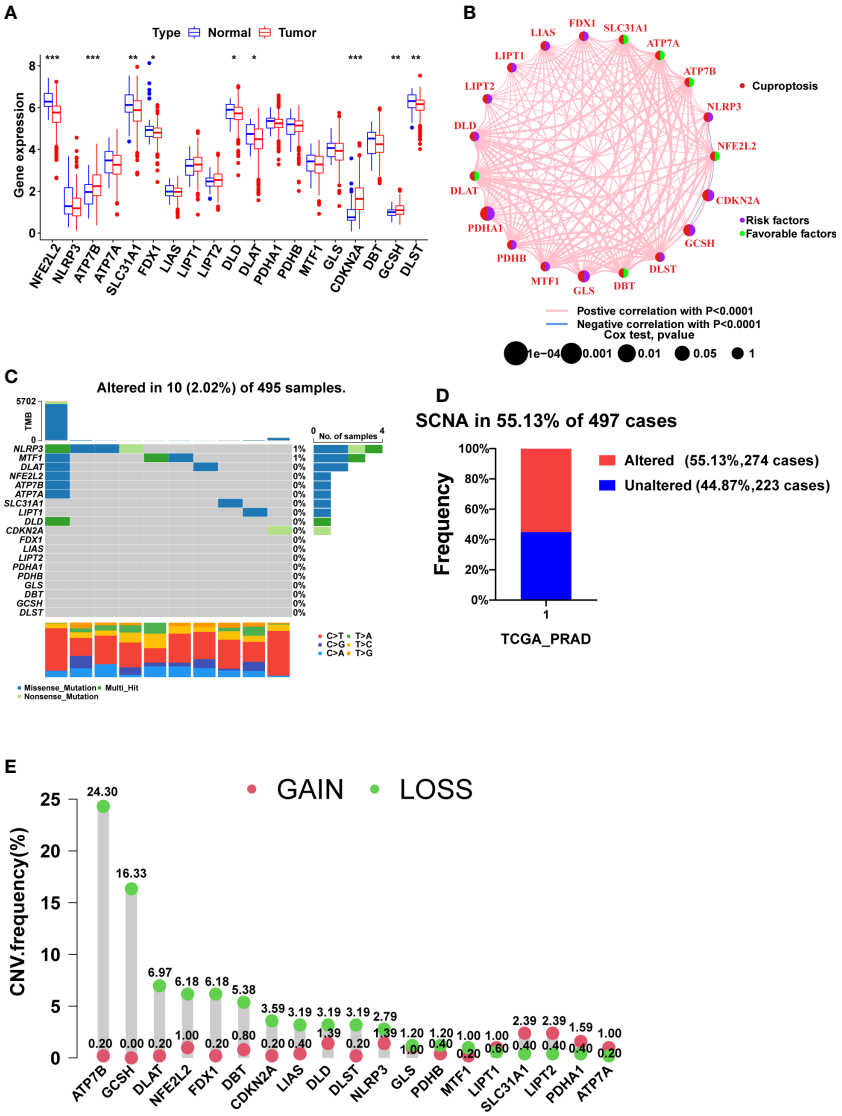


FIGURE 1
The expression, prognosis, and somatic alteration of CRGs in the TCGA PCa cohort. **(A)** The comparison of CRGs expression between tumor and normal tissues. **(B)** The PFS network of CRGs and co-expression relationship between CRGs in PCa. **(C)** The mutation frequency of CRGs in 495 PCa samples from the TCGA cohort. **(D)** Histogram of the SCNA frequency of CRGs in PCa. **(E)** Lollipop chart of the frequency of different SCNA types. (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

2.14 Statistical analysis

All statistical analyses were performed using R software (version 4.2.0), except for the statistical analysis of qPCR results, which were analyzed by the analysis of variance (ANOVA) method based on GraphPad Prism (version 8.2.1). The differences between two cuproptosis-related molecular subtypes and two risk groups were analyzed through the Wilcoxon rank sum test. KM analysis was applied to compare PFS. Univariate and multivariate cox regression analyses were carried out to obtain independent predictors for PCa. It was considered statistically significant if the p-value was less than 0.05 (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

3 Results

3.1 The expression, survival network and somatic alteration landscape of CRGs in TCGA cohort

Nine CRGs were differentially expressed between tumor and normal tissues, among which NFE2L2, SLC31A1, FDX1, DLD, DLAT, and DLST were lowly expressed in tumor tissues, and ATP7B, CDKN2A, and GCSH were highly expressed in tumor tissues (Figure 1A, $p<0.05$). Since FDX1, DLD, and DLAT are pro-

TABLE 2 The results of univariate Cox regression analysis and Kaplan–Meier survival analysis of CRGs in TCGA PCa cohort.

CRGs	HR	HR.95L	HR.95H	Unicox pvalue	KM pvalue
NFE2L2	0.832	0.625	1.107	0.207	0.003
NLRP3	1.166	0.863	1.576	0.318	0.011
ATP7B	0.899	0.693	1.166	0.421	0.032
ATP7A	0.938	0.713	1.233	0.644	0.028
SLC31A1	0.954	0.750	1.215	0.705	0.021
FDX1	1.379	0.828	2.296	0.217	0.063
LIAS	1.327	0.698	2.524	0.389	0.071
LIPT1	1.378	0.943	2.013	0.098	0.002
LIPT2	1.219	0.756	1.964	0.417	0.221
DLD	1.069	0.740	1.545	0.722	0.145
DLAT	0.977	0.761	1.255	0.858	0.102
PDHA1	2.583	1.379	4.840	0.003	<0.001
PDHB	1.072	0.679	1.692	0.767	0.338
MTF1	1.020	0.728	1.429	0.908	0.215
GLS	1.450	1.030	2.043	0.033	0.002
CDKN2A	1.289	1.008	1.649	0.043	0.002
DBT	0.794	0.593	1.065	0.123	0.007
GCSH	2.022	1.015	4.026	0.045	0.022
DLST	1.234	0.759	2.005	0.397	0.023

HR, Hazard ratio; Unicox, univariate Cox regression; KM, Kaplan–Meier curve analysis.

cuproptosis genes while CDKN2A is an anti-cuproptosis gene (23), PCa may be in a state of suppression of cuproptosis.

Figure 1B shows the relationship between PCa prognosis and CRGs as well as the mutual co-expression relationship between these CRGs. The univariate cox regression analysis showed that PDHA1, GLS, CDKN2A, and GCSH were significantly associated with poor prognosis (Figure 1B, Table 2, $p<0.05$). All of the co-expression relationships between CRGs were positive except NLRP3 and NFE2L2, NFE2L2 and CDKN2A, and CDKN2A and GCSH, which were negative co-expression relationships (Figure 1B). KM analysis found that patients with high expression of PDHA1 ($p<0.001$), GLS($p=0.002$), LIPT1($p=0.002$), CDKN2A($p=0.002$), NLRP3 ($p=0.011$), GCSH ($p=0.022$), and DLST ($p=0.023$) had significantly shorter PFS time (Supplementary Figures S1A-G), while patients with high expression of NFE2L2 ($p=0.003$), DBT ($p=0.007$), SLC31A1 ($p=0.021$), ATP7A ($p=0.028$) and ATP7B ($p=0.032$) had significantly longer PFS time (Supplementary Figures S1H-L). In summary, there is a complex co-expression relationship between CRGs in prostate cancer, and almost all CRGs are positively regulated among themselves. Furthermore, CRGs were closely related to the prognosis of prostate cancer.

Remarkably, CRGs were rarely mutated in PCa patients (only 2.02%) (Figure 1C), but SCNA of CRGs occurred in more than 55% of PCa patients (Figure 1D). CRGs, except for LIPT1, SLC31A1, LIPT2, PDHA1, and ATP7A, have a higher frequency of copy number loss than gain, with ATP7B and GCSH having the highest frequency of copy number loss but almost no copy number gain (Figure 1E, Supplementary Figure S2A). Taken together, SCNA, not mutation, was found to be the main cause of dysregulation of CRGs in PCa.

3.2 Identification of cuproptosis-related molecular subtypes in PCa

Four prognostic CRGs were identified through univariate cox regression analysis (Figure 1B, Table 2, $p<0.05$). Based on the expression levels of these CRGs, an unsupervised clustering approach was carried out to classify 497 PCa patients from the TCGA cohort into two cuproptosis-related subtypes, with 284 cases in cluster A and 213 cases in cluster B (Figure 2A, Supplementary Figures S2B-M). KM analysis indicated that cluster B had a poorer prognosis (Figure 2B $p=0.018$). Next, we compared the expression of CRGs and the distribution of clinical features between the two subtypes (Figure 2C). There were twelve CRGs differentially expressed across the two subtypes, and all were highly expressed in cluster B (Figure 2D, $p<0.05$). There was a difference in clinical characteristics between the two subtypes in terms of Gleason score, pathological T-stage, and pathological N-stage, with cluster B showing a higher proportion of patients with a high Gleason score ($p<0.001$), high pathological T-stage ($p<0.01$) and high pathological N-stage ($p<0.05$) (Table 3). In summary, CRGs can divide PCa into two subtypes with completely different prognostic and clinical characteristics.

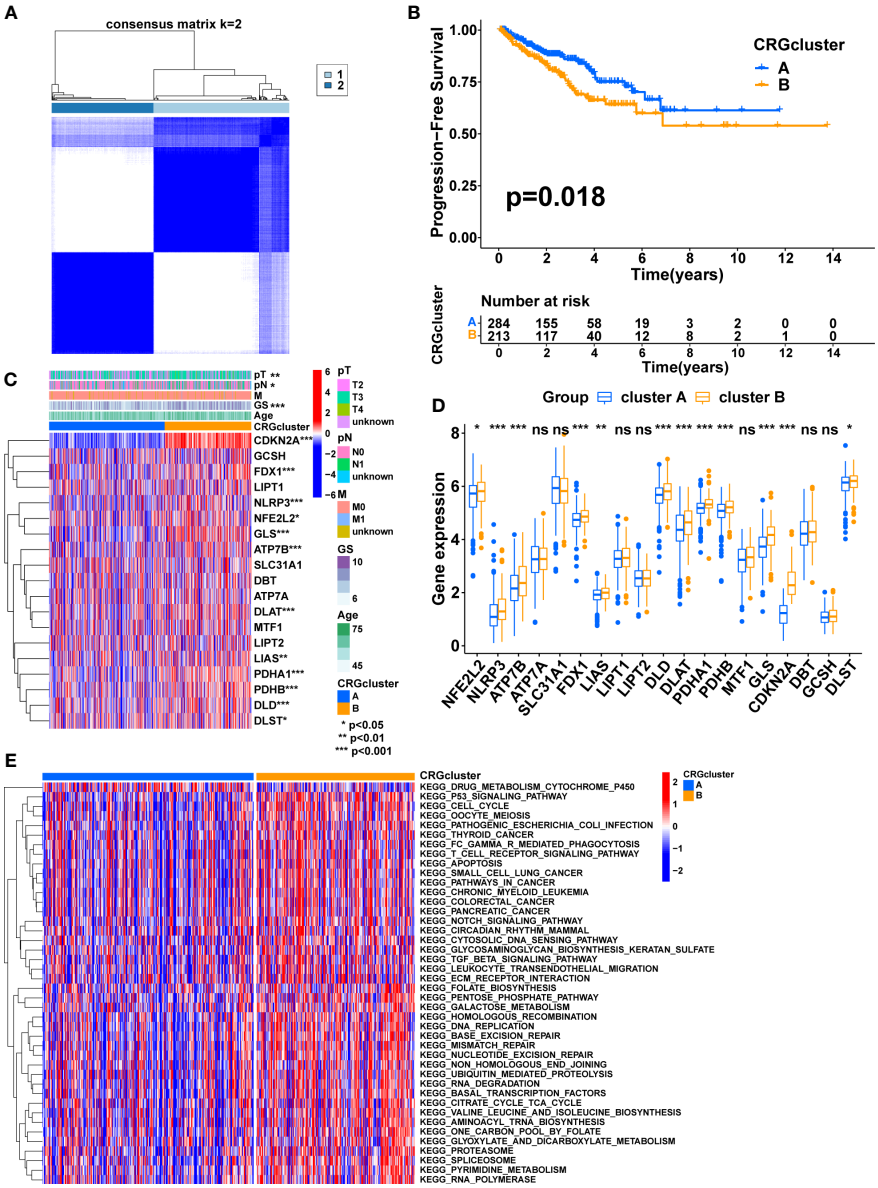


FIGURE 2
Consensus clustering of CRGs in PCa. **(A)** Consensus clustering matrix when k = 2. **(B)** The difference in PFS between the two clusters. **(C)** The heatmap shows the expression of CRGs between the two clusters and the correlations between the clusters and clinical features. **(D)** The comparison of CRGs expression between the two clusters. **(E)** The heatmap shows the result of GSVA between the two clusters. TNM, tumor node metastasis; p, pathology; GS, Gleason score. (*, p < 0.05; **, p < 0.01; ***, p < 0.001). ns, no significant.

According to these results, CRGs may be involved in tumor development *via* some underlying mechanisms. Therefore, GSVA was performed to explore the potential mechanisms. The result showed that most of the pathways involved in metabolism, immunity, and cancer, including the TCA cycle, FC gamma R-mediated phagocytosis, Leukocyte transendothelial migration, T cell receptor signaling pathway, P53 signaling pathway, pathways in cancer, Notch signaling pathway, TGF beta signaling pathway, and ECM-receptor interaction, were significantly enriched in cluster B, which may contribute to the poorer prognosis (Figure 2E, Supplementary Figure S2N).

3.3 The immune-related characteristics of cuproptosis-related subtypes

The ssGSEA algorithm was utilized to compare immune infiltration between the two subtypes. The high infiltration of Neutrophils characterized cluster A, whereas cluster B was characterized by the high infiltration of Activated CD4 T cells, Eosinophil, Immature dendritic cells, Regulatory T cells, Type 1 T helper cells, and Type 2 T helper cells (Figure 3A, p<0.05). Furthermore, the expression of MHC molecules between the two subtypes was compared. The expression levels of MHC molecules were higher in cluster B except for HLA-

TABLE 3 The distribution of clinical features of PCa patients between the two clusters.

Characteristics	N (%) Entire dataset (n=497)	N (%)		*P*
		Cluster A (n=284)	Cluster B (n=213)	
Age, years				0.2965
<=65	354(71.23)	208(73.24)	146(68.54)	
>65	143(28.77)	76(26.76)	67(31.46)	
Gleason score				0.0001
6	45(9.05)	34(11.97)	11(5.16)	
7	247(49.70)	156(54.93)	91(42.72)	
8	64(12.88)	36(12.68)	28(13.15)	
9	137(27.57)	56(19.72)	81(38.03)	
10	4(0.80)	2(0.70)	2(0.94)	
pT stage				0.0019
T2	187(37.63)	126(44.37)	61(28.64)	
T3	293(58.95)	152(53.52)	141(66.20)	
T4	10(2.01)	3(1.06)	7(3.29)	
unknown	7(1.41)	3(1.06)	4(1.88)	
pN stage				0.0201
N0	345(69.42)	208(73.24)	137(64.32)	
N1	79(15.90)	34(11.97)	45(21.13)	
unknown	73(14.69)	42(14.79)	31(14.55)	
M stage				0.1312
M0	455(91.55)	261(91.90)	194(91.08)	
M1	3(0.60)	0(0.00)	3(1.41)	
unknown	39(7.85)	23(8.10)	16(7.51)	

PCa, Prostate cancer; TNM, tumor node metastasis; p, pathology.

DRB5, HLA-DOA, HLA-C, HLA-J, HLA-G, HLA-DRB6, HLA-DQA2 and HLA-L (Figure 3B, p <0.05).

Subsequently, a series of evaluation indicators were used to determine whether cuproptosis-related subtypes were significantly associated with immunotherapy effects, including the expression of immune checkpoint molecules and genes that inhibit cancer-immunity cycles, TMB scores, and TIDE scores. There were 30 differentially expressed immune checkpoint molecules between subtypes. All of them were highly expressed in cluster B (p<0.05), including PD-1 (PDCD1), CTLA4, B7H3 (CD276), HAVCR2, and TIGIT (Figure 3C, Supplementary Figures S3A, B). 22 genes that inhibit the cancer-immunity cycle were differentially expressed between subtypes (p<0.05), and all of them, except ARG2 and TIMD4, were significantly overexpressed in cluster B (Figure 3D, Supplementary Figures S3C, D). Meanwhile, cluster B had a higher TMB score (Figure 3E, p<0.01) and TIDE score (Figure 3F, p<0.001). In summary, These results show a complex immune microenvironment for the different subtypes, with cluster B appearing to exhibit a more suppressed immune microenvironment.

3.4 Construction and validation of a cuproptosis-related signature

Firstly, 147 DEGs between the two cuproptosis-related subtypes were identified by differential analysis (Supplementary Figure S3E). Next, 63 DEGs associated with PFS were obtained *via* univariate cox regression (Figure 4A, P<0.05). Subsequently, we randomly divided 497 PCa patients from the TCGA cohort into a training group (n=249) and a validation group (n=248), and there was no significant difference in clinicopathological features between the two groups (Supplementary table S4, P>0.05). In the training group, we further screened the optimal prognostic biomarkers by LASSO regression analysis, and 11 DEGs were selected with 10-fold cross-validation (Figures 4B, C). Then, the model with the lowest Akaike information criterion (AIC) value was established through multivariate cox regression analysis. Finally, we generated a risk score model consisting of five DEGs, including B4GALNT4, FAM83D, COL1A1, CHRM3, and MYBPC1. Forest plots showed the association of expression levels of the five model genes with PFS,

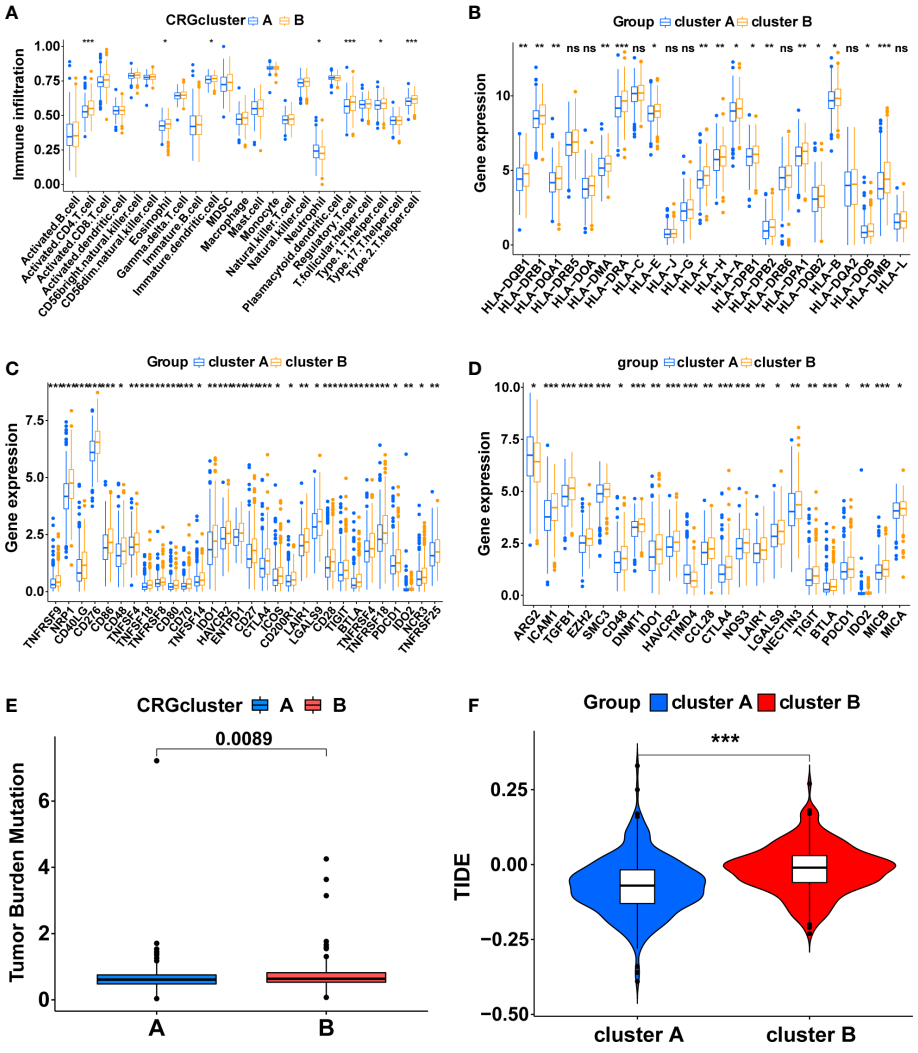


FIGURE 3
The immune-related characteristics of cuproptosis-related molecular subtypes in the TCGA cohort. **(A)** The difference in immune cell infiltration between the two clusters. **(B)** The comparison of MHC molecules expression between the two clusters. **(C)** Immune checkpoint molecules expression between the two clusters. **(D)** The expression level of the genes that inhibit the cancer-immunity cycle between the two clusters. The comparison of the TMB score **(E)** and TIDE score **(F)** between the two clusters. (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). ns, no significant.

where B4GALNT4 had the most considerable contribution to poorer prognosis (Figure 4A, Hazard ratio=1.559, p<0.01). The coefficient of each gene in the signature was exhibited in Figure 4D, and the risk score was calculated with the equation: CRRS= (0.4339∗B4GALNT4)+(0.2942∗FAM83D)+(0.2342∗COL1A1) +(−0.1351∗MYBPC1)+ (−0.4798∗CHRM3). The median risk score value of the training cohort was utilized to classify patients into high- and low-risk groups in the TCGA cohort.

Sankey diagrams illustrated the correlation between cuproptosis-related subtypes, risk score, and prognosis, and the patients with disease progression mainly were from the high-risk group (Figure 4E). The comparison of CRGs expression between the two risk groups is exhibited in Figure 4F. NLRP3, GLS, and CDKN2A were highly expressed in the high-risk group, while SLC31A1, PDHB, and DBT were lowly expressed in the high-risk group (Figure 4F, p<0.05). As expected, cluster A, with the better prognosis among the cuproptosis-related subtypes, had a lower risk score (Supplementary Figure S3F, p<0.001).

Then, we tested the performance of the signature in the TCGA cohort. KM analysis suggested that the high-risk patients had poorer PFS than the low-risk patients in the TCGA training (Figure 5A, p<0.001), test (Figure 5D, p<0.001), and all (Figure 5G, p<0.001) cohorts. We also visualized the risk score distribution and survival status in these cohorts. The results showed that a higher risk score was associated with a higher risk of disease progression and a shorter PFS period (Figures 5B, E, H). The model genes in the three cohorts also exhibited a similar expression pattern (Figures 5C, F, I). Then, the ROC curve was used to assess the performance of the signature. In the TCGA training cohort, the mean AUC values for predicting 1-, 3-and 5-year prognosis were 0.748, 0.766, and 0.772, respectively (Figure 5J). As for the TCGA test cohort, the average AUC values for 1-, 3- and 5-year prognostic

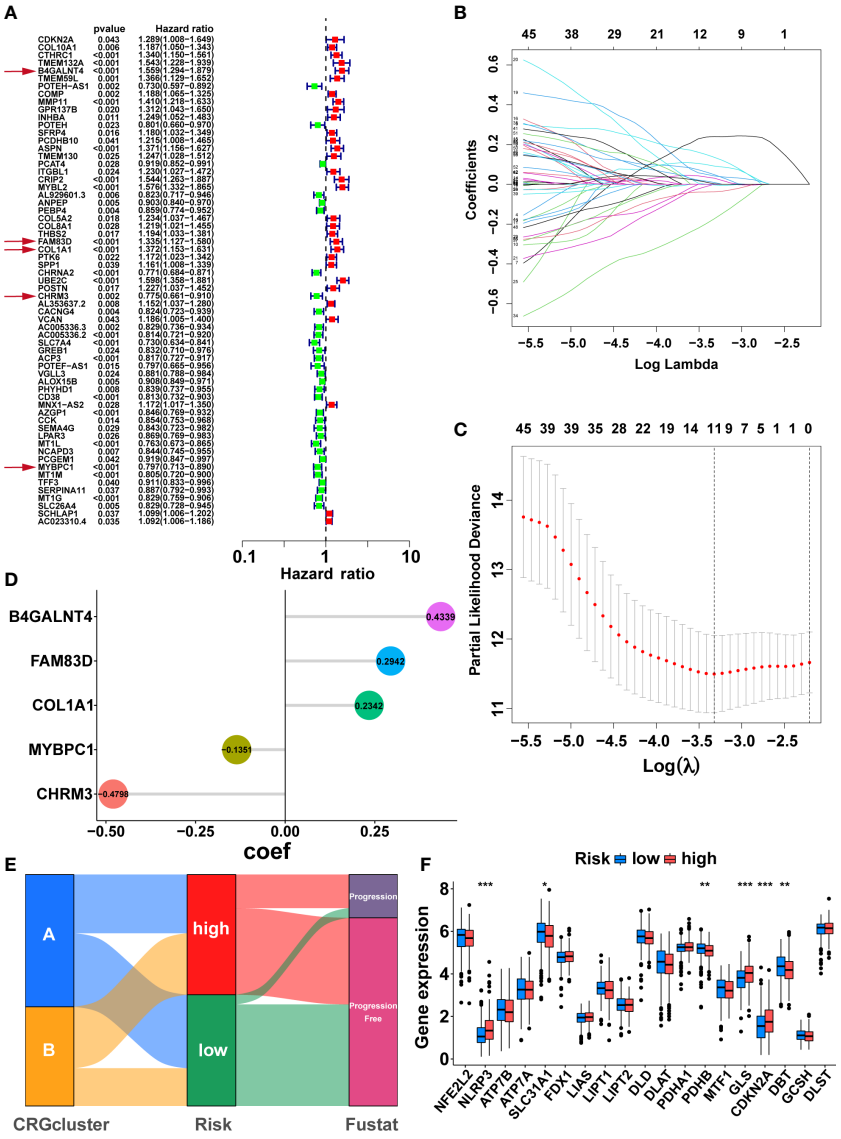


FIGURE 4
Development of the cuproptosis-related signature in the TCGA training cohort. **(A)** Sixty-three prognosis-related DEGs were identified by univariate Cox regression. The genes indicated by red arrows are the five genes involved in the construction of the prognostic model. **(B)** The horizontal axis represents the logarithm of the independent variable λ, and its coefficients are shown on the vertical axis. **(C)** The confidence interval corresponds to each lambda. **(D)** Coefficients of the five prognostic genes in the model. **(E)** Sankey diagrams displayed the correlation between cuproptosis-related subtypes, risk score, and prognosis. **(F)** The comparison of the expression levels of CRGs between two risk groups. (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

prediction were 0.719, 0.741, and 0.759, respectively (Figure 5K). In addition, the mean AUC values for predicting 1-, 3- and 5-year PFS were 0.736, 0.753, and 0.755 in the entire TCGA cohort (Figure 5L).

To further verify the generalizability of the signature, external validation was performed on eight completely independent datasets (DFKZ, MSKCC, CPGEA, GSE46602, GSE70768, GSE70769, GSE70770, and GSE54460), in which the CPGEA dataset was published in *Nature* by our team in 2020 (31), and we used the latest follow-up data in this study. Consistently, patients in the low-risk group had significantly longer PFS time in the eight cohorts, including the DFKZ cohort (n=81, p<0.001, Figure 6A), the MSKCC cohort (n=140, p<0.001, Figure 6B), the CPGEA cohort (n=125, p<0.001, Figure 6C), the GSE46602 cohort (n=36, p<0.001, Figure 6D), the GSE70768 cohort (n=111, p<0.001, Figure 6E), the GSE70769 cohort (n=92, p=0.01, Figure 6F), the GSE70770 cohort (n=203, p<0.001, Figure 6G), and the GSE54460 cohort (n=91, p=0.034, Figure 6H). Furthermore, the ROC curves demonstrated the good predictive performance of the signature in these datasets (Figure 6). In summary, this signature has good generalizability and application prospects.

Remarkably, it was verified that CRRS is an independent prognostic factor for PCa through univariate and multivariate cox regression analysis (Figures 7A-B, p<0.01). Finally, we developed a clinically applicable nomogram to predict 1-, 3-, and 5-year

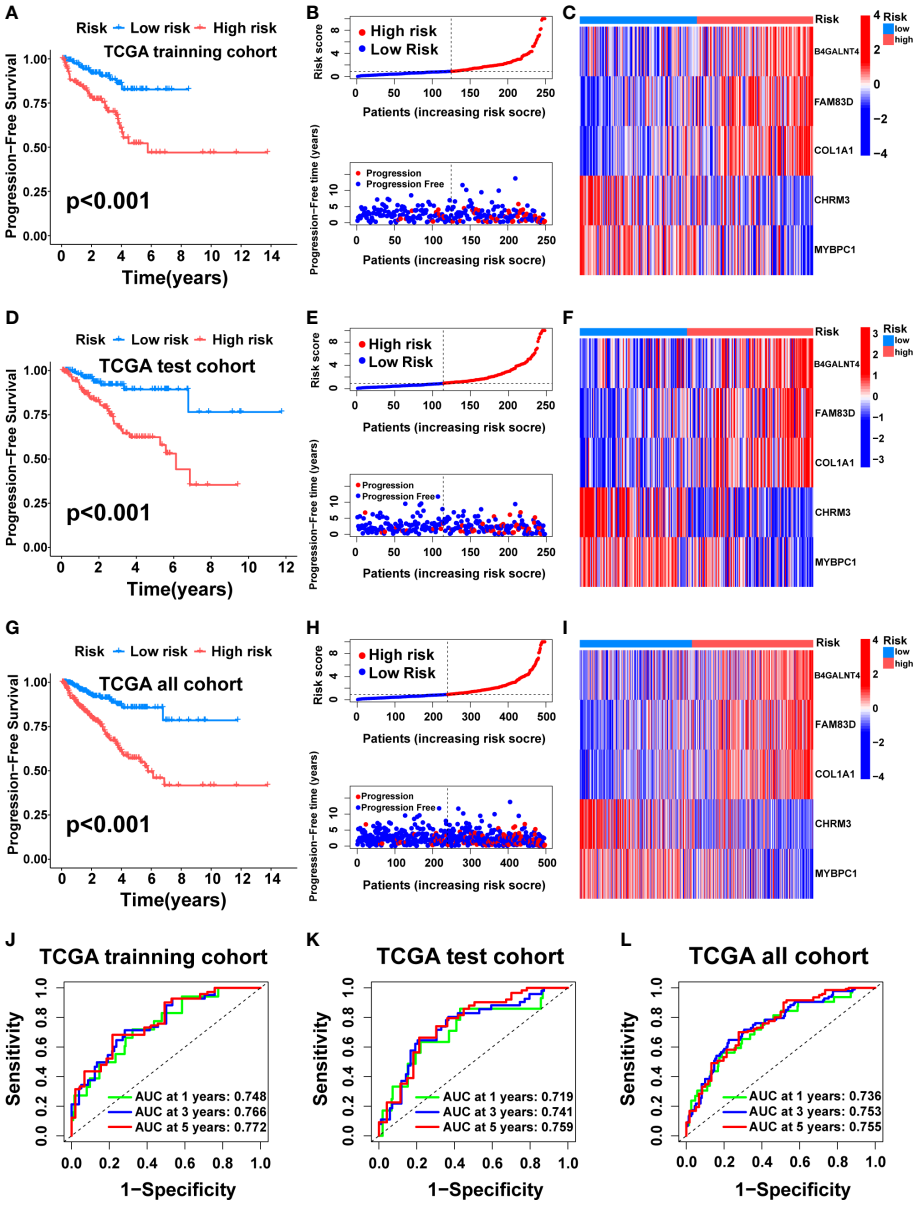


FIGURE 5
Construction and internal validation of the cuproptosis-related signature. For the TCGA training cohort: Kaplan–Meier curve **(A)**, risk score and survival status **(B)**, the expression heat map of the 5 model genes **(C)**, ROC curve, and AUC of the 5-gene signature **(J)**. For the TCGA test cohort: Kaplan–Meier curve **(D)**, risk score and survival status **(E)**, the expression heat map of the 5 model genes **(F)**, ROC curve, and AUC of 5-gene signature **(K)**. For the TCGA all cohort: Kaplan–Meier curve **(G)**, risk score and survival status **(H)**, the expression heat map of the 5 model genes **(I)**, ROC curve, and AUC of the 5-gene signature **(L)**.

prognosis for PCa patients (Figure 7C). The calibration curves illustrated good consistency between actual 1-, 3- and 5-year PFS rates and predicted PFS rates (Figure 7D).

3.5 The immune landscape of the signature

Previous studies have shown that tumor immune microenvironments are essential for tumor development (40, 41). Consequently, to explore the causes of poorer prognosis in the high-risk group, GSEA was conducted to investigate the enrichment of immune-related pathways and tumor-related pathways in the group. The result showed that many immune-related pathways were enriched in the high-risk group, including the B cell receptor signaling pathway, Natural killer cell mediated cytotoxicity, Neutrophil extracellular trap formation, T cell receptor signaling pathway, Th1 and Th2 cell differentiation, Th17 cell differentiation, and Toll–like receptor signaling pathway (Figure 8A). We also found that several classic tumor-related pathways were enriched in the high-risk group, including the Hippo signaling pathway, NF-

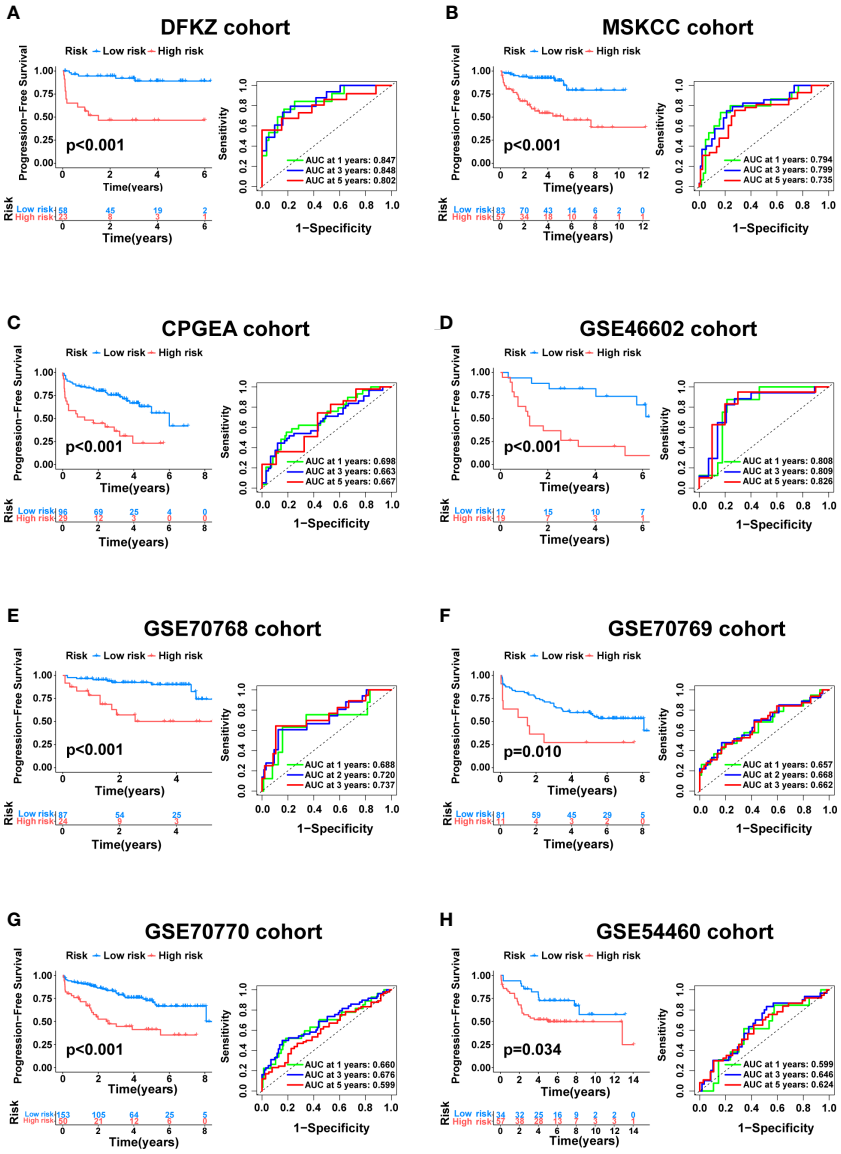


FIGURE 6
External validation of the cuproptosis-related signature. Kaplan–Meier curve as well as ROC curve and AUC of the signature in DFKZ cohort **(A)**, MSKCC cohort **(B)**, CPGEA cohort **(C)**, GSE46602 cohort **(D)**, GSE70768 cohort **(E)**, GSE70769 cohort **(F)**, GSE70770 cohort **(G)** and GSE54460 cohort **(H)**.

kappa B signaling pathway, p53 signaling pathway, PI3K-Akt signaling pathway, Rap1 signaling pathway, and Ras signaling pathway (Figure 8B).

Next, the correlation between this signature and the tumor immune microenvironment was further explored. The ssGSEA algorithm revealed higher immune cell infiltration and more active immune-related functions in the high-risk group. The immune cells that differentially infiltrated between the two risk groups were more infiltrated in the high-risk group except for Neutrophi, which was more infiltrated in the low-risk group (Figure 8C, p<0.05). The twelve immune-related functions that were differentially enriched between the two risk groups were all more active in the high-risk group (Figure 8D, p<0.05). Next, we explored the expression of MHC molecules and found that sixteen MHC molecules were differentially expressed between the two risk groups. Except for HLA-C, which was highly expressed in the low-risk group, the rest were highly expressed in the high-risk group (Figure 8E, p<0.05). Furthermore, the expression of immune checkpoint molecules and genes that inhibit the cancer-immunity cycle was also explored. A total of 35 immune checkpoint molecules were differentially expressed between the two risk groups. Except for CD44 and FGL1, which were highly expressed in the low-risk group, the rest were highly expressed in the high-risk group, including PD-1 (PDCD1), PDL1 (CD274), CTLA4, HAVCR2, B7H3(CD276), TIGIT and LAG3 (Figure 8F, p<0.05).

Finally, we compared the TME between the two risk groups through the ESTIMATE algorithm. The result revealed higher immune, stromal, and ESTIMATE estimation scores in the high-risk group (Figure 8G, p<0.001). On the IMvigor210 cohort, we performed a K-M analysis to assess the value of this signature in

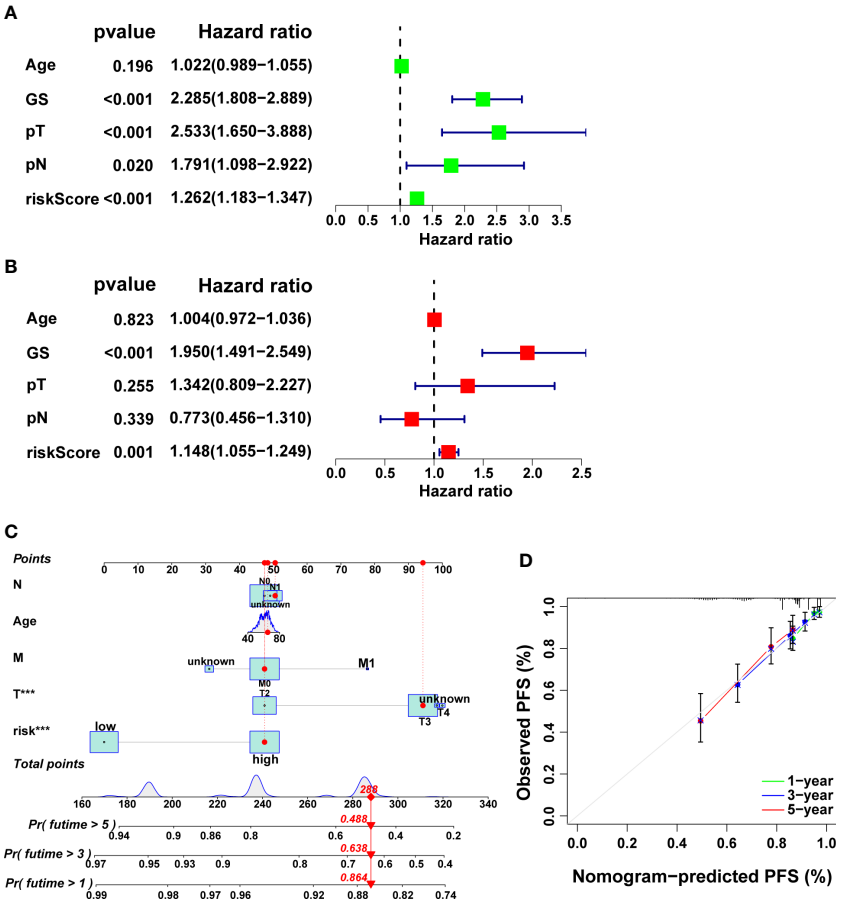


FIGURE 7
Independent prognostic analysis as well as the development and validation of a nomogram in the TCGA cohort. The results of univariate **(A)** and multivariate **(B)** Cox regression analysis. **(C)** The nomogram for predicting PFS in PCa. **(D)** Calibration plots of the nomogram.

predicting immune response to immunotherapy, which revealed that high-risk patients had a longer OS time than low-risk patients (Figure 8H, p=0.006).

3.6 Somatic mutation and TMB of the signature

SPOP (15%), TTN (10%), TP53 (6%), FOXA1 (3%), and KMT2D (4%) accounted for the highest mutation frequencies in the low-risk group, while SPOP (8%), TTN (10%), TP53(13%), FOXA1 (9%) and KMT2D (7%) had the highest mutation frequencies in the high-risk group (Figures 9A, B). Furthermore, the difference in TMB between the two risk groups was also compared. The high-risk group had higher TMB (Figure 9C, p<0.001), and TMB was positively correlated with risk score (Figure 9D, R = 0.22, p = 7e−07). KM analysis showed a shorter duration of PFS in patients with high TMB (Figure 9E, p<0.05). After combining with the signature, the prognosis of the high TMB + high-risk group was significantly poorer than that of the low TMB + low-risk group (Figure 9F, p<0.001). Finally, we found that the mutation frequencies of the five model genes were all low in PCa (Figure 9G).

3.7 Predicting chemotherapy response and screening small molecule drug

The differences in response to commonly used chemotherapeutic drugs between the two risk groups from TCGA were predicted *via* the GDSC dataset. We identified 53 chemotherapeutic drugs with significantly different IC50 values between the two risk groups, including 45 drugs with lower IC50 values in the high-risk group and 8 drugs with lower IC50 values in the low-risk group (Supplementary Table S5, Supplementary Figures S4, S5, p<0.001). Remarkably, the three most commonly used chemotherapy agents (Cisplatin, Docetaxel, and Bicalutamide) in PCa treatment and the copper ionophore Elesclomol that can induce cuproptosis showed significant differences in IC50 values between the two risk groups (23). Cisplatin, Docetaxel, and Eleclomol had lower IC50 values in the high-risk group, while Bicalutamide had a lower IC50 value in the low-risk group (Figures 10A-D, p<0.001).

Furthermore, we screened small-molecule drugs through the cMap database to identify potential treatment candidates for PCa patients. Based on the 312 upregulated genes and 107 downregulated genes generated by differential expression analysis between the two risk groups (Figure 10E, |logFC|>1, pvalue<0.05), the six most relevant small-molecule drugs (Purvalanol-A, Aminopurvalanol-A,

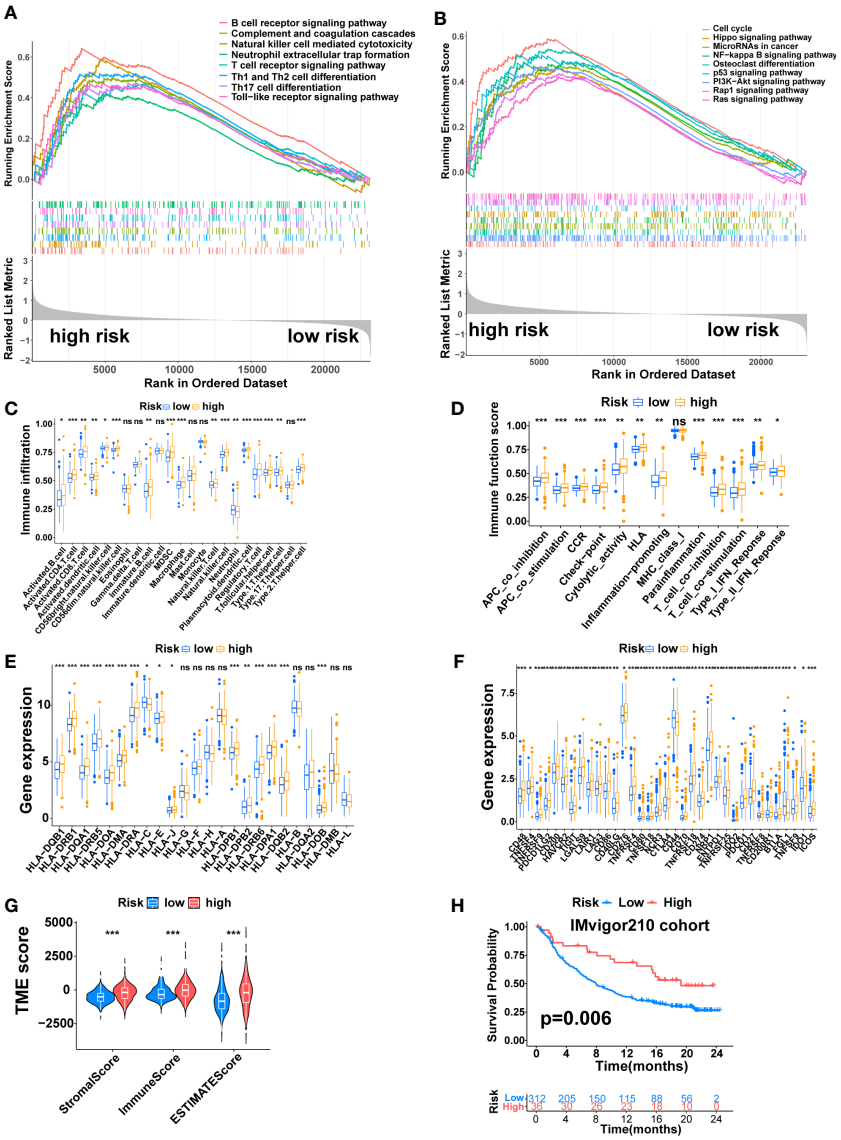


FIGURE 8
The Immune Landscape of the Signature. **(A)** Immune-related pathways enriched in the high-risk group. **(B)** Tumor-related pathways enriched in the high-risk group. **(C)** The difference in immune cell infiltration between the two risk groups. **(D)** The difference in immune-related functions or pathways between the two risk groups. **(E)** The comparison of MHC molecules expression between the two risk groups. **(F)** Immune checkpoint molecules expression between the two risk groups. **(G)** Stromal score, immune score, and estimate score between the two risk groups. **(H)** K-M analysis of the IMvigor210 cohort. (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). ns, no significant.

JAK3-inhibitor-VI, PHA-793887, Floxuridine, and Teniposide) were screened out. Their 3D structures were exhibited *via* the PubChem database (Figure 10F).

3.8 The expression and regulation of model genes in cell lines and the further experiment on B4GALNT4

To validate the results of the above analysis, the mRNA expression of the five model genes and the regulation of these genes in the presence of copper ions and copper ionophore Elesclomol were explored by qRT-PCR in various PCa cell lines (C4-2, PC3m, PC3, LNCaP). The results showed that B4GALNT4, FAM83D, COL1A1, and CHRM3 were stably expressed in the majority of PCa cell lines, while MYBPC1 was detected only in PC3 (Figures 11A–D). In addition, most of the model genes showed varying degrees of downregulation in the presence of Cu^{2+} and Elesclomol in most PCa cell lines, with B4GALNT4 and FAM83D being the most significant (Figures 11A-D, $p<0.05$), demonstrating the close association of these two genes with cuproptosis in prostate cancer cells.

Considering that B4GALNT4 contributed the most to poor prognosis, we conducted further research on B4GALNT4. Since B4GALNT4 has a high expression level in the C4-2 cell line, we constructed a stably transfected C4-2 cell line with the knockdown of B4GALNT4 (Figure 11E). Next, we performed proteomics and transcriptomics analyses using these stably transfected C4-2 cells

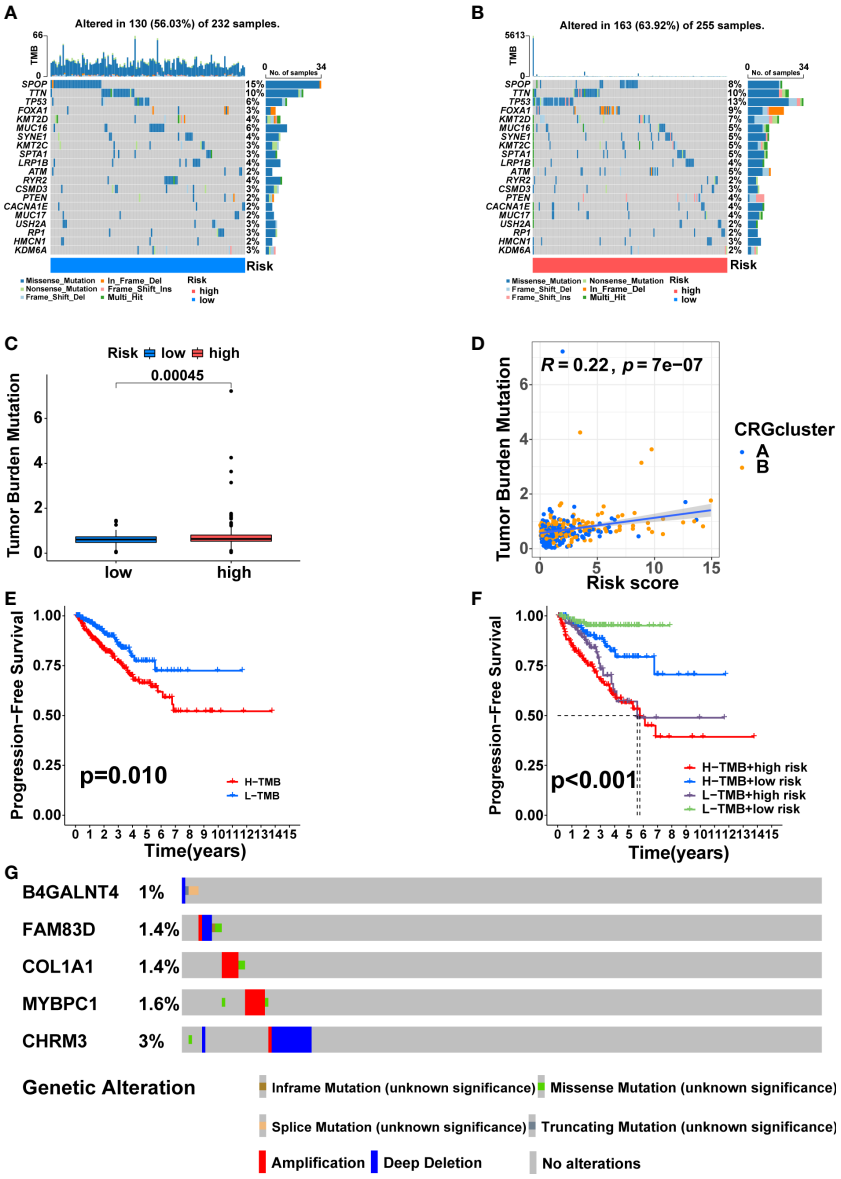


FIGURE 9
Somatic mutation and TMB based on the signature. Waterfall maps of the somatic mutations in the low-risk group **(A)** and the high-risk group **(B)**. **(C)** Difference of TMB between the two risk groups. **(D)** Correlation between risk score and TMB. **(E)** Comparison in PFS between high- and low-TMB groups. **(F)** Comparison in PFS based on TMB and risk score. **(G)** Mutation frequencies of the five model genes in PCa patients from the cBioPortal database.

(Figure 11F). In proteomics analysis, the CDKN2A protein level was significantly upregulated (Ratio<0.83), and the ATP7A protein level was significantly downregulated (Ratio>1.2) (Figure 11G). However, the transcriptomics analysis suggested that the RNA levels of these CRGs did not change significantly after the knockdown of B4GALNT4 (|logFoldChange|<1, Figure 11H). Additionally, GSEA analysis revealed that multiple cancer-related pathways were inhibited after the knockdown of B4GALNT4, including the PI3K−Akt signaling pathway, Rap1 signaling pathway, and Wnt signaling pathway. (Figure 11I). In summary, these results suggested that B4GALNT4 is a potential cuproptosis-related oncogene in PCa, which could be used as a target to treat PCa in combination with cuproptosis.

4 Discussion

Growing evidence suggests that genetic biomarkers have become increasingly crucial in highly personalized precision medicine (42). As tumor molecular biology advances, developing new predictive tools and therapeutic targets based on prognosis-related genes has become a promising field. These genes reflecting tumor progression at the molecular level not only contribute to more accurate personalized survival prediction and guide the choice of treatment regimens, but also help to develop molecular targets for precision treatment.

Cuproptosis, a newly discovered RCD form dependent on mitochondrial respiration, differs from any known RCD form (23). As a novel RCD form, cuproptosis has rapidly become a

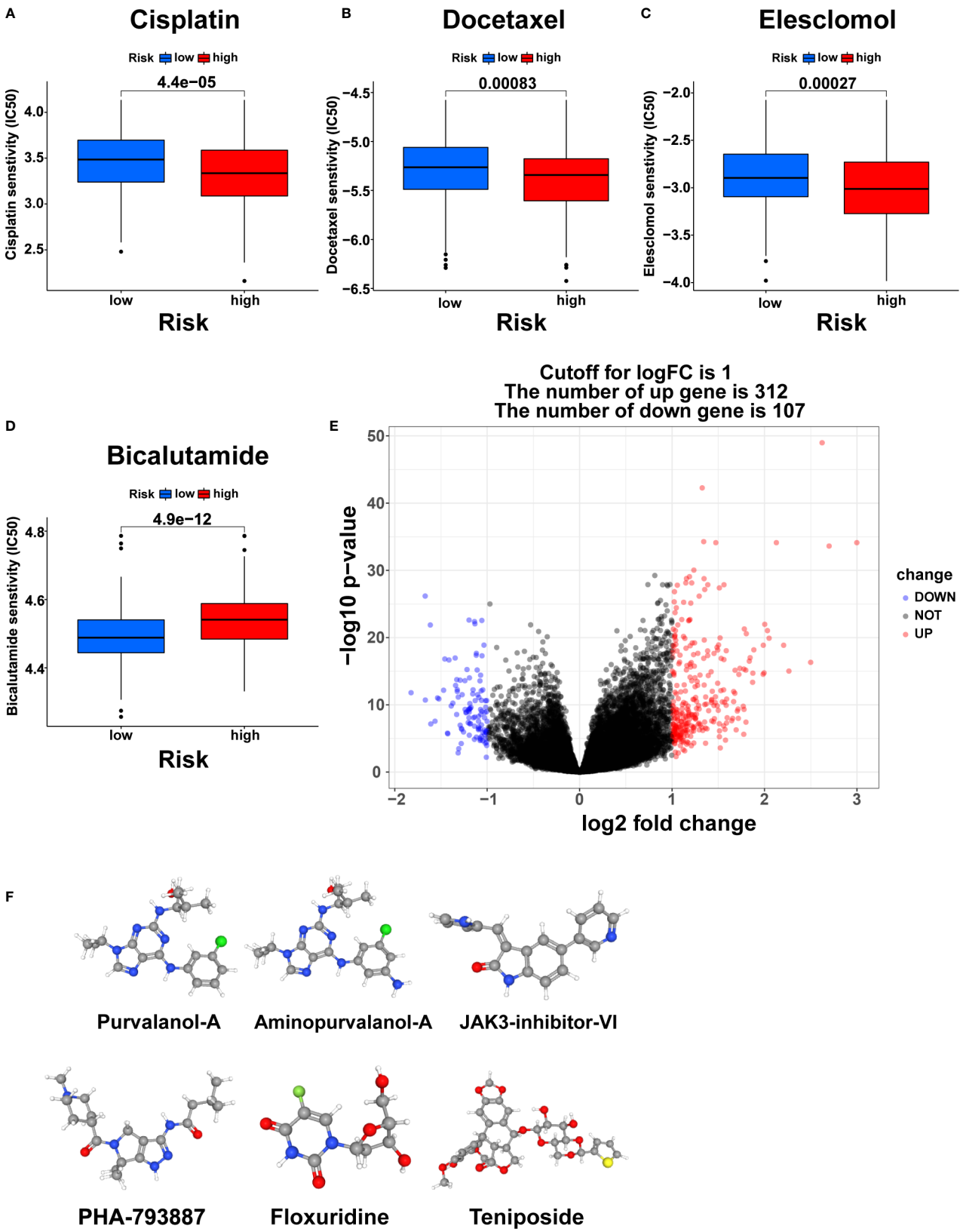


FIGURE 10
Chemotherapy response prediction and small molecule drug screening. The differences in the chemotherapy response of Cisplatin **(A)**, Docetaxel **(B)**, Elesclomol **(C)**, and Bicalutamide **(D)** between the two risk groups. **(E)** Volcano plot of DEGs between the two risk groups. **(F)** The 3D structure of six small molecule drugs screened out from the cMap database. IC50, the half maximal inhibitory concentration.

10.3389/fonc.2023.1162653

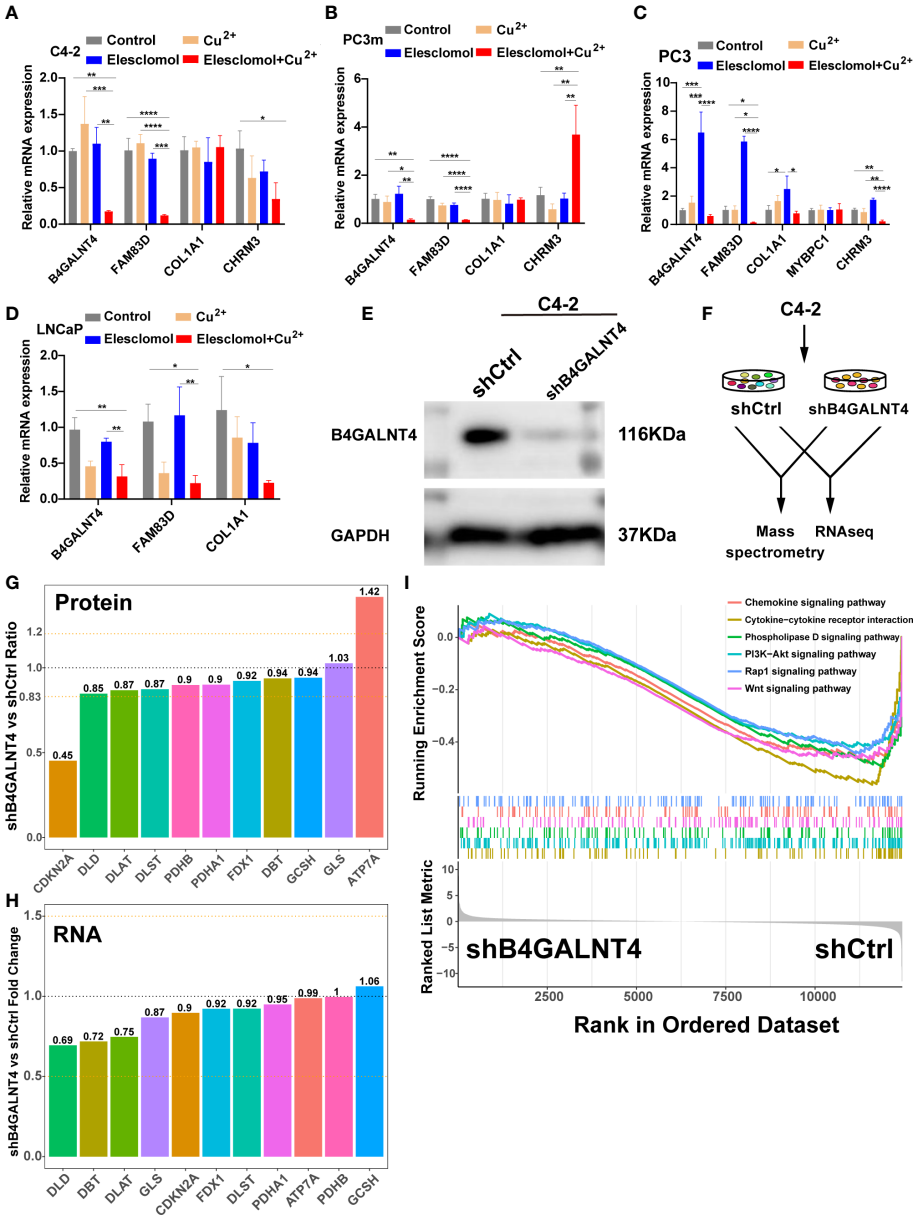


FIGURE 11
The expression and regulation of these model genes and further experiments on B4GALNT4. **(A–D)** qRT-PCR shows the expression and regulation of model genes in prostate cell lines treated with drugs that induce cuproptosis for 24 h (n = 3). CuCl2 (2mM), Elesclomol (20 nM), both CuCl2 (2mM) and Elesclomol (20 nM). **(E)** Western blot showing the knockdown effect of B4GALNT4 in C4-2. **(F)** Experimental scheme of proteomics and transcriptomics analysis on C4-2 stable cell lines with B4GALNT4 knockdown. **(G)** The changes in protein levels of CRGs after B4GALNT4 knockdown. **(H)** The changes in mRNA levels of CRGs after B4GALNT4 knockdown. NS, P >= 0.05; *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001. **(I)** GSEA demonstrated the enrichment of tumor-related pathways after B4GALNT4 knockdown.

research hotspot, providing additional references for drug development and refinement of clinical indicators (15). Current studies have shown that cuproptosis is associated with prognosis and TME in bladder, breast, and hepatocellular carcinoma, and a series of good prognostic models have been developed to predict tumor prognosis (24, 25, 27). However, in PCa, studies related to cuproptosis are still in the preliminary stage and most of them have focused on cuproptosis-related long non-coding RNA (lncRNA). Several studies have now reported that cuproptosis-related lncRNA have a better prognostic role in predicting PCa (43, 44). However, studies on cuproptosis-related coding genes in PCa are rarely reported, and the role of cuproptosis in PCa remains unknown.

In this study, PCa can be stratified into two molecular subtypes according to the expression of prognostic CRGs. The prognosis of the two subtypes was significantly different, and the PFS time of cluster A was significantly longer than that of cluster B. Twelve CRGs were highly expressed in cluster B, among which CDKN2A was the most significant. As an anti-cuproptosis gene, the significantly high expression of CDKN2A in cluster B may indicate an inhibitory state of cuproptosis in cluster B (23). In

addition, the analysis of clinicopathological features showed that cluster B had more advanced and malignant PCa cases. These results may explain the poorer prognosis of cluster B to some extent. Furthermore, we explored the underlying causes of these differences between the two clusters through GSVA. The result showed that the TCA cycle was significantly enriched in cluster B, which is enlightening considering the pivotal role of the TCA cycle in the process of cuproptosis.

The TME is a critical component of the growth of tumors. It comprises several types of cells, including tumor cells, infiltrating immune cells, and stromal cells. Tumor progression depends heavily on the crosstalk between these cells and between these cells and other non-cellular components (45). It has been revealed that the infiltration of different immune cells is closely associated with clinical outcomes of breast cancer, bladder cancer, and PCa (46–48). Therefore, the TME between cuproptosis-related subtypes was further compared. Patients in cluster B exhibited higher infiltration of immunosuppressive components, such as regulatory T (Treg) cells and activated CD4 T cells, whereas there was no difference in the proportion of anti-tumor immune cells, such as CD8 T cells and B cells, between the two clusters. Tumor-infiltrating Treg cells can inhibit anti-tumor immunity and promote cancer progression, which can cause adverse clinical outcomes, so it is considered the main obstacle to the successful application of immunotherapy (49–51). The recruitment and activation of CD4+ T lymphocytes are related to establishing a tumor immunosuppressive microenvironment (52). These previous findings suggest a tumor-promoting and anti-immune state in cluster B. Furthermore, most of the immune checkpoint genes (including PD-1 and CTLA4) and genes that inhibit the cancer-immunity cycle were also highly expressed in cluster B, which further indicated the immunosuppressive state in cluster B. PD-1 and CTLA4 were highly expressed in cluster B, suggesting that patients in cluster B may benefit more from anti-PD1/CTALA4 therapy. However, as indicated by TIDE analysis, anti-PD1/CTALA4 therapy was less effective in cluster B, which reflects the complexity of the TME and requires more in-depth research to elucidate the interactions between the various cellular and matrix components.

Although significant progress has been made in diagnosing and treating PCa in recent decades, PCa is currently the second leading cause of cancer death in Western countries (53). Lack of accurate prognostic prediction tools and drug resistance are two significant challenges in PCa treatment (54). The accurate prognostic prediction could determine whether patients benefit from more aggressive therapies, including neoadjuvant therapy, more intensive surgery, chemotherapy, radiotherapy, targeted therapy, and immunotherapy, which could be customized for individual patients to improve outcomes. Therefore, we established and tested a prognostic signature in this study to independently evaluate PCa patients' prognoses. The reliability and generalizability of the signature were verified in eight completely independent datasets involving a total of 879 PCa patients from multiple centers. Furthermore, a clinically applicable nomogram with high reliability for clinical practice was established. Interestingly, we found that pro-cuproptosis genes such as PDHB and SLC31A1 were lowly expressed in the high-risk group, while anti-cuproptosis genes such as GLS and CDKN2A were highly expressed in the high-risk group (23), indicating that PCa patients with high CRRS may be in an inhibited state of cuproptosis.

To further explore the mechanisms underlying the difference in prognosis between the two risk groups, we visualized pathway enrichment and immune landscape between the two groups. GSEA revealed that several classical cancer-related pathways, including the Hippo signaling pathway, NF-Kappa B signaling pathway, PI3K-Akt signaling pathway, and Ras signaling pathway, were enriched in the high-risk group. Among them, the Hippo signaling pathway and NF-Kappa B signaling pathway can promote metastasis and castration resistance of PCa (55–58). Studies have shown that the PI3K-Akt signaling pathway can interact with multiple cellular signaling cascades to promote PCa progression and influence ADT sensitivity in PCa cells (59). The interaction of the Ras signaling pathway and the Wnt signaling pathway can promote bone metastasis of PCa (60). Remarkably, several pro-tumor immune pathways, including T cell receptor signaling pathway, B cell receptor signaling pathway, Natural killer cell mediated cytotoxicity, Neutrophil extracellular trap formation, Th17 cell differentiation, and Toll–like receptor signaling pathway were also enriched in the high-risk group (61–63). These results explain, to some extent, the worse prognosis of the high-risk group.

Currently, immunotherapy has revolutionized the treatment strategy for many types of cancer (64, 65). However, due to the immune "cold" status of advanced PCa, which is usually characterized by poor T-cell infiltration, low mutational load, low MHC class I expression, and low PD-L1 expression (66, 67), the overall efficacy of single immunotherapy in cold tumors, including PCa, is poor (68, 69). In fact, PCa, as an indolent tumor, is an ideal model for cancer immunotherapy because it can provide sufficient time to form the anti-tumor immune response. With the approval of Sipuleucel-T for PCa treatment, tumor immunotherapy has achieved good efficacy in carefully selected PCa patients (70). In addition, combining tumor immunotherapy with ADT, chemotherapy, or DNA-damaging treatment can significantly promote the effect of immunotherapy, reflecting the excellent prospect of immunotherapy in treating PCa (70, 71). Therefore, apart from finding the optimum treatment combination, there is an urgent need to develop biomarkers that can predict tumor immune microenvironment and immunotherapy response, which are essential for the personalized treatment of patients with advanced PCa. Since the immune environment of TME is crucial for effective immunotherapy, we visualized the immune landscape of the two risk groups. Overall, patients in the high-risk group had higher immune cell infiltration, more active immune-related functions, higher expression of MHC molecules and immune checkpoint molecules (including PDL-1, PD-1, CTLA4, HAVCR2, B7H3, TIGIT, and LAG3), and higher immune scores. According to these findings, high-risk patients may experience a stronger immune response to tumor progression and may benefit more from immune checkpoint inhibitors (ICIs). Considering that high-risk patients have a higher TMB and that the immune system readily recognizes and kills tumor cells with high genomic instability (72), this again suggests that these patients may benefit more from immunotherapy. To further validate the role of CRRS

in predicting the response to immunotherapy, we performed a K-M analysis on the IMvigor210 cohort. As expected, patients in the high-risk group had longer OS than those in the low-risk group. Thus, CRRS may help to screen patients who may benefit more from immunotherapy.

Chemotherapy is a significant treatment for advanced PCa. It is of great importance to choose a suitable chemotherapy strategy. We found that high-risk patients were more sensitive to Cisplatin, Docetaxel, and Elesclomol, while low-risk patients were more sensitive to Bicalutamide. Since Cisplatin, Docetaxel, and Bicalutamide are the three commonly used chemotherapy drugs for PCa in clinical practice, CRRS may help to select the appropriate chemotherapeutic agents. Elesclomol is a copper ionophore that can induce cuproptosis in cells (23), to which high-risk patients are more sensitive, further confirming the previously mentioned inhibitory state of cuproptosis in these patients. In the future, Elesclomol may be used to treat PCa under the premise of a reliable predictive biomarker.

In addition, we predicted six potential compounds, including purvalanol-A, aminopurvalanol-A, JAK3-inhibitor-VI, PHA-793887, Floxuridine, and Teniposide, for the treatment of PCa using the cMap Database. PHA-793887 significantly inhibited the growth of abiraterone-resistant PCa cell lines and patient-derived xenograft-derived PCa models (73). Floxuridine variants have potential therapeutic value in p53-mutated and hormone-dependent PCa (74). Purvalanol A can enhance the cytotoxic effect of taxol on non-small cell lung cancer cells *in vitro* through Op18/stathmin (75). Previous studies have shown that JAK3-inhibitor-VI is a promising candidate for treating acute myeloid leukemia (76). Teniposide has good efficacy in breast cancer (77). Aminopurvalanol-a has not been reported. In subsequent studies, we will explore the effects of these drugs on PCa treatment.

Finally, in PCa cell lines, the expression of these model genes was validated. All model genes were stably expressed in several PCa cell lines, except MYBPC1, which was detected only in PC3. MYBPC1 encodes a member of the myosin-binding protein C family, which may be expressed primarily in non-tumor cells in the TME. In addition, B4GALNT4 and FAM83D were significantly downregulated after induction of cuproptosis in most PCa cell lines, suggesting that these two genes are closely associated with cuproptosis activity in PCa cells. Studies have shown that FAM83D is strongly associated with cancer development, proliferation, invasion, and metastasis (78, 79). Beta-1,4-N-acetylgalactosaminyltransferase 4 (B4GALNT4), as an N-acetylgalactosamine transferase, is involved in the post-translational regulation of genes through protein glycosylation modifications (80). B4GALNT4 is upregulated in various cancers, and its expression can enhance the malignant potential of cancers (81). Considering that B4GALNT4 contributed the most to poor prognosis, we further investigated the model gene B4GALNT4. After the knockdown of B4GALNT4, the protein level of anti-cuproptosis CDKN2A was significantly down-regulated (23), indicating that the knockdown of B4GALNT4 might promote cuproptosis in PCa. Therefore, the up-regulation in the protein level of copper exporters ATP7A was probably due to the increase of copper ions in the cells after the enhancement of cuproptosis activity caused by the knockdown of B4GALNT4, and the compensatory up-regulation of ATP7A occurred in the cells to maintain the homeostasis of copper ions. Remarkably, the mRNA levels of the above CRGs were not significantly changed after the knockdown of B4GALNT4, suggesting that B4GALNT4 may regulate CRGs through post-transcriptional protein modifications. Meanwhile, transcriptomics analysis suggested that the knockdown of B4GALNT4 inhibited several classical pro-cancer pathways, including PI3K–Akt signaling and Wnt signaling pathways, indicating a pro-carcinogenic role of B4GALNT4 in PCa.

There are some limitations to our study. First of all, the signature was only validated with retrospective data. In the future, more prospective studies are required to verify its clinical value. Secondly, this study just investigated the relationship between the signature and TME as well as immunotherapy, only suggesting a possible correlation between them. Therefore, a clinical trial with sufficient samples to assess the value of this signature in guiding immunotherapy is required in the future. Thirdly, the value of the model for a personalized selection of chemotherapy drugs requires to be validated in later clinical trials, and the therapeutic effect of the screened potential small molecule compounds also needs to be further investigated. Lastly, further experiments *in vivo* and *in vitro* are required to investigate the role of the five model genes in PCa cuproptosis and tumorigenesis.

5 Conclusion

In conclusion, this study distinguished molecular subtypes based on CRGs in PCa and constructed a robust prognostic signature. The cuproptosis-related molecular subtypes and the prognostic signature could be used to predict the prognosis of PCa. Moreover, this signature may help to identify PCa patients who benefit more from anticancer immunotherapy and guide the choice of chemotherapy or targeted agents for patients with advanced PCa. In addition, we validated and explored the expression and regulation of model genes at the cellular level, respectively. Furthermore, the role of B4GALNT4 in cuproptosis and tumorigenesis in PCa was further explored through proteomics and transcriptomics analysis. In summary, our systematic study of CRGs will help to understand their role and value in PCa, and the signature can provide a reference for the clinical judgment of prognosis and selection of treatment options. Furthermore, we identified a potential cuproptosis-related oncogene in PCa, which could be a potential target to treat PCa in combination with cuproptosis.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Author contributions

Conceptualization, JZ. Data curation, SJ. Funding acquisition, XG. Investigation, DG. Methodology, SJ. Project administration, XG. Resources, MQ. Software, WZ. Supervision, XS, MQ, CY and XG. Validation, DG. Writing – original draft, JZ. Writing – review & editing, YW. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the National Natural Science Foundation of China [Grant Nos. 82272793 and 82203309], and the National key research and development program of China [Grant Nos. 2020YFC2002704].

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1162653/full#supplementary-material

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: Cancer J Clin* (2021) 71:209–49. doi: 10.3322/caac.21660

2. Lalonde E, Ishkanian AS, Sykes J, Fraser M, Ross-Adams H, Erho N, et al. Tumour genomic and microenvironmental heterogeneity for integrated prediction of 5-year biochemical recurrence of prostate cancer: a retrospective cohort study. *Lancet Oncol* (2014) 15:1521–32. doi: 10.1016/s1470-2045(14)71021-6

3. Wang G, Zhao D, Spring DJ, DePinho RA. Genetics and biology of prostate cancer. *Genes Dev* (2018) 32:1105–40. doi: 10.1101/gad.315739.118

4. Shao N, Wang Y, Jiang WY, Qiao D, Zhang SG, Wu Y, et al. Immunotherapy and endothelin receptor antagonists for treatment of castration-resistant prostate cancer. *Int J Cancer* (2013) 133:1743–50. doi: 10.1002/ijc.28162

5. Mansinho A, Macedo D, Fernandes I, Costa L. Castration-resistant prostate cancer: mechanisms, targets and treatment. *Adv Exp Med Biol* (2018) 1096:117–33. doi: 10.1007/978-3-319-99286-0_7

6. Sharifi N, Gulley JL, Dahut WL. Androgen deprivation therapy for prostate cancer. *Jama* (2005) 294:238–44. doi: 10.1001/jama.294.2.238

7. Cornford P, Bellmunt J, Bolla M, Briers E, De Santis M, Gross T, et al. EAU-ESTRO-SIOG guidelines on prostate cancer. part II: treatment of relapsing, metastatic, and castration-resistant prostate cancer. *Eur Urol* (2017) 71:630–42. doi: 10.1016/j.eururo.2016.08.002

8. Rycaj K, Li H, Zhou J, Chen X, Tang DG. Cellular determinants and microenvironmental regulation of prostate cancer metastasis. *Semin Cancer Biol* (2017) 44:83–97. doi: 10.1016/j.semcancer.2017.03.009

9. Kirby M, Hirst C, Crawford ED. Characterising the castration-resistant prostate cancer population: a systematic review. *Int J Clin Pract* (2011) 65:1180–92. doi: 10.1111/j.1742-1241.2011.02799.x

10. Fizazi K, Flaig TW, Stöckle M, Scher HI, de Bono JS, Rathkopf DE, et al. Does Gleason score at initial diagnosis predict efficacy of abiraterone acetate therapy in patients with metastatic castration-resistant prostate cancer? an analysis of abiraterone acetate phase III trials. *Ann oncology: Off J Eur Soc Med Oncol* (2016) 27:699–705. doi: 10.1093/annonc/mdv545

11. Loeb S, Vonesh EF, Metter EJ, Carter HB, Gann PH, Catalona WJ. What is the true number needed to screen and treat to save a life with prostate-specific antigen testing? *J Clin oncology: Off J Am Soc Clin Oncol* (2011) 29:464–7. doi: 10.1200/jco.2010.30.6373

12. A J, Zhang B, Zhang Z, Hu H, Dong JT. Novel gene signatures predictive of patient recurrence-free survival and castration resistance in prostate cancer. *Cancers* (2021) 13 (4):917. doi: 10.3390/cancers13040917

13. Peng F, Liao M, Qin R, Zhu S, Peng C, Fu L, et al. Regulated cell death (RCD) in cancer: key pathways and targeted therapies. *Signal transduction targeted Ther* (2022) 7:286. doi: 10.1038/s41392-022-01110-y

14. Tang D, Kang R, Berghe TV, Vandenabeele P, Kroemer G. The molecular machinery of regulated cell death. *Cell Res* (2019) 29:347–64. doi: 10.1038/s41422-019-0164-5

15. Tang S, Zhao L, Wu XB, Wang Z, Cai LY, Pan D, et al. Identification of a novel cuproptosis-related gene signature for prognostic implication in head and neck squamous carcinomas. *Cancers* (2022) 14 (16):3986. doi: 10.3390/cancers14163986

16. Qi X, Li Q, Che X, Wang Q, Wu G. Application of regulatory cell death in cancer: based on targeted therapy and immunotherapy. *Front Immunol* (2022) 13:837293. doi: 10.3389/fimmu.2022.837293

17. Gong Y, Fan Z, Luo G, Yang C, Huang Q, Fan K, et al. The role of necroptosis in cancer biology and therapy. *Mol Cancer* (2019) 18:100. doi: 10.1186/s12943-019-1029-8

18. Ruiz LM, Libedinsky A, Elorza AA. Role of copper on mitochondrial function and metabolism. *Front Mol Biosci* (2021) 8:711227. doi: 10.3389/fmolb.2021.711227

19. Li Y. Copper homeostasis: emerging target for cancer treatment. *IUBMB Life* (2020) 72:1900–8. doi: 10.1002/iub.2341

20. Kahlson MA, Dixon SJ. Copper-induced cell death. *Sci (New York N.Y.)* (2022) 375:1231–2. doi: 10.1126/science.abo3959

21. Oliveri V. Selective targeting of cancer cells by copper ionophores: an overview. *Front Mol Biosci* (2022) 9:841814. doi: 10.3389/fmolb.2022.841814

22. Miao Y, Liu J, Liu X, Yuan Q, Li H, Zhang Y, et al. Machine learning identification of cuproptosis and necroptosis-associated molecular subtypes to aid in prognosis assessment and immunotherapy response prediction in low-grade glioma. *Front Genet* (2022) 13:951239. doi: 10.3389/fgene.2022.951239

23. Tsvetkov P, Coy S, Petrova B, Dreishpoon M, Verma A, Abdusamad M, et al. Copper induces cell death by targeting lipoylated TCA cycle proteins. *Sci (New York N.Y.)* (2022) 375:1254–61. doi: 10.1126/science.abf0529

24. Song Q, Zhou R, Shu F, Fu W. Cuproptosis scoring system to predict the clinical outcome and immune response in bladder cancer. *Front Immunol* (2022) 13:958368. doi: 10.3389/fimmu.2022.958368

25. Sha S, Si L, Wu X, Chen Y, Xiong H, Xu Y, et al. Prognostic analysis of cuproptosis-related gene in triple-negative breast cancer. *Front Immunol* (2022) 13:922780. doi: 10.3389/fimmu.2022.922780

26. Lv H, Liu X, Zeng X, Liu Y, Zhang C, Zhang Q, et al. Comprehensive analysis of cuproptosis-related genes in immune infiltration and prognosis in melanoma. *Front Pharmacol* (2022) 13:930041. doi: 10.3389/fphar.2022.930041

27. Zhang Z, Zeng X, Wu Y, Liu Y, Zhang X, Song Z. Cuproptosis-related risk score predicts prognosis and characterizes the tumor microenvironment in hepatocellular carcinoma. *Front Immunol* (2022) 13:925618. doi: 10.3389/fimmu.2022.925618

28. Xu Y, Liu SY, Zeng L, Ma H, Zhang Y, Yang H, et al. An enzyme-engineered nonporous Copper(I) coordination polymer nanoplatform for cuproptosis-based synergistic cancer therapy. *Advanced materials (Deerfield Beach Fla.)* (2022) 34: e2204733. doi: 10.1002/adma.202204733

29. Gerhauser C, Favero F, Risch T, Simon R, Feuerbach L, Assenov Y, et al. Molecular evolution of early-onset prostate cancer identifies molecular risk markers and clinical trajectories. *Cancer Cell* (2018) 34:996–1011.e1018. doi: 10.1016/j.ccell.2018.10.016

30. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, et al. Integrative genomic profiling of human prostate cancer. *Cancer Cell* (2010) 18:11–22. doi: 10.1016/j.ccr.2010.05.026

31. Li J, Xu C, Lee HJ, Ren S, Zi X, Zhang Z, et al. A genomic and epigenomic atlas of prostate cancer in Asian populations. *Nature* (2020) 580:93–9. doi: 10.1038/s41586-020-2135-x

32. Mortensen MM, Høyer S, Lynnerup AS, Ørntoft TF, Sørensen KD, Borre M, et al. Expression profiling of prostate cancer tissue delineates genes associated with recurrence after prostatectomy. *Sci Rep* (2015) 5:16018. doi: 10.1038/srep16018

33. Ross-Adams H, Lamb AD, Dunning MJ, Halim S, Lindberg J, Massie CM, et al. Integration of copy number and transcriptomics provides risk stratification in prostate cancer: a discovery and validation cohort study. *EBioMedicine* (2015) 2:1133–44. doi: 10.1016/j.ebiom.2015.07.017

34. Long Q, Xu J, Osunkoya AO, Sannigrahi S, Johnson BA, Zhou W, et al. Global transcriptome analysis of formalin-fixed prostate cancer specimens identifies biomarkers of disease recurrence. *Cancer Res* (2014) 74:3228–37. doi: 10.1158/0008-5472.Can-13-2699

35. Mariathasan S, Turley SJ, Nickles D, Castiglioni A, Yuen K, Wang Y, et al. TGFβ attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. *Nature* (2018) 554:544–8. doi: 10.1038/nature25501

36. Bindea G, Mlecnik B, Tosolini M, Kirilovsky A, Waldner M, Obenauf AC, et al. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. *Immunity* (2013) 39:782–95. doi: 10.1016/j.immuni.2013.10.003

37. Qiu C, Shi W, Wu H, Zou S, Li J, Wang D, et al. Identification of molecular subtypes and a prognostic signature based on inflammation-related genes in colon adenocarcinoma. *Front Immunol* (2021) 12:769685. doi: 10.3389/fimmu.2021.769685

38. Xu WX, Zhang J, Hua YT, Yang SJ, Wang DD, Tang JH. An integrative pan-cancer analysis revealing LCN2 as an oncogenic immune protein in tumor microenvironment. *Front Oncol* (2020) 10:605097. doi: 10.3389/fonc.2020.605097

39. Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. *Immunity* (2013) 39:1–10. doi: 10.1016/j.immuni.2013.07.012

40. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* (2011) 144:646–74. doi: 10.1016/j.cell.2011.02.013

41. Joyce JA, Pollard JW. Microenvironmental regulation of metastasis. *Nat Rev Cancer* (2009) 9:239–52. doi: 10.1038/nrc2618

42. Rebello RJ, Oing C, Knudsen KE, Loeb S, Johnson DC, Reiter RE, et al. Prostate cancer. *Nat Rev Dis Primers* (2021) 7:9. doi: 10.1038/s41572-020-00243-0

43. Jiang S, Li Z, Dou R, Lin Z, Zhang J, Zhang W, et al. Construction and validation of a novel cuproptosis-related long noncoding RNA signature for predicting the outcome of prostate cancer. *Front Genet* (2022) 13:976850. doi: 10.3389/fgene.2022.976850

44. Cheng X, Zeng Z, Yang H, Chen Y, Liu Y, Zhou X, et al. Novel cuproptosis-related long non-coding RNA signature to predict prognosis in prostate carcinoma. *BMC Cancer* (2023) 23:105. doi: 10.1186/s12885-023-10584-0

45. Wu T, Dai Y. Tumor microenvironment and therapeutic response. *Cancer Lett* (2017) 387:61–8. doi: 10.1016/j.canlet.2016.01.043

46. Ali HR, Chlon L, Pharoah PD, Markowetz F, Caldas C. Patterns of immune infiltration in breast cancer and their clinical implications: a gene-Expression-Based retrospective study. *PloS Med* (2016) 13:e1002194. doi: 10.1371/journal.pmed.1002194

47. Efstathiou JA, Mouw KW, Gibb EA, Liu Y, Wu CL, Drumm MR, et al. Impact of immune and stromal infiltration on outcomes following bladder-sparing trimodality therapy for muscle-invasive bladder cancer. *Eur Urol* (2019) 76:59–68. doi: 10.1016/j.eururo.2019.01.011

48. Rodrigues DN, Rescigno P, Liu D, Yuan W, Carreira S, Lambros MB, et al. Immunogenomic analyses associate immunological alterations with mismatch repair defects in prostate cancer. *J Clin Invest* (2018) 128:5185. doi: 10.1172/jci125184

49. Facciabene A, Motz GT. Coukos, g. T-regulatory cells: key players in tumor immune escape and angiogenesis. *Cancer Res* (2012) 72(9):2162–71. doi: 10.1158/0008-5472.Can-11-3687

50. Liu C, Workman CJ, Vignali DA. Targeting regulatory T cells in tumors. *FEBS J* (2016) 283:2731–48. doi: 10.1111/febs.13656

51. Arce Vargas F, Furness AJS, Solomon I, Joshi K, Mekkaoui L, Lesko MH, et al. Fc-optimized anti-CD25 depletes tumor-infiltrating regulatory T cells and synergizes with PD-1 blockade to eradicate established tumors. *Immunity* (2017) 46:577–86. doi: 10.1016/j.immuni.2017.03.013

52. Zhang Y, Yan W, Mathew E, Bednar F, Wan S, Collins MA, et al. CD4+ T lymphocyte ablation prevents pancreatic carcinogenesis in mice. *Cancer Immunol Res* (2014) 2:423–35. doi: 10.1158/2326-6066.Cir-14-0016-t

53. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA: Cancer J Clin* (2022) 72:7–33. doi: 10.3322/caac.21708

54. Mottet N, van den Bergh RCN, Briers E, Van den Broeck T, Cumberbatch MG, De Santis M, et al. EAU-EANM-ESTRO-ESUR-SIOG guidelines on prostate cancer-2020 update. part 1: screening, diagnosis, and local treatment with curative intent. *Eur Urol* (2021) 79:243–62. doi: 10.1016/j.eururo.2020.09.042

55. Salem O, Hansen CG. The hippo pathway in prostate cancer. *Cells* (2019) 8 (4):370. doi: 10.3390/cells8040370

56. Varzavand A, Hacker W, Ma D, Gibson-Corley K, Hawayek M, Tayh OJ, et al. α3β1 integrin suppresses prostate cancer metastasis *via* regulation of the hippo pathway. *Cancer Res* (2016) 76:6577–87. doi: 10.1158/0008-5472.Can-16-1483

57. Chen L, De Menna M, Groenewoud A, Thalmann GN, Kruithof-de Julio M, Snaar-Jagalska BEA. NF-κB-Activin a signaling axis enhances prostate cancer metastasis. *Oncogene* (2020) 39:1634–51. doi: 10.1038/s41388-019-1103-0

58. Thomas-Jardin SE, Dahl H, Nawas AF, Bautista M, Delk NA. NF-κB signaling promotes castration-resistant prostate cancer initiation and progression. *Pharmacol Ther* (2020) 211:107538. doi: 10.1016/j.pharmthera.2020.107538

59. Shorning BY, Dass MS, Smalley MJ, Pearson HB. The PI3K-AKT-mTOR pathway and prostate cancer: At the crossroads of AR, MAPK, and WNT signaling. *Int J Mol Sci* (2020) 21(12):4507. doi: 10.3390/ijms21124507

60. Lin SR, Mokgautsi N, Liu YN. Ras and wnt interaction contribute in prostate cancer bone metastasis. *Molecules (Basel Switzerland)* (2020) 25(10):2380. doi: 10.3390/molecules25102380

61. Dong C, Dang D, Zhao X, Wang Y, Wang Z, Zhang C. Integrative characterization of the role of IL27 in melanoma using bioinformatics analysis. *Front Immunol* (2021) 12:713001. doi: 10.3389/fimmu.2021.713001

62. Liu S, Liu F, Zhang B, Yan P, Rowan BG, Abdel-Mageed AB, et al. CD4(+) T helper 17 cell response of aged mice promotes prostate cancer cell migration and invasion. *Prostate* (2020) 80:764–76. doi: 10.1002/pros.23990

63. Xiao Y, Cong M, Li J, He D, Wu Q, Tian P, et al. Cathepsin c promotes breast cancer lung metastasis by modulating neutrophil infiltration and neutrophil extracellular trap formation. *Cancer Cell* (2021) 39:423–437.e427. doi: 10.1016/j.ccell.2020.12.012

64. Zou W, Wolchok JD, Chen L. PD-L1 (B7-H1) and PD-1 pathway blockade for cancer therapy: mechanisms, response biomarkers, and combinations. *Sci Trans Med* (2016) 8(328):328rv4. doi: 10.1126/scitranslmed.aad7118

65. Khalil DN, Smith EL, Brentjens RJ, Wolchok JD. The future of cancer treatment: immunomodulation, CARs and combination immunotherapy. *Nat Rev Clin Oncol* (2016) 13:273–90. doi: 10.1038/nrclinonc.2016.25

66. Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point. *Nature* (2017) 541:321–30. doi: 10.1038/nature21349

67. Hegde PS, Karanikas V, Evers S. The where, the when, and the how of immune monitoring for cancer immunotherapies in the era of checkpoint inhibition. *Clin Cancer research: an Off J Am Assoc Cancer Res* (2016) 22:1865–74. doi: 10.1158/1078-0432.Ccr-15-1507

68. Herbst RS, Soria JC, Kowanetz M, Fine GD, Hamid O, Gordon MS, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature* (2014) 515:563–7. doi: 10.1038/nature14011

69. Rescigno P, de Bono JS. Immunotherapy for lethal prostate cancer. *Nat Rev Urol* (2019) 16:69–70. doi: 10.1038/s41585-018-0121-y

70. Bilusic M, Madan RA, Gulley JL. Immunotherapy of prostate cancer: facts and hopes. *Clin Cancer research: an Off J Am Assoc Cancer Res* (2017) 23:6764–70. doi: 10.1158/1078-0432.Ccr-17-0019

71. Gamat M, McNeel DG. Androgen deprivation and immunotherapy for the treatment of prostate cancer. *Endocrine-related Cancer* (2017) 24:T297–t310. doi: 10.1530/erc-17-0145

72. Liu L, Bai X, Wang J, Tang XR, Wu DH, Du SS, et al. Combination of TMB and CNA stratifies prognostic and predictive responses to immunotherapy across metastatic cancer. *Clin Cancer research: an Off J Am Assoc Cancer Res* (2019) 25:7413–23. doi: 10.1158/1078-0432.Ccr-19-0558

73. Qin S, Gao H, Kim W, Zhang H, Gu Y, Kalari KR, et al. Biomarkers for predicting abiraterone treatment outcome and selecting alternative therapies in castration-resistant prostate cancer. *Clin Pharmacol Ther* (2022) 111:1296–306. doi: 10.1002/cpt.2582

74. Cattaneo-Pangrazzi RM, Schott H, Wunderli-Allenspach H, Rothen-Rutishauser B, Guenthert M, Schwendener RA. Cell-cycle arrest and p53-independent induction of apoptosis *in vitro* by the new anticancer drugs 5-FdUrd-P-FdCydOct and dCydPam-P-FdUrd in DU-145 human prostate cancer cells. *J Cancer Res Clin Oncol* (2000) 126:247–56. doi: 10.1007/s004320050339

75. Chen X, Liao Y, Long D, Yu T, Shen F, Lin X. The Cdc2/Cdk1 inhibitor, purvalanol a, enhances the cytotoxic effects of taxol through Op18/stathmin in non-small cell lung cancer cells. *vitro. Int J Mol Med* (2017) 40:235–42. doi: 10.3892/ijmm.2017.2989

76. Chen Y, Guo Y, Zhao W, Tina Ho WT, Fu X, Zhao ZJ. Identification of an orally available compound with potent and broad FLT3 inhibition activity. *Oncogene* (2016) 35:2971–8. doi: 10.1038/onc.2015.362

77. Nielsen D, Boas J, Engelholm SA, Hansen OP, Dombernowsky P. Teniposide in advanced breast cancer. a phase II trial in patients with no prior chemotherapy. *Ann oncology: Off J Eur Soc Med Oncol* (1992) 3:377–8. doi: 10.1093/oxfordjournals.annonc.a058210

78. Mu Y, Zou H, Chen B, Fan Y, Luo S. FAM83D knockdown regulates proliferation, migration and invasion of colorectal cancer through inhibiting FBXW7/Notch-1 signalling pathway. *Biomedicine pharmacotherapy = Biomedecine pharmacotherapie* (2017) 90:548–54. doi: 10.1016/j.biopha.2017.03.073

79. Hua YQ, Zhang K, Sheng J, Ning ZY, Li Y, Shi WD, et al. Fam83D promotes tumorigenesis and gemcitabine resistance of pancreatic adenocarcinoma through the wnt/β-catenin pathway. *Life Sci* (2021) 287:119205. doi: 10.1016/j.lfs.2021.119205

80. Uhler R, Popa-Wagner R, Kröning M, Brehm A, Rennert P, Seifried A, et al. Glyco-engineered HEK 293-f cell lines for the production of therapeutic glycoproteins with human n-glycosylation and improved pharmacokinetics. *Glycobiology* (2021) 31:859–72. doi: 10.1093/glycob/cwaa119

81. Baba H, Kanda M, Sato Y, Sawaki K, Shimizu D, Koike M, et al. Expression and malignant potential of B4GALNT4 in esophageal squamous cell carcinoma. *Ann Surg Oncol* (2020) 27:3247–56. doi: 10.1245/s10434-020-08431-8

10.3389/fonc.2023.1162653

Glossary

PCa	Prostate cancer
RCD	Regulated cell death
CRGs	cuproptosis-related genes
TCGA	The Cancer Genome Atlas
DEGs	Differentially expressed genes
TME	The tumor microenvironment
ssGSEA	Single-sample gene set enrichment analysis
PFS	progression-free survival
ADT	Androgen deprivation therapy
CRPC	Castration-resistant PCa
TCA	tricarboxylic acid
UCSC	University of California, Santa Cruz
DFKZ	The German Cancer Research Center, Deutsches Krebsforschungszentrum
MSKCC	The Memorial Sloan Kettering Cancer Center
CPGEA	Chinese Prostate Cancer Genome and Epigenome Atlas
GEO	Gene Expression Omnibus
TMB	Tumor mutation burden
SCNA	somatic copy number alterations
K-M	Kaplan-Meier
GSVA	Gene Set Variation Analysis
GSEA	Gene Set Enrichment Analysis
MHC	Major histocompatibility complex
TIDE	Tumor Immune Dysfunction and Exclusion
LASSO	Least absolute shrinkage and selection operator
CRRS	Cuproptosis-related risk score
AUC	Area under the curve
ROC	Receiver operating characteristic
GDSC	Genomics of Drug Sensitivity in Cancer
IC50	The Half Maximal Inhibitory concentration
cMap	Connectivity Map
ANOVA	Analysis of variance
AIC	Akaike information criterion.

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 03 May 2023
DOI 10.3389/fonc.2023.1130680

OPEN ACCESS

EDITED BY
Ran Xu,
Central South University, China

REVIEWED BY
Zheng Chen,
First Affiliated Hospital of Jinan University, China
Katharina Boehm,
University Hospital Carl Gustav Carus, Germany

*CORRESPONDENCE
Xiaoping Zhang
xzhang@hust.edu.cn
Hailong Ruan
hlruan2010@126.com

†These authors have contributed equally to this work

RECEIVED 23 December 2022
ACCEPTED 19 April 2023
PUBLISHED 03 May 2023

CITATION
Miao Q, Wei Z, Liu C, Ye Y, Cheng G, Song Z, Chen K, Zhang Y, Chen J, Yue C, Ruan H and Zhang X (2023) Overall survival and cancer-specific survival were improved in local treatment of metastatic prostate cancer.
Front. Oncol. 13:1130680.
doi: 10.3389/fonc.2023.1130680

Overall survival and cancer-specific survival were improved in local treatment of metastatic prostate cancer

Qi Miao[1,2†], Zhihao Wei[1,2†], Chenchen Liu[1,2†], Yuzhong Ye[1,2], Gong Cheng[1,2], Zhengshuai Song[3], Kailei Chen[1,2], Yunxuan Zhang[1,2], Jiawei Chen[1,2], Changjie Yue[1,2], Hailong Ruan[1,2*] and Xiaoping Zhang[1,2*]

[1]Department of Urology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [2]Institute of Urology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [3]Department of Urology, The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Background: For metastatic prostate cancer (mPCa), radical prostatectomy (RP) and radiation therapy (RT) may improve overall survival (OS) and cancer-specific survival (CSS). Compared with RT, RP shows significant advantages in improving patient outcomes. External beam radiation therapy (EBRT) even slightly elevates CSM with no statistical difference in OS compared with no local treatment (NLT).

Objective: To evaluate OS and CSS after local treatment (LT) (including RP and RT) versus NLT in mPCa.**Design, setting, and participants**Within the Surveillance, Epidemiology and End Results (SEER) database (2000-2018), 20098 patients with metastatic prostate cancer were selected in this study, of which 19433 patients had no local treatment, 377 patients with radical prostate treatment, and 288 patients with RT.

Outcome measurements and statistical analysis: Multivariable competing risks regression analysis after propensity score matching (PSM) was used to calculate CSM. Multivariable Cox regression analysis was used to identify the risk factors. Kaplan-Meier methods were used to calculate OS.

Results and limitations: A total of 20098 patients were included: NLT (n = 19433), RP (n=377) and RT (n=288). In a competing risk regression analysis after PSM (ratio 1:1), RP resulted in a significantly lower CSM (hazard ratio [HR] 0.36, 95% confidence interval [CI] 0.29-0.45) than NLT, while RT showed a slightly lower CSM (HR 0.77, 95% CI 0.63-0.95). In a competing risk regression analysis after PSM (ratio 1:1), RP led to a lower CSM (HR 0.56, 95% CI 0.41-0.76) versus RT. As for all-cause mortality (ACM), RP (HR 0.37, 95% CI 0.31-0.45) and RT (HR 0.66, 95% CI 0.56-0.79). also showed a downward trend. In terms of OS, RP and RT significantly improved the survival probability compared with NLT, with the effect of RP being more pronounced. Obviously, older age, Gleason scores ≥8, AJCC T3-T4 stage, AJCC N1, AJCC M1b-M1c were all associated with higher CSM ($P < 0.05$). The same results held true for ACM. The limitation of this article is that it

is not possible to assess the effect of differences in systemic therapy on CSM in mPCa patients and clinical trials are needed to verify the results.

Conclusions: For patients with mPCa, both RP and RT are beneficial to patients, and the efficacy of RP is better than RT from the perspective of CSM and ACM. Older age, higher gleason scores and the more advanced AJCC TNM stage all put patients at higher risk of dying.

Patient summary: A large population-based cancer database showed that in addition to first-line therapy (hormonal treatment), RP and radiotherapy can also benefit patients with mPCa.

KEYWORDS

metastatic prostate cancer, radical prostatectomy, external beam radiation therapy, radiotherapy, cancer-specific survival

1 Introduction

Prostate cancer is the second most commonly diagnosed cancer and the sixth leading cause of cancer death among men worldwide (1). In 2022, the number of estimated new cases of prostate cancer in the United State is 268490, and the number of estimated deaths is 34500 (2). Although prostate cancer is an indolent tumor, many patients progress to intermediate or high-risk localized, locally advanced, or metastatic cancer.

RP and RT are important options in the principle treatments of localized prostate cancer (3). For locally advanced disease and metastatic prostate cancer, continuous androgen deprivation therapy (ADT) is the first line of treatment (4–6). However, whether ADT in combination with local treatment (RP or radiotherapy) for primary tumor will benefit patients remains controversial.

Currently, RT is usually reserved for symptomatic lesions for mPCa. However, several studies have shown systemic benefits of RT in addition to local symptom control, such as reducing tumor oxygenation leading to tumor cell apoptosis and necrosis (7). RP has been shown to be feasible and safe for men with mPCa, although the survival benefit is less certain (8).

In two previous retrospective studies based on SEER database, both RP and RT improved CSM in patients with mPCa, but one of them lacked propensity score matching, and the other had a limited sample size of treatment group (RP: 313 patients, RT:161 patients) and ignored the effect of local treatment on non-cancer-specific mortality or all-cause mortality (9, 10). Another retrospective analytic cohort study also showed that prostate RT was associated with improved OS (11). These studies were limited to retrospective analysis and were plagued by sample size. Interestingly, two randomized-controlled-trials (RCTs) (STAMPEDE and HORRAD) showed that RT in patients with metastatic prostate cancer did not improve OS (7, 12). In addition, multiple studies have suggested an OS benefit and lower CSM for RP in mPCa (13, 14).

In this study, we enrolled latest patients with mPCa and compared the association of different local treatments with CSM, and OS in competing risk regression analysis and multivariable cox regression analysis after propensity score matching. Additionally, we have innovatively identified the effect of EBRT on CSM and OS in metastatic prostate cancer.

2 Methods

2.1 Patient selection

20098 patients with M1a-M1c (sixth edition of American Joint Committee on Cancer [AJCC] Cancer Staging Manual) metastatic prostate cancer who excluded autopsy and death certificate from reported sources were identified from the SEER (18 registers, 2000-2018) database (15). Complete follow-up data and positive histology were ensured for each patient. Of these patients, 19433 did not receive local therapy and 665 received local treatment, including 377 who underwent RP (surgery site code 50, 70 and 80) and 288 who received RT (including brachytherapy, radioisotopes and combination of beam with implants or isotopes). Age, race, Gleason score, prostate-specific antigen (PSA) values and AJCC TNM staging of each patient were included in the analysis.

Patients were stratified based on RP or RT or NLT. Patients treated with beam radiation were excluded based on the lack of beam radiation organ site-specific records within SEER. Patients treated with endoscopic therapy were also excluded. Incidentally, when patients receiving beam radiation were included in the analysis, we found that radiotherapy (for *in situ* or metastases)

Abbreviations: ACM, all-cause mortality; CI, confidence interval; CSS, cancer-specific survival; CSM, cancer-specific mortality; EBRT, external beam radiation therapy; HR, hazard ratio; LT, local treatment; mPCa, metastatic prostate cancer; NLT, no local treatment; OS, overall survival; PSA, prostate specific antigen; RP, radical prostatectomy; RT, radiotherapy.

(HR 1.06, 95%CI 1.00-1.11) slightly increased the CSM of patients with mPCa.

2.2 Propensity score matching

The sample matching between the NLT and LT was achieved *via* the "MatchIt" packages in R software (ratio 1:1), which based on the nearest-neighbor matching (16). Characteristics used to calculate propensity scores included age, race, year of diagnosis, Gleason score, PSA value, AJCC.T, AJCC.N, and AJCC.M stage, which have been found to be independent risk factors in previous studies (17). Following matching, the treatment effect was evaluated in subsequent analyses between NLT (n= 665) and LT (n= 665) who were successfully matched. Similarly, PSM (ratio 1:1) also identified suitable samples of RP (n= 288) and RT (n= 288) for analysis.

2.3 Establishment of nomogram

The multivariable Cox regression analysis was performed to screen the indicators affecting OS and predict their weights. By using the "rms" R packages, a nomogram with the independent indicators such as age, race, Gleason score, PSA value, AJCC.T, AJCC.N, AJCC.M stage, RP and RT was established for predicting OS in mPCa (18).

2.4 Survival analysis

The differences in OS between the NLT, RP and RT were represented by the Kaplan-Meier curve using the "survival" R package (19).

2.5 Statistical analysis

Competing risk regression analysis was used to calculate the cumulative incidence of CSM and independent factors associated with CSM were identified by using stepwise multivariable competing risk regression analysis (20). Pearson chi-square analysis was used to determine variables differing among NLT and LT. Statistical significance was defined as $p < 0.05$. All statistical analyses were conducted using R 4.1.2 (https://www.r-project.org/).

3 Results

3.1 mPCa benefits from LT

The median age of 19433 patients without local treatment was 71 years, compared with RP (63 years) and RT (67 years) (Table 1). According to Gleason scores, the highest proportion of NLT was Gleason score = 7 (34.6%), corresponding to LT of 29.3%, but the rate for Gleason score ≥ 8 of NLT (6%) was lower than LT (14.3%). The proportion of PSA values > 20 (40.6%) was significantly higher than those with a PSA value ≤ 20 (10.4%) in patients with NLT, whereas the opposite composition was observed in patients with LT (PSA > 20: 18.0%, PSA ≤ 20:30.2%). The rate for AJCC.T stage T3-T4 (55.5%) was higher than T1-T2 (21.6%) in NLT patients, and the same was true for LT patients (T1-T2: 9.8%, T3-T4: 50.8%). As can be seen in the AJCC.N staging, the ratio of N0 was much higher than that of N1 in both NLT (N0: 51.9%, N1: 24.0%) and LT (N0: 62.6%, N1: 25.1%) patients. Finally, in AJCC.M staging, NLT patients were composed as follows, M1a: 6%, M1b: 68.6%, M1c: 21.3%, while LT patients were composed as follows, M1a: 11.1%, M1b: 65.6%, M1c: 20.0%. Specific to the classification under LT patients, there was a roughly consistent trend in the composition of Gleason score, AJCC.T stage, AJCC.N stage and AJCC.M stage between RP and RT. The difference was PSA value, with RP patients having the higher proportion of PSA ≤ 20 (41.1%) and RT patients having the higher proportion of PSA > 20 (23.3%). In a total of 20098 enrolled patients with mPCa, the number of cancer-specific deaths in NLT, RP and RT were 12489, 111, 146, respectively.

Following propensity score matching, there was no statistical differences in all clinical characteristics between the NLT and LT patients, but residual statistically significantly differences remained for age, PSA value, AJCC.T stage and AJCC.N stage between RP and RT patients. After propensity score matching, the number of cancer-specific deaths in the NLT, RP, RT groups were 397, 87 and 146, respectively, and the CSM rate of NLT, RP, RT were 59.7%, 30.2% and 50.7%, respectively (Table 1).

In a multivariable competing risk regression analysis after PSM, both RP (HR 0.36, 95%CI 0.29-0.45, P<0.001) and RT (HR 0.77, 95%CI 0.63-0.95, P<0.05) had lower CSM rate compared to NLT (Table 2). In addition to no local treatments that could increase CSM, the white race (vs Asian or Pacific Islander: HR 0.689, 95%CI 0.49-0.97), Gleason score ≥ 8 (vs Gleason ≤ 6, HR 2.06, 95%CI 1.12-3.80, P< 0.05), PSA value >20 (vs PSA ≤ 20, HR 1.38 95%CI 1.06-1.80, P< 0.05), T3-T4 (vs T1-T2, HR 1.64, 95%CI 1.37-1.97, P< 0.001), N1 (vs N0, HR 1.43, 95%CI 1.16-1.76, P< 0.001) and M1b (vs M1a, HR 1.90, 95%CI 1.39-2.58, P< 0.001) - M1c (vs M1a, HR 2.46, 95%CI 1.76-3.43, P< 0.001) were associated with higher CSM. Besides CSM, OS was also a clinically non-negligible prognostic indicator. A multivariable Cox regression analysis after PSM drew conclusions close to competing risk regression analysis, older age (HR 1.02, 95%CI 1.02-1.03, P< 0.001), Gleason ≥ 8 (HR 2.30, 95%CI 1.36-3.89, P< 0.05), T3-T4 (HR 1.52, 95%CI 1.29-1.78, P<0.001), N1 (HR 1.39, 95%CI 1.16-1.67, P< 0.001), M1b (HR 1.65, 95%CI 1.27-2.14, P< 0.001) and M1c (HR 2.21, 95%CI 1.66-2.94, P<0.001) impaired OS, while Asian or Pacific Islander (HR 0.59,95%CI 0.43-0.82, P<0.05), PSA ≤ 20 (HR 0.62, 95%CI 0.49-0.79, P<0.001), RP (HR 0.37, 95%CI 0.31-0.45, P<0.001) and RT (HR 0.66, 95%CI 0.56-0.79, P<0.001) improved OS of patients with mPCa (Figure 1A). We then constructed a nomogram to predict 1-year, 3-year, and 5-year OS using independent prognostic indictors (Figure 1B).

Interestingly, in the initial analysis, we found that CSM of radiotherapy (including beam radiation: for *in situ* or metastases) (HR 1.06, 95%CI 1.00-1.11, P< 0.05) was slightly elevated compared to non-treatment, possibly suggesting that beam radiation for

10.3389/fonc.2023.1130680

TABLE 1 Characteristics of patients with and without propensity score matching.

Variables	No local treatment (n = 19433;%)	Local treatment (n = 665;%)	P value [a]	Propensity score-adjusted no local treatment (n = 665;%)	Propensity score-adjusted local treatment (n = 665;%)	P value [b]
Median age, yr (IQR)	71.00 (62.00, 79.00)	64.00 (58.00, 70.00)	<0.001	63.00 (57.00, 70.00)	64.00 (58.00, 70.00)	0.346
Race, n (%)			0.349			0.923
White	13943 (71.7)	497 (74.7)		486 (73.1)	497 (74.7)	
African American	4200 (21.6)	128 (19.2)		137 (20.6)	128 (19.2)	
Other	1225 (6.3)	37 (5.6)		39 (5.9)	37 (5.6)	
Unknown	65 (0.3)	3 (0.5)		3 (0.5)	3 (0.5)	
Year of diagnosis, n (%)			0.601			0.784
2004	1265 (6.5)	41 (6.2)		51 (7.7)	41 (6.2)	
2005	1308 (6.7)	46 (6.9)		56 (8.4)	46 (6.9)	
2006	1354 (7.0)	56 (8.4)		58 (8.7)	56 (8.4)	
2007	1394 (7.2)	46 (6.9)		49 (7.4)	46 (6.9)	
2008	1454 (7.5)	55 (8.3)		55 (8.3)	55 (8.3)	
2009	1485 (7.6)	51 (7.7)		52 (7.8)	51 (7.7)	
2010	1606 (8.3)	53 (8.0)		61 (9.2)	53 (8.0)	
2011	1619 (8.3)	63 (9.5)		57 (8.6)	63 (9.5)	
2012	1734 (8.9)	48 (7.2)		31 (4.7)	48 (7.2)	
2013	1905 (9.8)	55 (8.3)		49 (7.4)	55 (8.3)	
2014	2022 (10.4)	79 (11.9)		75 (11.3)	79 (11.9)	
2015	2287 (11.8)	72 (10.8)		71 (10.7)	72 (10.8)	
Gleason score, n (%)			<0.001			0.291
≤6	196 (1.0)	28 (4.2)		33 (5.0)	28 (4.2)	
7	6732 (34.6)	195 (29.3)		178 (26.8)	195 (29.3)	
≥8	1171 (6.0)	95 (14.3)		79 (11.9)	95 (14.3)	
Unknown	11334 (58.3)	347 (52.2)		375 (56.4)	347 (52.2)	
PSA, ng/ml, n (%)			<0.001			0.68
>20	7895 (40.6)	120 (18.0)		118 (17.7)	120 (18.0)	
≤20	2013 (10.4)	201 (30.2)		188 (28.3)	201 (30.2)	
Unknown	9525 (49.0)	344 (51.7)		359 (54.0)	344 (51.7)	
AJCC T stage, n (%)			<0.001			0.71
T1-T2	4199 (21.6)	60 (9.0)		65 (9.8)	60 (9.0)	
T3-T4	10792 (55.5)	329 (49.5)		338 (50.8)	329 (49.5)	
TX	4442 (22.9)	276 (41.5)		262 (39.4)	276 (41.5)	
AJCC N stage, n (%)			<0.001			0.372

(Continued)

TABLE 1 Continued

Variables	No local treatment (n = 19433;%)	Local treatment (n = 665;%)	P value [a]	Propensity score-adjusted no local treatment (n = 665;%)	Propensity score-adjusted local treatment (n = 665;%)	P value [b]
N0	10086 (51.9)	416 (62.6)		440 (66.2)	416 (62.6)	
N1	4672 (24.0)	167 (25.1)		148 (22.3)	167 (25.1)	
NX	4675 (24.1)	82 (12.3)		77 (11.6)	82 (12.3)	
AJCC M stage, n (%)			<0.001			0.991
M1a	1165 (6.0)	73 (11.0)		74 (11.1)	73 (11.0)	
M1b	13379 (68.8)	436 (65.6)		438 (65.9)	436 (65.6)	
M1c	4143 (21.3)	133 (20.0)		132 (19.8)	133 (20.0)	
M1NOS	746 (3.8)	23 (3.5)		21 (3.2)	23 (3.5)	
Cancer-specific death, n (%)	12489 (NA)	257 (NA)	NA	397 (NA)	257 (NA)	NA

IQR, interquartile range; AJCC, American Joint Committee on Cancer; NLT, no local treatment; LT, local treatment; NA, not applicable; PSA, prostate specific antigen.
[a]Comparing NLT versus LT (unmatched).
[b]Comparing NLT versus LT (propensity score-adjusted cohorts).
proportions presented are of the corresponding subgroups.

TABLE 2 Multivariable competing risks regression analysis after PSM of patients with metastatic prostate cancer, stratified according to treatment type (NLT vs LT).

Variables	Local treatment versus no local treatment	
	HR (95% CI)	p value
Type of treatment		
No local therapy	**Ref.**	
Radiotherapy	**0.77 (0.63-0.95)**	**0.01**
Radical prostatectomy	**0.36 (0.29-0.45)**	**<0.001**
Age (yr)	1.00 (1.00-1.02)	0.12
Race		
White	**Ref.**	
African American	0.88 (0.71-1.07)	0.20
Other	**0.69 (0.49-0.97)**	**0.03**
Year of diagnosis		
2004	**Ref.**	
2005	0.87 (0.59-1.28)	0.47
2006	0.93 (0.62-1.38)	0.72
2007	1.10 (0.73-1.66)	0.65
2008	1.14 (0.78-1.67)	0.51
2009	1.28 (0.87-1.86)	0.21
2010	0.83 (0.47-1.45)	0.51
2011	1.00 (0.58-1.73)	0.99
2012	1.31 (0.69-2.47)	0.41
2013	0.75 (0.41-1.39)	0.36
2014	0.61 (0.34-1.11)	0.11
2015	0.69 (0.38-1.25)	0.22
Gleason score		
≤6	**Ref.**	
7	1.42 (0.76-2.68)	0.27
≥8	**2.06 (1.12-3.80)**	**0.02**
PSA		
≤20	**Ref.**	
>20	**1.38 (1.06-1.80)**	**0.01**
AJCC.T		
T1-T2	**Ref.**	
T3-T4	**1.64 (1.37-1.97)**	**<0.001**
AJCC.N		
N0	**Ref.**	
N1	**1.43 (1.16-1.76)**	**<0.001**

(Continued)

10.3389/fonc.2023.1130680

TABLE 2 Continued

Variables	Local treatment versus no local treatment	
	HR (95% CI)	p value
AJCC.M		
M1a	**Ref.**	
M1b	**1.90 (1.39-2.58)**	**<0.001**
M1c	**2.46 (1.76-3.43)**	**<0.001**
M1NOS	**2.85 (1.66-4.90)**	**<0.001**

CI, confidence interval; HR, hazard ratio; Ref., reference;
AJCC, American Joint Committee on Cancer.
The meaning of the bold values is p<0.05.

metastatic prostate cancer, especially for metastases, may not be beneficial in improving CSM but rather increase the risk of cancer-specific death (Tables S1, S2). However, no statistically significant difference was found between radiotherapy (including beam radiation: for *in situ* or metastases) (HR 0.97, 95%CI 0.93-1.02, P= 0.24) and non-treatment in multivariate Cox regression analysis after PSM, while RP (HR 0.38, 95%CI 0.33-0.45, P<0.001) still substantially improved OS (Figure S1).

3.2 RP is superior to RT in mPCa

After confirming that local treatment was helpful in improving CSM and OS in patients, we further investigated the differences between RP and RT. Obviously, a competing risk regression (RP vs RT) after PSM showed that RP (HR 0.56, 95%CI 0.41-0.76, P< 0.001) highlighted therapeutic advantages over RT (Tables 3, 4). There was no difference between races in improving CSM when RP versus RT. Additionally, CSM was also higher in presence of PSA> 20 (vs PSA≤ 20: HR 2.07, 95%CI 1.27-3.38, P< 0.05), T3-T4 (vs T1-T2: HR 1.78, 95%CI 1.25-2.54, P< 0.05), N1 (vs N0: HR 1.58, 95%CI 1.11-2.25, P< 0.05) and M1b (vs M1a: HR 2.22, 95%CI 1.25-3.93, P< 0.05)-M1c (vs M1a: HR 3.11, 95%CI 1.72-5.63, P<0.001). A multivariate Cox regression after PSM (RP vs RT) was performed to determine independent factors affecting patients OS (Figure 2A). The results showed that prognosis was worse in patients with older age (HR 1.04, 95%CI 1.02-1.05, P< 0.001), T3-T4 (HR 1.75, 95%CI 1.31-2.33, P< 0.001) and M1b (HR 1.69, 95%CI 1.06-2.68, P< 0.05)-M1c (HR 2.11, 95%CI 1.30-3.41, P<0.05). Similarly, a nomogram was plotted to predict 1-year, 3-year, and 5-year OS with mPCa who underwent RP or RT (Figure 2B).

The median follow-up of 1330 patients enrolled after PSM was 42 months (IQR, 20.00-76.75). A cumulative incidence curve of CSM and competing mortality was presented in Figure 3A. Compared with NLT, RP could reduce both of CSM and competing mortality of patients, and the efficacy of reducing CSM was particularly significant, while RT could slightly reduce CSM of patients, but had no advantage in improving mortality from other causes. Figure 3B revealed the differences in OS between NLT, RP and RT. Consistent with above conclusion, both RP and RT could improve the OS of patients with mPCa, with RP being better.

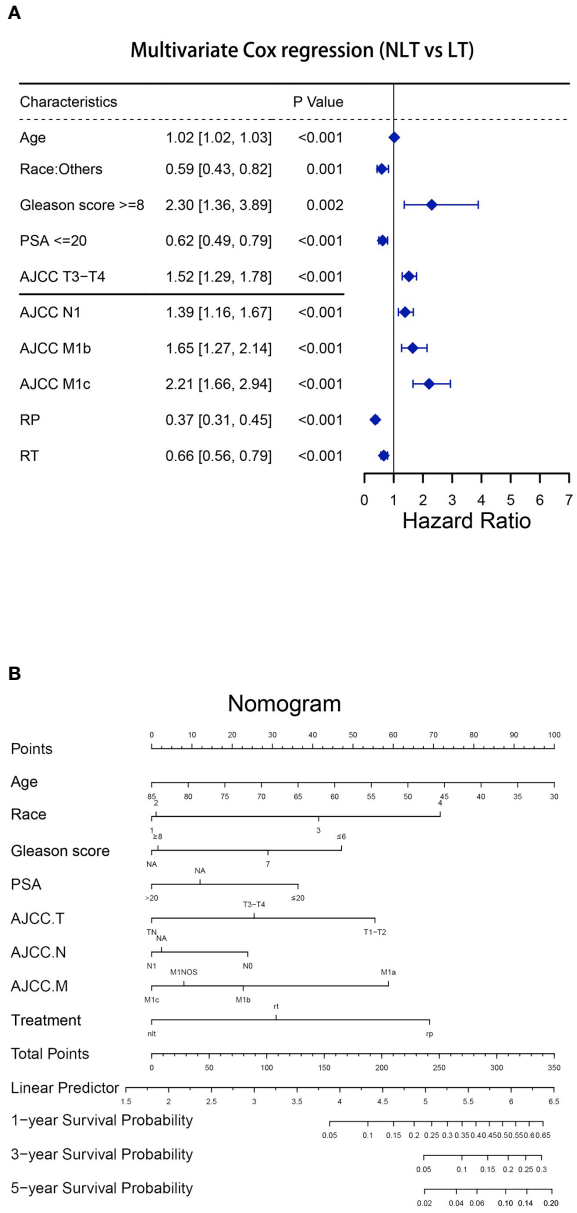


FIGURE 1
Establishment of the nomogram to predict the survival for patients with mPCa (NLT vs LT). **(A)**Multivariate Cox regression analysis of patients with mPCa for independent risk factors (NLT vs LT). **(B)** Establishment of the nomogram predicting survival of patients with mPCa (NLT vs LT).

4 Discussion

For metastatic prostate cancer, continuous androgen deprivation therapy remains the most effective treatment. In recent years, increasing studies have focused on ADT combined with other therapies to further improve patient outcomes (5, 21). Several large RCTs have been conducted with ADT combined with chemotherapy agents and combined with the new hormonal treatments (such like abiraterone and enzalutamide), and the data showed that the combination therapy was truly effective in improving OS (4, 22, 23). However, the significance of local

10.3389/fonc.2023.1130680

TABLE 3 Characteristics of LT patients with and without propensity score matching.

Variables	Radical prostatectomy (n = 377;%)	Radiotherapy (n = 288;%)	P value [a]	Propensity score-adjusted radical prostatectomy (n = 288;%)	Propensity score-adjusted radiotherapy (n = 288;%)	P value [b]
Median age, yr (IQR)	63.00 (57.00, 68.00)	67.00 (60.00, 74.00)	<0.001	63.00 (58.00, 68.00)	67.00 (60.00, 74.00)	<0.001
Race, n (%)			0.071			0.52
White	296 (78.5)	201 (69.8)		215 (74.7)	201 (69.8)	
African American	61 (16.2)	67 (23.3)		56 (19.4)	67 (23.3)	
Other	18 (4.8)	19 (6.6)		15 (5.2)	19 (6.6)	
Unknown	2 (0.5)	1 (0.3)		2 (0.7)	1 (0.3)	
Year of diagnosis, n (%)			<0.001			0.428
2004	14 (3.7)	27 (9.4)		14 (4.9)	27 (9.4)	
2005	25 (6.6)	21 (7.3)		24 (8.3)	21 (7.3)	
2006	23 (6.1)	33 (11.5)		22 (7.6)	33 (11.5)	
2007	23 (6.1)	23 (8.0)		21 (7.3)	23 (8.0)	
2008	28 (7.4)	27 (9.4)		26 (9.0)	27 (9.4)	
2009	28 (7.4)	23 (8.0)		25 (8.7)	23 (8.0)	
2010	29 (7.7)	24 (8.3)		22 (7.6)	24 (8.3)	
2011	34 (9.0)	29 (10.1)		28 (9.7)	29 (10.1)	
2012	35 (9.3)	13 (4.5)		18 (6.2)	13 (4.5)	
2013	39 (10.3)	16 (5.6)		20 (6.9)	16 (5.6)	
2014	46 (12.2)	33 (11.5)		37 (12.8)	33 (11.5)	
2015	53 (14.1)	19 (6.6)		31 (10.8)	19 (6.6)	
Gleason score, n (%)			<0.001			0.156
≤6	17 (4.5)	11 (3.8)		15 (5.2)	11 (3.8)	
7	122 (32.4)	73 (25.3)		75 (26.0)	73 (25.3)	
≥8	68 (18.0)	27 (9.4)		42 (14.6)	27 (9.4)	
Unknown	170 (45.1)	177 (61.5)		156 (54.2)	177 (61.5)	
PSA, ng/ml, n (%)			<0.001			0.001
>20	53 (14.1)	67 (23.3)		51 (17.7)	67 (23.3)	
≤20	155 (41.1)	46 (16.0)		84 (29.2)	46 (16.0)	
Unknown	169 (44.8)	175 (60.8)		153 (53.1)	175 (60.8)	
AJCC T stage, n (%)			<0.001			<0.001
T1-T2	16 (4.2)	44 (15.3)		16 (5.6)	44 (15.3)	
T3-T4	138 (36.6)	191 (66.3)		138 (47.9)	191 (66.3)	
TX	223 (59.2)	53 (18.4)		134 (46.5)	53 (18.4)	
AJCC N stage, n (%)			<0.001			<0.001
N0	224 (59.4)	192 (66.7)		184 (63.9)	192 (66.7)	

(Continued)

TABLE 3 Continued

Variables	Radical prostatectomy (n = 377;%)	Radiotherapy (n = 288;%)	P value [a]	Propensity score-adjusted radical prostatectomy (n = 288;%)	Propensity score-adjusted radiotherapy (n = 288;%)	P value [b]
N1	127 (33.7)	40 (13.9)		79 (27.4)	40 (13.9)	
NX	26 (6.9)	56 (19.4)		25 (8.7)	56 (19.4)	
AJCC M stage, n (%)			0.019			0.101
M1a	50 (13.3)	23 (8.0)		40 (13.9)	23 (8.0)	
M1b	252 (66.8)	184 (63.9)		180 (62.5)	184 (63.9)	
M1c	66 (17.5)	67 (23.3)		59 (20.5)	67 (23.3)	
M1NOS	9 (2.4)	14 (4.9)		9 (3.1)	14 (4.9)	
Cancer-specific death, n (%)	111 (NA)	146 (NA)	NA	87 (28.2)	146 (66.5)	NA

IQR, interquartile range; AJCC, American Joint Committee on Cancer; NLT, no local treatment; LT, local treatment; NA, not applicable; PSA, prostate specific antigen.
[a]Comparing RP versus RT (unmatched).
[b]Comparing RP versus RT (propensity score-adjusted cohorts).
proportions presented are of the corresponding subgroups.

TABLE 4 Multivariable competing risks regression analysis after PSM of patients with metastatic prostate cancer, stratified according to treatment type (RP vs RT).

Variables	Radical prostatectomy versus radiotherapy	
	HR (95% CI)	p value
Type of treatment		
Radiotherapy	**Ref.**	
Radical prostatectomy	**0.56 (0.41-0.76)**	**<0.001**
Age (yr)	**1.03 (1.02-1.05)**	**<0.001**
Race		
White	**Ref.**	
African American	1.40 (0.99-1.87)	0.058
Other	0.59 (0.35-1.01)	0.054
Year of diagnosis		
2004	**Ref.**	
2005	0.84 (0.44-1.63)	0.61
2006	0.64 (0.31-1.30)	0.22
2007	1.01 (0.50-2.01)	0.98
2008	1.07 (0.57-2.01)	0.84
2009	1.16 (0.60-2.23)	0.67
2010	0.93 (0.36-2.39)	0.88
2011	1.63 (0.69-3.86)	0.27
2012	1.67 (0.65-4.34)	0.29
2013	0.92 (0.36-2.36)	0.86
2014	0.85 (0.35-2.09)	0.73
2015	1.15 (0.46-2.92)	0.76
Gleason score		
≤6	**Ref.**	
7	1.08 (0.32-3.65)	0.91
≥8	2.29 (0.75-6.96)	0.15
PSA		
≤20	**Ref.**	
>20	**2.07 (1.27-3.38)**	**0.0035**
AJCC.T		
T1-T2	**Ref.**	
T3-T4	**1.78 (1.25-2.54)**	**0.0014**
AJCC.N		
N0	**Ref.**	
N1	**1.58 (1.11-2.25)**	**0.011**
AJCC.M		
M1a	**Ref.**	
M1b	**2.22 (1.25-3.93)**	**0.006**
M1c	**3.11 (1.72-5.63)**	**<0.001**
M1NOS	**2.84 (1.26-6.42)**	**0.012**

(Continued)

TABLE 4 Continued

CI, confidence interval; HR, hazard ratio; Ref., reference; AJCC, American Joint Committee on Cancer.
The meaning of the bold values is p<0.05.

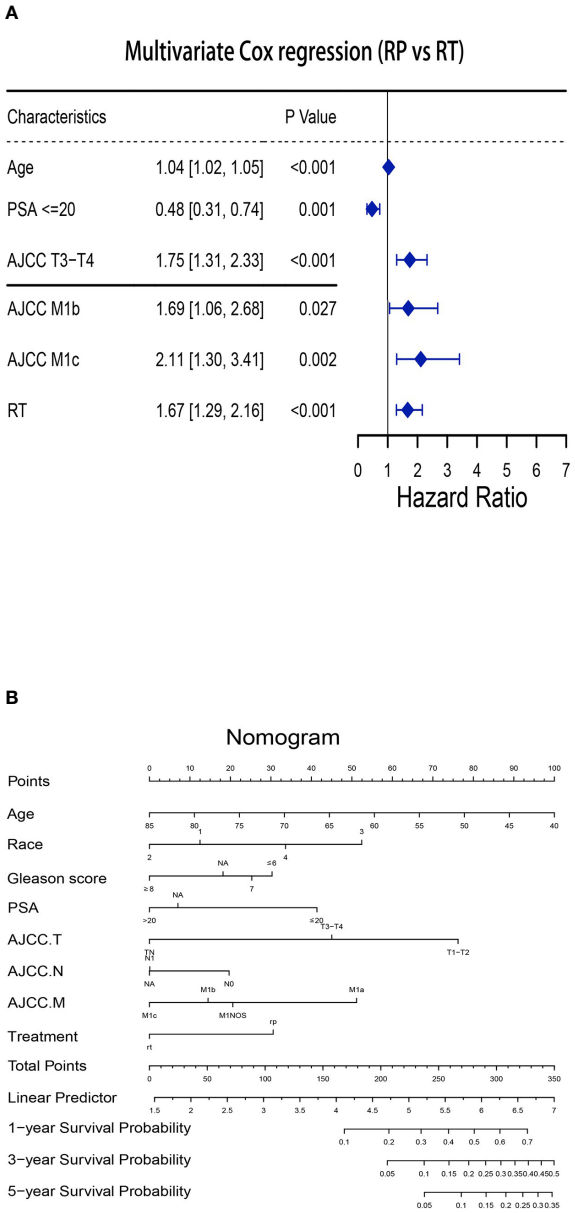


FIGURE 2
Establishment of the nomogram to predict the survival for patients with mPCa (RP vs RT). **(A)** Multivariate Cox regression analysis of patients with mPCa for independent risk factors (RP vs RT). **(B)** Establishment of the nomogram predicting survival of patients with mPCa (RP vs RT).

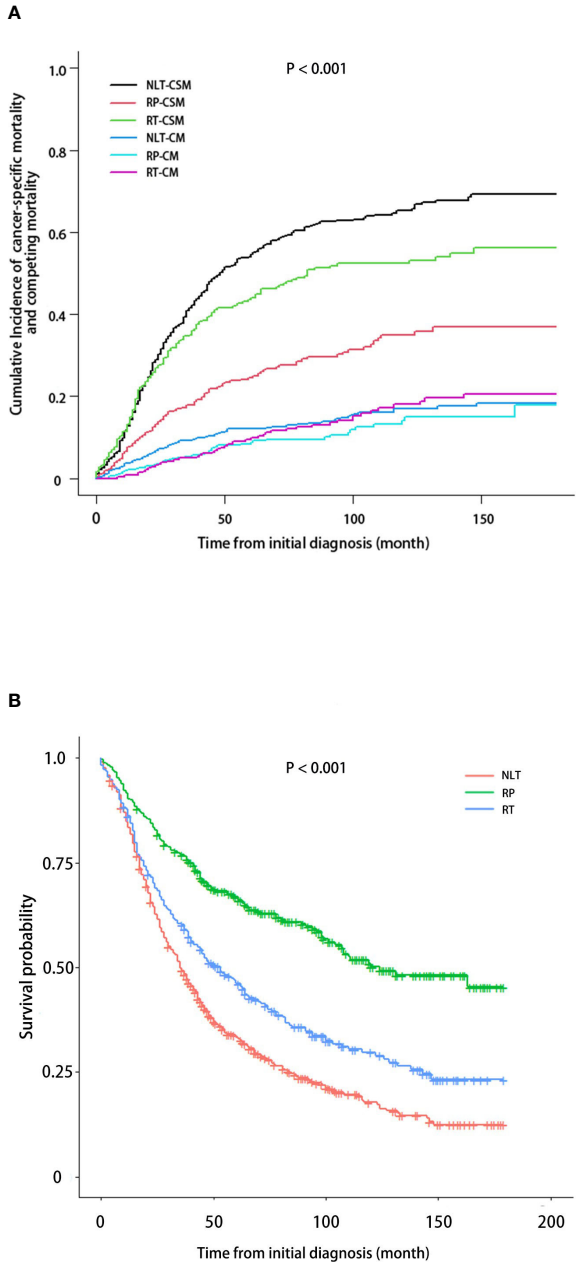


FIGURE 3
CSM and OS in patients with mPCa based on treatment received. **(A)** Cumulative incidence of CSM, accounting for the competing risk of non-PCa. **(B)** Kaplan-Meier analysis demonstrating OS.

treatment for metastatic prostate cancer remains unclear. Of note, in a randomized controlled phase 3 trial (STAMPEDE), radiotherapy improved failure-free survival in the standard of care group compared with standard of care plus radiotherapy (HR 0.76, 95% CI 0.68–0.84, P< 0·0001), but did not improve OS (HR 0.92, 95%CI 0.80–1.06; P= 0·266) (12). A similar conclusion to this study is the HORRAD trial, a multicenter RCT recruiting 432 patients with prostate-specific antigen (PSA) >20 ng/ml and primary bone mPCa (7). This trail showed no significant difference in OS (HR 0.90, 95% CI: 0.70–1.14, P = 0.4). These studies only addressed the effect of local treatment of RP on OS and ignored differences in CSM. However, a retrospective study of 13,692 patients with metastatic prostate cancer from 2004 to 2013 based on the SEER database showed that RP significantly reduced CSM (HR 0.48, 95% CI 0.35-0.66) compared with NLT (9). Similarly, another retrospective study of 8185 prostate cancer patients from 2004 to 2010 based on the SEER database showed that RP reduced CSM (HR 0.68, 95%CI 0.49-0.93), but the researcher did not perform propensity score matching on the patients prior to analysis (10). We could see that the above studies came to seemingly opposite conclusions, and it was clear that these studies only focused on the CSM or OS of patients without analyzing them individually, and both retrospective studies were limited to small sample size. Through these

studies we remained in doubt as to whether patients with mPCa should receive local radiation therapy. A trial of 1538 patients with metastatic prostate cancer between 1998 and 2010 based on Munich Cancer Registry (MCR) showed that RP was effective in improving OS, but this study lacked of analysis of CSM and only 5% of the patients in this study received RP (14). In addition, a retrospective cohort study included 1809 men with biopsy Gleason score 9-10 prostate cancer indicated that no significant differences in CSM and OS were found between men treated with ERBT or RP (24). In summary, these studies are insufficient for us to draw robust conclusion. Our work is the first to compare the effects of different local treatment on CSM and OS in patients with mPCa, while considering the impact of EBRT on patient outcomes.

Our study was conducted on the basis of eliminating the clinical characteristics that differed between NLT and LT, attributing the difference to then variable of treatment. Moreover, we were not limited to analyzing OS of patients, we also performed an analysis of CSM. After including the latest patients with metastatic prostate cancer, we noted that, consistent with previous retrospective studies, both RP and RT reduced CSM. Besides, both RP and RT improved OS. We found that clinical characteristics independently associated with increased CSM in patients who received local therapy included older age, higher PSA value, higher Gleason score, and more advanced AJCC.TNM staging (25, 26). Obviously, the existing research evidence suggested that these factors are directly related to the worse prognosis of patients, and in line with the fact that the patients with these features also benefit less from RP and RT, suggesting that we should aggressively perform local treatment earlier for low-risk prostate cancer patients to reap greater benefits. Also, with full consideration for the patients' basal physical condition, active administration of RP will obtain greater benefits compared with RT. However, it cannot be ignored that in specific cases, the choice of treatment should also consider the feasibility of surgery, the sequelae of surgery, the quality of life of patients and so on (27).

Interestingly, we got some noteworthy findings in the initial analysis. Despite the belief that EBRT could alleviate symptoms, patients receiving EBRT even had a slightly elevated CSM compared with NLT, and there was no significant difference in OS. In contrast to Amar U. Kishan et al., RP still benefited patients in the long term despite Gleason score ≥ 8 (4). This result raised a new question about whether EBRT was a wise choice for patients with mPCa. Clearly, for patients with mPCa, ADT combined with RP was superior to ADT combined with local brachytherapy, and EBRT was the least favorable option.

Our research also has many unavoidable limitations. First, our study is only a retrospective study. Second, the data we used were all from SEER database, variables unavailable from SEER undoubtedly limited our analysis. Third, it can be seen that many missing values appeared in the process of our analysis, especially the PSA value, which in turn is a credible independent risk factor (28). Fourth, the SEER database does not provide information on the specific extent of metastasis, which affects the prognosis and efficacy of the patient. Finally, the SEER database lacks EBRT coding for specific sites, which may lead to the inclusion of some patients in the wrong treatment stratification, thereby reducing the reliability of the findings.

5 Conclusion

Our study demonstrated that LT can improve CSM and OS in patents with mPCa compared with NLT, and RP has more advantages in reducing CSM and improving OS than RT. Younger age, lower PSA value, lower Gleason score and clinical stage are associated with a greater benefit for LT. And similarly, these patients are associated with a greater benefit for RP than for RT. In addition, we provide evidence that EBRT even slightly elevated CSM compared with NLT, while OS was not statistically different.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

Conceptualization, QM, CL and JC. Data curation, ZW. Formal analysis, ZW, QM. Funding acquisition, XZ and HR. Investigation, CL and CY. Methodology, CL and YY. Project administration, QM, ZW and ZS. Resources, YY and KC. Software, QM, ZW. Supervision, YZ. Validation, HR and XZ. Visualization, GC. Writing – original draft, QM. Writing – review & editing, XZ. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the National Natural Science Foundation of China (82002704, 81972630, 81902588, 81874090 & 82002706).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1130680/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Establishment of the nomogram to predict the survival for patients with mPCa (NLT vs RP/BR). **(A)** Multivariate Cox regression analysis of patients with mPCa for independent risk factors (NLT vs RP/BR). **(B)** Establishment of the nomogram predicting survival of patients with mPCa (NLT vs RP/BR).

References

1. Culp MB, Soerjomataram I, Efstathiou JA, Bray F, Jemal. A. Recent global patterns in prostate cancer incidence and mortality rates. *Eur Urol* (2020) 77(1):38–52. doi: 10.1016/j.eururo.2019.08.005

2. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin* (2022) 72(1):7–33. doi: 10.3322/caac.21708

3. Scardino P. Update: NCCN prostate cancer clinical practice guidelines. *J Natl Compr Canc Netw* (2005) 3 Suppl 1:S29–33.

4. Kyriakopoulos CE, Chen Y-H, Carducci MA, Liu G, Jarrard DF, Hahn. NM, et al. Chemohormonal therapy in metastatic hormone-sensitive prostate cancer: long-term survival analysis of the randomized phase III E3805 CHAARTED trial. *J Clin Oncol* (2018) 36(11):1080–7. doi: 10.1200/JCO.2017.75.3657

5. Cornford P, Bellmunt J, Bolla M, Briers E, De Santis M, Gross T, et al. EAU-ESTRO-SIOG guidelines on prostate cancer. part II: treatment of relapsing, metastatic, and castration-resistant prostate cancer. *Eur Urol* (2017) 71(4):630–42. doi: 10.1016/j.eururo.2016.08.002

6. Ryan CJ, Smith MR, Fizazi K, Saad F, Mulders PFA, Sternberg CN, et al. Abiraterone acetate plus prednisone versus placebo plus prednisone in chemotherapy-naive men with metastatic castration-resistant prostate cancer (COU-AA-302): final overall survival analysis of a randomized, double-blind, placebo-controlled phase 3 study. *Lancet Oncol* (2015) 16(2):152–60. doi: 10.1016/S1470-2045(14)71205-7

7. Boeve L, Hulshof MCCM, Vis A, Zwinderman AH, Twisk JWR, Witjes WPJ, et al. Effect on survival of androgen deprivation therapy alone compared to androgen deprivation therapy combined with concurrent radiation therapy to the prostate in patients with primary bone metastatic prostate cancer in a prospective randomised clinical trial: data from the HORRAD trial. *Eur Urol* (2019) 75(3):410–8. doi: 10.1016/j.eururo.2018.09.008

8. Teo MY, Rathkopf DE, Kantoff P. Treatment of advanced prostate cancer. *Annu Rev Med* (2019) 70:479–99. doi: 10.1146/annurev-med-051517-011947

9. Leyh-Bannurah SR, Gazdovich S, Budäus L, Zaffuto E, Briganti A, Abdollah F, et al. Local therapy improves survival in metastatic prostate cancer. *Eur Urol* (2017) 72 (1):118–24. doi: 10.1016/j.eururo.2017.03.020

10. Culp SH, Schellhammer PF, Williams MB. Might men diagnosed with metastatic prostate cancer benefit from definitive treatment of the primary tumor? *A SEER-based study Eur Urol* (2014) 65(6):1058–66. doi: 10.1016/j.eururo.2013.11.012

11. Morgan SC, Holmes OE, Craig J, Grimes S, Malone S. Long-term outcomes of prostate radiotherapy for newly-diagnosed metastatic prostate cancer. *Prostate Cancer Prostatic Dis* (2021) 24(4):1041–7. doi: 10.1038/s41391-021-00339-y

12. Parker CC, James ND, Brawley CD, Clarke NW, Hoyle AP, Ali A, et al. Radiotherapy to the primary tumour for newly diagnosed, metastatic prostate cancer (STAMPEDE): a randomised controlled phase 3 trial. *Lancet* (2018) 392(10162):2353–66. doi: 10.1016/S0140-6736(18)32486-3

13. Heidenreich A, Pfister D. Radical cytoreductive prostatectomy in men with prostate cancer and oligometastatic disease. *Curr Opin Urol* (2020) 30(1):90–7. doi: 10.1097/MOU.0000000000000691

14. Gratzke C, Engel J, Stief CG. Role of radical prostatectomy in metastatic prostate cancer: data from the Munich cancer registry. *Eur Urol* (2014) 66(3):602–3. doi: 10.1016/j.eururo.2014.04.009

15. Harlan LC, Hankey BF. The surveillance, epidemiology, and end-results program database as a resource for conducting descriptive epidemiologic and clinical studies. *J Clin Oncol* (2003) 21(12):2232–3. doi: 10.1200/JCO.2003.94.023

16. Elze MC, Gregson J, Baber U, Williamson E, Sartori S, Mehran R, et al. Comparison of propensity score methods and covariate adjustment: evaluation in 4 cardiovascular studies. *J Am Coll Cardiol* (2017) 69(3):345–57. doi: 10.1016/j.jacc.2016.10.060

17. Chang AJ, Autio KA, Roach M 3rd, Scher HI. High-risk prostate cancer-classification and therapy. *Nat Rev Clin Oncol* (2014) 11(6):308–23. doi: 10.1038/nrclinonc.2014.68

18. Balachandran VP, Gonen M, Smith JJ, DeMatteo RP. Nomograms in oncology: more than meets the eye. *Lancet Oncol* (2015) 16(4):e173–80. doi: 10.1016/S1470-2045(14)71116-7

19. Ranstam J, Cook JA. Kaplan-Meier Curve. *Br J Surg* (2017) 104(4):442. doi: 10.1002/bjs.10238

20. Gillam MH, Ryan P, Graves SE, Miller LN, de Steiger RN, Salter A, et al. Competing risks survival analysis applied to data from the Australian orthopaedic association national joint replacement registry. *Acta Orthop* (2010) 81(5):548–55. doi: 10.3109/17453674.2010.524594

21. Katipally RR, Pitroda SP, Juloori A, Chmura SJ, Weichselbaum RR. The oligometastatic spectrum in the era of improved detection and modern systemic therapy. *Nat Rev Clin Oncol* (2022) 19(9):585–99. doi: 10.1038/s41571-022-00655-9

22. James ND, Sydes MR, Clarke NW, Mason MD, Dearnaley DP, Spears MR, 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(10024):1163–77. doi: 10.1016/S0140-6736(15)01037-5

23. Gravis G, Fizazi K, Joly F, Oudard S, Priou F, Esterni B, et al. Androgen-deprivation therapy alone or with docetaxel in non-castrate metastatic prostate cancer (GETUG-AFU 15): a randomised, open-label, phase 3 trial. *Lancet Oncol* (2013) 14 (2):149–58. doi: 10.1016/S1470-2045(12)70560-0

24. Kishan AU, Prostatectomy R. External beam radiotherapy, or external beam radiotherapy with brachytherapy boost and disease progression and mortality in patients with Gleason score 9-10 prostate cancer. *JAMA* (2018) 319(9):896–905. doi: 10.1001/jama.2018.0587

25. Humphreys MR, Fernandes KA, Sridhar SS. Impact of age at diagnosis on outcomes in men with castrate-resistant prostate cancer (CRPC). *J Cancer* (2013) 4 (4):304–14. doi: 10.7150/jca.4192

26. Bertaglia V, Tucci M, Fiori C, Aroasio E, Poggio M, Buttigliero C, et al. Effects of serum testosterone levels after 6 months of androgen deprivation therapy on the outcome of patients with prostate cancer. *Clin Genitourin Cancer* (2013) 11(3):325–330.e1. doi: 10.1016/j.clgc.2013.01.002

27. Grypari IM, Zolota V, Tzelepi V. Radical or not-So-Radical prostatectomy: do surgical margins matter? *Cancers (Basel)* (2021) 14(1):13. doi: 10.3390/cancers14010013

28. Satkunasivam R, Kim AE, Desai M, Nguyen MM, Quinn DI, Ballas L, et al. Radical prostatectomy or external beam radiation therapy vs no local therapy for survival benefit in metastatic prostate cancer: a SEER-Medicare analysis. *J Urol* (2015) 194(2):378–85. doi: 10.1016/j.juro.2015.02.084

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 10 May 2023
DOI 10.3389/fonc.2023.1182727

OPEN ACCESS

EDITED BY
Sifeng Qu,
Shandong University, China

REVIEWED BY
Hongtao Xiao,
University of Electronic Science and Technology of China, China
Anqi Li,
The Ohio State University, United States

*CORRESPONDENCE
Haolong Li
Haolong.Li@ucsf.edu

RECEIVED 09 March 2023
ACCEPTED 24 April 2023
PUBLISHED 10 May 2023

CITATION
Shin HJ, Hua JT and Li H (2023) Recent advances in understanding DNA methylation of prostate cancer. *Front. Oncol.* 13:1182727. doi: 10.3389/fonc.2023.1182727

Recent advances in understanding DNA methylation of prostate cancer

Hyun Jin Shin[1,2], Junjie T. Hua[1,2] and Haolong Li[1,2]*

[1]Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, San Francisco, CA, United States, [2]Department of Radiation Oncology, University of California, San Francisco, San Francisco, CA, United States

Epigenetic modifications, such as DNA methylation, is widely studied in cancer. DNA methylation patterns have been shown to distinguish between benign and malignant tumors in various cancers, including prostate cancer. It may also contribute to oncogenesis, as it is frequently associated with downregulation of tumor suppressor genes. Aberrant patterns of DNA methylation, in particular the CpG island hypermethylator phenotype (CIMP), have shown associative evidence with distinct clinical features and outcomes, such as aggressive subtypes, higher Gleason score, prostate-specific antigen (PSA), and overall tumor stage, overall worse prognosis, as well as reduced survival. In prostate cancer, hypermethylation of specific genes is significantly different between tumor and normal tissues. Methylation patterns could distinguish between aggressive subtypes of prostate cancer, including neuroendocrine prostate cancer (NEPC) and castration resistant prostate adenocarcinoma. Further, DNA methylation is detectable in cell-free DNA (cfDNA) and is reflective of clinical outcome, making it a potential biomarker for prostate cancer. This review summarizes recent advances in understanding DNA methylation alterations in cancers with the focus on prostate cancer. We discuss the advanced methodology used for evaluating DNA methylation changes and the molecular regulators behind these changes. We also explore the clinical potential of DNA methylation as prostate cancer biomarkers and its potential for developing targeted treatment of CIMP subtype of prostate cancer.

KEYWORDS

epigenetic regulation, DNA methylation, 5mC, CIMP, mCRPC

1 Introduction

Prostate cancer is the most common cancer in men, making up 29% of new cases, and one of the top cancer-related causes of death in men in the United States (1). In prostate cancer, androgen receptor (AR) is a key oncogenic driver, is often found amplified in the gene body and enhancer upstream of AR and is associated with aggressive progression of disease. While androgen-deprivation therapy (ADT) is the first line treatment for patients with locally advanced or metastatic prostate cancer, the disease often progresses to the

castration resistant prostate cancer (CRPC) stage. Newly developed AR targeted therapies, such as enzalutamide and abiraterone, have shown promise as effective treatments for advanced prostate cancer. Yet, the resistant tumor invariably occurs, leading the disease to its terminal stage. To understand disease progression and treatment resistance, majority of studies have focused on genetic alterations of the key drivers such as *AR* gene, *AR* co-factors (e.g. *NCOA2*, *EP300*, and *FOXA1*), *ETS* gene fusions (e.g. *TMPRSS2-ERG* fusion), *SPOP* mutations, mutations affecting gene expression, and chromatin regulation (e.g. *KDM6A/UTX*, *MLL2*, *MLL3*, *CHD1*, and *EZH2*) (2, 3). Some of these genetic changes can be used as prognostic markers, such as mutations in *BRCA1*, *BRCA2*, *HOXB13*, *ATM*, and *CHEK2*, dysregulation of *PTEN*, *TMPRSS2-ERG* fusion, and high overall number of somatic copy number aberrations, which are associated with poor prognosis (4). In addition to *AR* signaling driven tumors, subtypes such as neuroendocrine prostate cancer (NEPC) are *AR*-independent, thus making ADT ineffective (5). NEPC is an aggressive histologic subtype of prostate cancer associated with poor prognosis. NEPC tumors share some common genetic aberrations as prostate adenocarcinoma, such as *TMPRSS2-ERG* fusion and loss of *RB1* and *TP53*, but often do not express *AR* and downstream *AR*-regulated targets such as PSA and prostate-specific membrane antigen (PSMA) (6). Beyond genomic and transcriptomic subtypes of prostate cancer, epigenetic alterations are also found to play a critical role in prostate cancer progression and treatment resistance.

DNA methylation is an epigenetic process that involves the addition of methyl groups to DNA. The most predominant type of DNA methylation, termed 5-methylcytosine (5mC), typically happens on cytosines of CpG dinucleotide sequences and are usually associated with DNA inactivation. DNA methylation is modulated by DNA methyltransferase (DNMT) and ten eleven translocation (TET) enzymes, which are writers and erasers of 5mC, respectively. More specifically, DNMT1 maintains DNA methylation and prefers hemimethylated DNA, and DNMT3A and DNMT3B are responsible for *de novo* DNA methylation. Meanwhile, the TET family enzymes, TET1, TET2, and TET3, convert 5mC back to unmethylated cytosine in a series of steps with functionally important intermediates. 5mC is first converted to 5-hydroxymethylcytosine (5hmC), then 5-formylcytosine, followed by 5-carboxylcytosine, and finally back to cytosine. A portion of total methylation modifications consists of 5hmC. Unlike 5mC, 5hmC is enriched at transcriptionally active regions and associated with expression of many genes. Further, IDH1 and IDH2, which are not directly involved in methylation, modulates methylation by affecting TET2 function. IDH1 and IDH2 produces α-ketoglutarate, an obligatory substrate for TET. Most CpG dinucleotides in the genome are highly methylated, with the exception of CpG islands, CpG shores (± 2 kbp around islands), and CpG shelves (± 2kbp around shores), which show variable methylation level (7). CpG islands are regions of DNA with high concentration of CpG dinucleotides and are typically associated with cis-regulatory regions such as promoters. DNA methylation at promoters leads to gene repression by blocking transcription factors from binding and/or by recruiting methyl-CpG binding domain proteins that subsequently recruit and synergize with chromatin remodelers and histone deacetylases to establish a silenced chromatin state for long-term transcriptional repression (8–10). Similarly, DNA methylation at enhancers is reported to repress enhancer activity (11). While DNA methylation is essential for mammalian development and aging, aberrant methylation patterns are significant contributors to oncogenesis (12). Specifically, global DNA hypomethylation and hypermethylation of specific CpG islands is frequently observed in cancer, leading to expression of normally silenced repetitive elements and repression of tumor suppressors and DNA repair genes, respectively (13–15). Interestingly, these CpG hypermethylation profiles are found to be highly tumor-type-specific and may serve as potential biomarkers (15). By clustering cancer samples based on methylation levels at specific loci, a subtype of tumors characterized by hypermethylation of CpG island methylation has been identified and termed the CpG island methylator phenotype (CIMP).

The presence of a CIMP subtype is widely accepted in several cancer types, such as colorectal and breast cancer. However, whether CIMP is a pan-cancer phenomenon is still unclear, as are the exact molecular mechanisms driving CIMP (16). Most early findings on CIMP have been solely based on selective loci that lack consistencies between studies and cancer types, which hindered pan-cancer interpretation. Recent improvement of sequencing technologies and development of novel sequencing approaches, particularly whole-genome bisulfite sequencing, plays a significant role in providing a pan-cancer CIMP definition (17). Furthermore, integrative analyses across different sequencing approaches have accelerated our understanding of potential molecular mechanisms behind CIMP. Clinically, CIMP subtype is often associated with differential tumor prognosis, aggressiveness, and survival across different cancer types, which highlights the potential for the development of methylation-based prognostic biomarkers. Furthermore, demethylating agents are showing promise as novel cancer treatments (16, 18, 19). However, whether CIMP-associated hypermethylation is causal in tumorigenesis and cancer progression has remained largely elusive until recently (20). Further, other epigenetic changes are believed to be signs of disease progression in prostate cancer. For instance, changes like chromatin accessibility, SWI/SNF, histone marks, and DNA methylation, are distinguishing features of NEPC (5). This review will highlight recent biological and clinical findings of CIMP and other changes in methylation patterns in prostate cancer and discuss its clinical potentials.

2 DNA methylation and cancer

Aberrant DNA methylation patterns, specifically CIMP, is well established in multiple cancers, including colorectal cancer, gastric cancer, glioma, breast cancer, and leukemia. CIMP has first been identified in colorectal cancer through the detection of colorectal cancer-specific methylation in selective CpG island regions, including *p16* and *THBS1* (18). This concept has subsequently been validated by various studies which examined additional regions (21–25). CIMP is now a well-established molecular subtype of colorectal cancer that is associated with specific genetic

and clinicopathological features and tumorigenic pathways. Notably, CIMP colorectal cancer is associated with *BRAF V600E* and *KRAS* mutations, *CDX2* loss, as well as low chromosomal aberrations and high microsatellite instability (MSI), which is reported to be driven by methylation of *hMLH1* gene (18, 21, 23–26). Colorectal cancer can be further categorized into three molecularly distinct subclasses based on CIMP status: CIMP-high (CIMP-H) tumors associated with MSI and *BRAF* mutations, CIMP-low tumors associated with *KRAS* mutations, and CIMP-negative tumors associated with high *p53* mutations (20, 23). However, beyond these associations, the causal relationship between CIMP and these key driver mutations in colorectal cancer are largely unclear. Recent studies have found that aberrant DNA methylation occurs at early stages of colorectal cancer development and may sensitize colorectal cells to *BRAF V600E*-driven tumorigenic transformations into colorectal cancer (25, 26).

CIMP has been identified in other types of cancer but lacks a clear definition. In gastric cancer, CIMP is identified based on hypermethylation of specific genes, most commonly *MINT1*, *MINT2*, *MINT12*, *MINT25*, *MINT31*, *hMLH1*, and *p16* (27–34). While many studies' CIMP markers include these genes, others do not; and more recent studies use whole-genome sequencing to identify CIMP. Meanwhile, CIMP is identified in breast cancer by hypermethylation of a few genes, including tumor suppressor genes *BRCA1*, *p16*, *APC* (35, 36). Moreover, in blood cancer, CIMP has been initially identified in acute myeloid leukemia and acute lymphoblastic leukemia based on hypermethylation of specific genes such as *CDH1*, *CDH13*, and *sFRP1* (37, 38). However, these sites, as well as few additional CpG sites, have only been validated in some but not all studies, suggesting extensive heterogeneity between studies (39–41). Overall, there has also been a lack of consensus on the definition of CIMP in leukemia in terms of which specific CpG sites were used as biomarkers to identify CIMP. More recently, several studies have applied genome-wide DNA methylation arrays along with a panel of 1,293 CpG sites to classify CIMP in leukemia to standardize the field (42, 43). Using this approach, CIMP has been defined by comparing methylation levels across a wider range of CpG sites rather than a methylation of specific genes, providing a clearer definition of CIMP. Overall, there is a lack of consensus on the definition of CIMP in various cancers in terms of specific sites used as CIMP markers, but new methods of detecting methylation allow identification of CIMP based on methylation of a greater number of CpG islands.

Despite the lack of a consistent definition of CIMP across cancer types in early studies, there is a consensus between multiple studies within each cancer type that CIMP is associated with distinct molecular features (Table 1). In gastric cancer, CIMP is associated with MSI, Epstein-Barr virus (EBV)-associated gastric cancer, and *H. pylori* infection (27, 28, 32–34, 44–47). Unlike

TABLE 1 Hypermethylated genes and associated molecular features of CIMP in various cancer types.

Cancer Type	Frequently hypermethylated genes	Associated molecular features
Colorectal cancer	• *P16* • *THBS1*	• *BRAF V600E* and *KRAS* mutations • *CDX1* loss • Low chromosomal aberrations • Microsatellite instability (MSI)
Gastric cancer	• *MINT1* • *MINT2* • *MINT12* • *MINT25* • *MINT31* • *hMLH1* • *p16*	• MSI • Epstein-Barr virus (EBV)-associated gastric cancer • *H. pylori* infection
Breast cancer	• *BRCA1* • *P16* • *APC*	• Presence of estrogen receptor and progesterone receptor status • Invasive lobular breast cancer • Copy number alterations
Acute myeloid leukemia and lymphoblastic leukemia	• *CDH1* • *CDH13* • *sFRP1*	• *TET2*, *IDH1*, and *IDH2* mutations • I-CIMP associated with *IDH1/2* mutations • A-CIMP enriched in *CEBPA* and *WT1* mutations
Glioma	• *TMS1/ASC*	• Methylation of *MGMT* • *IDH1* mutations • *Gene copy variations*
Prostate cancer	• *RARβ* • *GSTP1* • *CDH13* • *RASSF1A* • *APC* • *p16* • *DAPK* • *FHIT* • *MGMT* • *CDH1*	• Significantly higher methylation levels at recurrent hypomethylated regions in mCRPC • RNA expression of oncogenic driver genes such as *AR*, *MYC*, and ERG in mCRPC • Less likely to have *ETS* fusions or *TP53* biallelic inactivation in mCRPC • Mutations in *TET2*, *IDH1*, *BRAF*, and *DNMT3B* in mCRPC • Downregulation of tumor suppressor genes

colorectal cancer CIMP, gastric CIMP is not associated with *p53* and *KRAS* mutations (28, 32, 48, 49). Methylation patterns in breast cancer show association with molecular subtypes of breast cancer. Most notably, CIMP is associated with the presence of estrogen receptor (ER) and progesterone receptor (PR) status (36, 50, 51). CIMP is also associated with invasive lobular breast cancer, which displays higher frequency of hypermethylation than invasive ductal carcinoma (52, 53). Also, CIMP shows association with copy number alterations, which could help further classify breast cancer subtypes (53, 54). CIMP in leukemia is associated with distinct molecular features, including *TET2*, *IDH1* and *IDH2* mutations in acute myeloid leukemia (43, 55–58). Furthermore, in acute myeloid leukemia, CIMP can be further divided into two categories, I-CIMP associated with *IDH1/2* mutations and A-CIMP enriched in *CEBPA* and *WT1* mutations (59). These molecular features suggest an association between hypermethylation and leukemogenesis, but a causal relationship has yet to be established. Moreover, in glioma, hypermethylation of promoter associated CpG islands of genes such as *TMS1/ASC* is commonly reported (60–62). CIMP has then been identified in grade IV gliomas, or glioblastomas, and in lower grade gliomas and found to be associated with specific molecular and clinical features (63–65). Significantly, CIMP is associated with methylation of O^6-methylguanine DNA methyltransferase (*MGMT*) gene and *IDH1* mutations (63, 65–67). Also, a study has found IDH1 can induce DNA hypermethylation that mimics CIMP subtypes in lower grade gliomas, suggesting a causal relationship (65). In addition, CIMP status is also associated with gene copy variations (68, 69).

3 DNA methylation in prostate cancer

3.1 Methods for DNA methylation detection

Methylation-specific PCR (MS PCR) was most commonly used in early studies to analyze DNA methylation patterns in CpG island and determine CIMP status. For MS PCR, DNA is first purified and treated with sodium bisulfite, which converts cytosine cytosine to uracil but not 5-methylcytosine. Then, PCR is performed with two primer pairs for detectable methylated and unmethylated DNA. A different method to analyze DNA methylation patterns is using methyl-CpG binding domain (MBD). MBD preferentially binds methylated DNA and can be used to enrich methylated genomic DNA fragments and create libraries (70). This library can be analyzed using real-time PCR, tiling microarrays, and next-generation sequencing. DNA methylation alterations in prostate cancer samples can also be analyzed by sequencing sodium-bisulfite-converted genomic DNA (e.g. Illumina HumanMethylation27, MethylPlex-next-generation sequencing, MethylationEPIC BeadChip), which allows more quantitative accuracy and detection sensitivity, high efficiency, and a wide spectrum for analysis (71). Similar to MS PCR, for this method, DNA is treated with sodium bisulfite, then subsequent PCR and specific methylation primers are used to sequence and identify the methylated genomic regions (71). Another method to analyze DNA methylation patterns is using methylated DNA immunoprecipitation sequencing (MeDIP). In this method, genomic DNA is sonicated and immunoprecipitated using antibodies specific to 5mC, and resulting fragments are amplified, prepped, and sequenced. Similarly, hydroxymethylated DNA immunoprecipitation sequencing (hMeDIP-seq) can be used to analyze patterns of hydroxymethylation, using the same methods as MeDIP-seq, but using antibodies specific to 5hmC instead.

DNA methylation analysis methods each have their strengths and limitations. For example, array-based methods are useful for profiling DNA methylation changes across large regions of the genome, but they have limited coverage of CpG sites (72). Reduced representation bisulfite sequencing (RRBS) can provide high coverage of CpG sites, but its ability to read the entire genome is limited. Whole-genome bisulfite sequencing (WGBS) offers the highest resolution and can be performed on single nuclei, but the method would not distinguish 5mc and 5hmc (73). A combined 5mc and 5hmc detection method such as WGBS and oxidative WGBS (oxWGBS) could provide a more comprehensive view of DNA methylation changes, but this has not yet been extensively studied in the context of prostate cancer.

3.2 Patterns of CIMP in prostate cancer

Patterns in DNA methylation in prostate cancer are distinct from other cancer types mentioned above but share some common features. CIMP in prostate cancer is often determined by checking methylation status of several loci using MS PCR, then later confirmed using bilsulfite DNA sequencing (74). With newer methods of detecting DNA methylation at a more global scale, CIMP can be determined by cancer-specific differentially methylated regions rather than through checking DNA methylation of specific genes. As in leukemia, CIMP in prostate cancer can be identified as groups with higher methylation levels when comparing these differentially methylated regions. Genes commonly hypermethylated in prostate cancer include tumor suppressor genes involved in DNA damage repair, cell adhesion, apoptosis, cell cycle control, signal transduction, and hormonal responses, such as *RARβ*, *GSTP1*, *CDH13*, *RASSF1A*, *APC*, *p16*, *DAPK*, *FHIT*, *MGMT*, and *CDH1* (70, 74–78). Some hypermethylated genes in prostate cancer are also hypermethylated in other types of cancer, such as *p16* in colorectal cancer (18), gastric cancer (34), and leukemia; and *CDH13*, *APC*, and *CDH1* in leukemia (35–38). When compared to samples from benign prostatic hyperplasia (BPH) and nonmalignant tissues, samples from prostate cancer showed higher levels of methylation (74, 76). Further, methylation of tumor suppressor genes *GSTP1*, *APC*, and *MGMT* is strongly associated with their downregulation, suggesting an important role for DNA methylation in driving carcinogenesis and disease progression (70, 78). Because increase in methylation may be an age-related event, Kang et al. have examined methylation status of *APC*, *COX2*, *DAPK*, *CDH1*, *GSTP1*, *MGMT*, *p14*, *p16*, *RASSF1A*, *RUNX3*, and *THBS1* from non-neoplastic prostate samples of mostly older men. They have found that there is very low or no promoter methylation in these samples, suggesting

hypermethylation of specific loci in prostate cancer is likely not an age-related event, but rather a tumor-related one (78).

While initial studies on methylation in prostate cancer have been limited due to the focus on evaluating methylation levels through MS PCR of select loci in a small number of prostate cancer samples, newer methods of analyzing methylation levels have emerged and allowed to analyze methylation in prostate cancer in a broader spectrum of an increasing number of tumor samples. Using the MBD approach, Aryee et al. have generated a library of methylated genomic DNA fragments and hybridized the library to Affymetrix SNP 6.0 high-density oligonucleotide microarrays and found DNA methylation alterations are maintained across all metastases within the same individual, and that regions with high consistency of hypermethylation across metastases within individuals show enrichment for cancer-related genes (70). Variability in genome-wide methylation patterns in benign, low-grade, and high-grade prostate cancer have also been analyzed using MDB-isolated genome sequencing (MiGS). This has revealed variations in methylation patterns that can distinguish between benign, low-grade, and high-grade prostate cancer samples. Further, by integrating DNA methylation data with RNA-seq and survival data, they have shown hypermethylation regions are in gene promoters and at intergenic regions that are enriched for DNA-protein binding sites (79). In addition, they have shown that downregulation of genes where DNA methylation and expression are well correlated is associated with poor outcome (79).

Using the sodium-bisulfite sequencing method, it has been shown that methylation pattern alterations are more frequent in prostate cancer and in benign prostate tissues adjacent to tumor, compared to age-matched organ-donor prostates (80). In addition, overall promoter CpG island methylation is significantly increased in localized and metastatic cancer tissues, and differentially methylated regions are cancer-specific (81). Also, by profiling DNA methylation in plasma samples of patients with metastatic prostate cancer over 9 months, Silva et al. show that methylation patterns within an individual are consistent with clinical progression, including disease progression and therapeutic response (82). By integrating methylome analysis with whole genome sequencing (WGS) and transcriptome sequencing (mRNA-seq), Gerhauser et al. describe four molecular subgroups of prostate cancer of different aggressiveness. Subgroup 1 represents normal basal and luminal prostate epithelium. Subgroup 2 is associated with high immune cell content but low T-luminal cell content, high GS, and shorter time to biochemical recurrence. Subgroup 3 represents an intermediate-risk group, and Subgroup 4 is associated with a high fraction of normal-like luminal cells and a known gene signature associated with less-aggressive prostate cancer (83). Hypermethylator phenotype has also been identified in metastatic castration resistant prostate cancer (mCRPC) using whole-genome bisulfite sequencing paired with deep whole-genome and transcriptome sequencing (84). This subtype has significantly higher methylation levels at recurrent hypomethylated regions (HMRs) and overall fewer HMRs at CpG island, shores, shelves, and in CpG open seas (regions outside islands, shelves, and shores) (84). There is also increased hypermethylation at differentiation and cancer genes (70). More specifically, in mCRPC, methylation is associated with RNA expression of oncogenic driver genes such as *AR*, *MYC*, and *ERG* (84). Moreover, key AR-associated genes, such as KLK3, NKX3-1, and FOLH1 are correlated with DNA methylation independent of DNA changes (84). They also have found that this subtype of tumors is less likely to have *ETS* fusions or *TP53* biallelic inactivation, is not significantly associated with anatomic site of biopsy, and contains mutually exclusive mutations in *TET2*, *IDH1*, *BRAF*, and *DNMT3B* (84). In another study using enhanced reduced-representation bisulfite sequencing (eRRBS) on patient tumor samples, Beltran et al. show there is a strong epigenetic segregation between castration resistant neuroendocrine prostate cancer and castration resistant prostate adenocarcinoma. Notably, they have found hypermethylation and reduced expression of *SPDEF*, a tumor suppressor gene, in castration resistant neuroendocrine prostate cancer. This has been validated in the neuroendocrine prostate cancer cell line NCI-H660, as compared to prostate adenocarcinoma cell line LNCaP (85).

Furthermore, MeDIP sequencing of 51 tumor and 53 benign prostate samples has revealed there are more than 147,000 cancer-associated epigenetic alterations, there are significant global methylation pattern differences associated with *TMPRSS2-ERG* rearrangement status, and hypermethylation of miR-26a can be involved in *ERG* rearrangement-independent *EZH2* activation (86). Further, another study using the same technique on samples from plasma DNA of patients with localized and metastatic prostate cancer has found that there is global hypermethylation in metastatic samples and hypomethylation in the pericentromeric regions (87). It also has shown that there is hypermethylation of the promoter of *NR3C1*, a glucocorticoid receptor gene, that is associated with decreased immune signature (87).

Beyond the hypermethylator phenotype, there are other methylation changes that may hold significance in prostate cancer. For example, somatic mutations and putative regulatory regions are frequently located in regions that are differentially hypomethylated (84). Not only is methylation silencing of tumor suppressors a significant event in progression of cancer, cancer-associated hypomethylation in oncogenic genes leading to their overexpression in mCRPC is also important (84). Multiple expression associated HMRs (eHMR) have been identified near AR, including AR promoter, AR enhancer, and additional loci upstream and downstream of AR (84). Although AR promoter is hypomethylated in all tissues, other eHMR are only identified in mCRPC samples but not in benign or primary PCa samples (84). The number of these hypomethylated eHMR loci is positively associated with AR expression. Additionally, eHMR loci found in AR gene body is positively associated with AR expression, representing novel intergenic regulatory regions of AR that can potentially contribute to ADT-resistance (84). 5hmC levels have also been shown to be associated with various clinical features of prostate cancer using hMeDIP-seq. 5hmC marks activation of cancer drivers and downstream targets such as *AR*, *EZH2*, *CDK1*, *TBX3*, *HOXA13*, *FOXA1*, and *HOXB13* (88). There is also a progressive increase in 5hmC levels in genes in proliferative and oncogenic pathways during tumor progression, and 5hmC patterns can accurately track dedifferentiation and lineage plasticity to

neuroendocrine and gastrointestinal lineages (88). Further, 5hmC patterns in cell-free DNA are able to be detected and used to accurately estimate ct-fraction and find specific gene activation of driver genes *TOP2A* and *EZH2* that are not altered at the DNA level (88). Overall, in addition to patterns in 5mC methylation, hypomethylation and 5hmC patterns show potential to be used as a prognostic biomarker that can differentiate various subtypes of prostate cancer that genetic changes alone cannot.

4 Molecular drivers of CIMP

The development of CIMP in cancer has been attributed to various genomic and environmental factors, which differs depending on the cancer type. Most notably, protein-coding mutations in *BRAF* and *IDH1* have been shown to establish CIMP (20, 65). In the case of CIMP-high colorectal cancer, spontaneous aging-like promoter hypermethylation makes organoids more sensitive to transformation by *BRAF V600E* mutation, which leads to CIMP (20). *BRAF V600E* mutation may lead to CIMP in a pathway that involves MAFG, which binds promoters of *MLH1* and other CIMP-related genes and recruits corepressor complex, leading to hypermethylation and gene silencing (89). In CIMP-low colorectal cancer, KRAS upregulates zinc-finger DNA-binding protein, ZNF304, which binds promoters and recruits a corepressor complex with DNMT1, leading to DNA hypermethylation (90). Contrastingly, in glioma and leukemia, *IDH1* mutations that result in 2-hydroxyglutarate production disrupts TET2 function and establishes CIMP and global DNA hypermethylation (58, 65). TET2 loss of function mutation itself is also associated with similar epigenetic defects as *IDH1* mutants, and *TET2* knockouts are also frequently associated with hypermethylation (58, 91–93). In addition, mutations in *DNMT3A* and *DNMT3B* and knock outs are also frequently associated with hypomethylation, while overexpression of *DNMT3B* is associated with hypermethylation in gastric and breast cancer cell lines (91, 94–100). As mentioned previously, mCRPC tumors of the hypermethylator subtype contain mutually exclusive mutations in *TET2*, *IDH1*, *BRAF*, and *DNMT3B*, suggesting mutations in these proteins may contribute to hypermethylation (84). Further, Kobayashi et al. have shown there is increased expression of *DNMT3A2*, *DNMT3B*, and *EZH2* in tumors, and transient *DNMT3B1* and *DNMT3B2* overexpression in primary prostate cells results in increased methylation of some CpG sites that show increased methylation in tumors (101). Furthermore, in AML, TET2, IDH1, and DNMT3B do not seem to affect each other in terms of methylation pattern and regulation of downstream genes, but IDH1 and DNMT3A do (58, 102). More specifically, co-occurrence of *DNMT3A* and *IDH1* mutations show epigenetic patterns different from those of either *IDH1* or *DNMT3A* mutation, upregulation of RAS signaling and unique sensitivity to MEK inhibition and appear to be associated with either worse clinical outcome or show no difference in EFS or OS (103–105). In addition, DNMT3A and TET2 also seem to affect one another, showing different methylation patterns and phenotypes (106, 107). However, it is not clear if this is the case in prostate cancer, especially considering Zhao et al. have found mutations in *TET2*, *IDH1*, and *BRAF* were mutually exclusive in mCRPC samples. While mutations in *TET2*, *IDH1*, *DNMT3A*, and *DNMT3B* may play a role in establishing distinct methylation patterns found in prostate cancer, further study is required to establish a causal relationship and see how the various proteins involved in methylation interact with and affect one another.

Aside from gene mutations, several other factors such as EBV infection, aging and hypoxia also contribute to methylation changes. In multiple studies, EBV infection of epithelial cells *in vitro* directly induces global hypermethylation of the host genome, around the transcription start site, and results in gene silencing (108). It is partly driven by EBV latency protein, latent membrane protein 2A (LMP2A), which upregulates expression of *DNMT1* and downregulates TET1 and TET2, as well as LMP1, which upregulates *DNMT1*, *DNMT3A*, and *DNMT3B* (108). In addition, it has been previously demonstrated that aging is associated with increased CpG island hypermethylation in colon mucosa (18, 109). Methylation of CpG island on the ER gene becomes progressively more pronounced with age, even in the early stages of tumor formation (109). This methylation of CpG island is associated with transcription repression, and in some cases, less *ER* expression (109). Moreover, re-expression of ER gene showed growth inhibition of colon carcinoma cells (109). Together, these findings suggest reduced ER expression, which is associated with age-related hypermethylation of CpG island on the *ER* gene, may be an early event that predisposes to sporadic colorectal tumorigenesis. Furthermore, hypoxia has been shown to increase hypermethylation at gene promoters in murine breast tumors (110). Mechanistically, hypoxia inhibits oxygen-dependent catalytic activities of the TET family methylation erasers, leading to the accumulation of methylation (110, 111). Oxidative stress from inflammation can also induce CpG island hypermethylation in tumors (112, 113). Specifically, oxidative stress generates 7,8-dihydro-8-guanine, which recruits DNMT1 that interacts with MSH2-MSH6 protein and methylates DNA promoters (112–114). JAK2, which is also associated with CIMP, localizes to the nucleus, interacts with MSH2-MSH6 upon oxidative stress induction and helps drive oxidative stress-induced interaction of MSH2-MSH6 with DNMT1 and consequently, global methylation (115).

5 Clinical implications

5.1 Biomarker

CIMP has been found to independently associate with patient survival in several cancer types, including kidney renal clear cell carcinoma, hepatocellular carcinoma, leukemia, gastric cancer, breast cancer, and adrenocortical carcinoma (30, 32, 39, 42, 43, 53, 116–119). CIMP is associated with worse prognosis in colorectal cancer, breast cancer, renal cell carcinoma, and adrenocortical carcinoma (26, 30, 41–43, 118, 120–124). CIMP status is also associated with other cancer type-specific clinical characteristics. In colorectal cancer, CIMP-H status is associated with female gender, proximal tumor location, higher tumor grade, older age,

poor differentiation, and MSI (23, 26, 117). Moreover, colorectal cancer diagnosed within 5 years after colonoscopy is more likely to have CIMP and MSI than cancer diagnosed after 5 years (125). In breast cancer, CIMP is associated with high grade and increased metastatic risk (53, 118). Further, methylation in serum is also associated with breast cancer and recurrence risk of rural sporadic breast cancer, showing potential of CIMP to be detectable in serum of breast cancer patients and allow distinction between tumor and normal samples with at least 90% specificity and sensitivity (35, 36, 126). Classification of glioma based on combination of CIMP status and copy number alteration status is associated with survival (63, 68, 69). In addition, CIMP is associated with better overall survival, as well as low-grade glioma and improved outcome (63–65, 68, 69, 127, 128). Studies have also found that upon recurrence, there is a shift from CIMP high to CIMP low (26, 41).

However, clinical features of CIMP in gastric cancer and leukemia remain ambiguous. Some studies have shown that CIMP is associated with better overall survival and progression-free survival while others have concluded that CIMP is associated with higher stage, lymph node metastasis, and worse survival (27–34, 49, 120, 121, 129). This discrepancy is likely due to heterogeneity between studies in both CIMP markers and patient samples. Majority of studies showing better prognosis identifies CIMP by a set of genes that included *MINT1*, *MINT2*, *MINT12*, *MINT25*, and *MINT31*. In acute myeloid leukemia, some studies have found A-CIMP patients are associated with longer overall survival than CIMP-negative patients while I-CIMP patients are not (59, 130). There is also evidence of increase in methylation at relapse (38). Contrarily, recent studies using genome-wide approaches have found CIMP patients are associated with better overall and disease-free survival in both T and B cell acute lymphoblastic leukemia, with shorter response to treatments in T cell acute lymphoblastic leukemia (42, 43, 131, 132). Clinical features of CIMP acute lymphoblastic leukemia are ambiguous, possibly also due to the lack of consensus on the definition of CIMP. Early studies with CIMP defined by selective CpG sites found CIMP patients are associated with worse disease-free and overall survival (37, 39). Similarly, inconclusive results from gastric cancer perhaps are due to differences in what genes are used to identify CIMP, which further warrants meta-analysis of global methylation data in the future to identify CIMP. Regardless, specific differentially methylated regions can also be used to distinguish different subtypes (50, 53, 133). In gastric cancer, CIMP, as defined within each study, is associated with EBV, methylation increases with tumor progression, and CIMP status in combination with *TP53* hotspot mutation status forms subgroups with distinct overall and progression free survival (28, 29, 31, 34, 47, 134).

In prostate cancer, the hypermethylator phenotype is associated with clinical features of poor prognosis. Multiple genes, such as *GSTP1*, *APC*, *MDR1*, *MGMT*, and *RASSF1A*, show higher methylation frequency in prostate cancer samples compared to BPH and non-neoplastic prostate samples (74, 78). Additionally, high methylation of *RARβ*, *RASSF1A*, *GSTP1*, *CDH13*, *APC*, *RUNX3*, *MDR1*, and *cyclin D2* is associated with high Gleason score and high PSA (74–76, 78). Methylation score, determined by statistical analysis comparing methylation status of various genes of benign prostatic hyperplasia and prostate cancer, is also found to be associated with high pT and other advanced pathological features, and can also distinguish organ-confined cancers from locally advanced cancer (74). Through MS PCR, Yegnasubramanian et al. also show that hypermethylation patterns of *GSTP1*, *APC*, *RASSF1A*, *PTGS2*, and *MDR1* is able to be used to distinguish primary prostate cancer from benign prostate tissues, hypermethylation of CpG island at *EDNRB* is correlated with tumor grade and stage of primary prostate cancers, and hypermethylation of CpG island of *PTGS2* is associated with increased risk of recurrence (135). Beyond these specific loci, analysis using Illumina HumanMethylation27 platform has identified 87 CpG sites with increased DNA methylation in 83/87 tumor samples, making them the most predictive diagnostic methylation biomarkers that can predict either tumor state or benign adjacent state of prostate cancer (101). Also, by integrating clinical follow-up data, it has been shown that there are prognostic DNA methylation alterations that correlate with biochemical recurrence of tumor (101). Furthermore, hypermethylation changes are highly maintained across anatomically distinct metastases within an individual, highlighting the potential of methylation status to be used as a longitudinal biomarker for clinically advanced prostate cancer (70). Methylation patterns have also been shown to be able to distinguish between castration resistant adenocarcinoma and neuroendocrine prostate cancer, and Berchuck et al. has been able to build a model that predicts the presence of NEPC using MeDIP-seq with 100% sensitivity and 90% specificity (5, 136). Furthermore, methylation changes have also been detectable in cell-free DNA (cfDNA), showing potential of methylation patterns to be used to develop liquid biomarkers. Liquid biomarkers, including circulating tumor cells, tumor cell fragments, nucleic acids, and proteins, are more readily accessible through any bodily fluids such as urine and blood, making them more easily obtainable than biopsies of prostate cancer metastases. As such, ability to detect methylation patterns in cfDNA and use it to distinguish specific clinical features holds significant clinical implications. To do so, cfDNA are isolated from plasma samples of patients with localized and metastatic prostate cancer, isolated, then profiled using bisulfite sequencing, MeDIPseq, or 5hmC sequencing (5hmC-seq). cfDNA global methylation patterns within each individual are temporally stable throughout the disease course, can distinguish metastatic from localized samples with 0.989 prediction accuracy, and can be used to build a model that can predict presence of NEPC and discriminate NEPC from castration resistant prostate adenocarcinoma (82, 87, 136). Moreover, using methylation sensitive restriction enzyme-qPCR analyses in liquid biopsies from mCRPC patients responsive and non-responsive to different therapies, Dillinger et al. has found higher methylation of specific loci in non-responsive patients before and after abiraterone treatment and identified 23 individual marker genes for which methylation was a negative prognostic factor for disease recurrence (137). In addition, Wu et al. have shown by sequencing plasma DNA from mCRPC patients receiving abiraterone or enzalutamide pre and post chemotherapy, there is hypomethylation of segments of AR binding sequences that are associated with AR copy number gain and more aggressive clinical

course (138). And as previously mentioned, 5hmC patterns with prognostic value can also be detected in cfDNA of mCRPC patients (88). Overall, methylation alterations show potential, even as a liquid biopsy, to serve to work in conjunction with genetic alterations for clinical biomarker development.

Using bisulfite sequencing, MeDIP-seq, or hMeDIP-seq are useful tools for scientific research. It allows exploration of mechanisms and its potential as a clinical prognostic biomarker. However, to be used in a clinical setting, the cost for running these sequencing methods should be considered. Currently, there is effort to develop targeted panels of DNA methylation to help reduce cost. In addition, there are commercial efforts to use different methods beyond pulling down methylated or hydroxymethylated DNA using antibodies to analyze DNA methylation patterns (139).

5.2 Treatment options

As CIMP tumors are hypermethylated, several DNA methyltransferase inhibitors, such as decitabine and azacitidine, are being evaluated in pre-clinical and clinical settings (34, 128, 140–142). DNA methyltransferase inhibitors azacitidine and decitabine are FDA-approved and show potential as a new therapeutic anticancer treatment. Treatment with decitabine has shown to slow tumor growth, decrease cell proliferation, and induce tumor suppressors in breast cancer cell lines in *in vitro* and *in vivo* studies using mice induced with human breast cancer cell lines (141, 142). Similarly, decitabine administered with talazoparib decreases tumor growth and increases overall survival in ovarian and breast cancer models (140). Further, in gastric cancer, as EBV-induced hypermethylation targets and silences key tumor suppressor genes including *APC*, *RASSF1*, *BRCA1*, *THBS1*, and *CDKN2A*, DNA methyltransferase inhibitors may also serve as a new therapeutic treatment for patients with EBV-positive gastric cancer (34, 129). There are also some concerns with using demethylation through the use of DNA methyltransferase inhibitors as a new treatment method, as demethylation induces pro-metastatic genes and increases invasiveness of non-invasive breast cancer (141, 142). However, an *in vitro* study by Chik et al. shows that depletion of DNMT1 suppresses cell growth but does not induce invasiveness while depletion of DNMT3a does not change cell transformation and increases cell invasiveness, demonstrating that specific DNMT1 inhibitors, azacitidine and decitabine, may avoid adverse effects (142). Current clinical trials on DNA methyltransferase inhibitors include studies on side effects and best dose of decitabine with nivolumab in treating colorectal cancer, efficacy of treatment of azacitidine in recurrent *IDH1*-mutant gliomas and finding maximum tolerated dose of azacitidine with capecitabine and oxaliplatin in treating metastatic colorectal cancer. They measure maximum tolerated dose, overall response rate, adverse events, and progression free and overall survival after treatment with decitabine or azacitidine for a month to 1 year.

Azacitidine has been explored as a new therapeutic drug for prostate cancer treatment in combination with chemotherapy or anti-androgen therapy. Azacitidine shows antiproliferative effects in 22Rv1 and PC3 cell lines, and *in vivo*, 0.8 mg/kg intraperitoneal injection of azacitidine reduced tumor proliferation and induced apoptosis in PC3 and 22Rv1 xenografts (143). Additionally, azacitidine shows synergistic effects with docetaxel and cisplatin, sensitizing both PC3 and 22Rv1 xenografts to docetaxel and cisplatin treatments and causing tumor growth delay without complete regression (143). This combination treatment was superior to either treatment alone and tolerable in mice (143). Azacitidine has also been tried in a clinical setting, to determine if it can reverse docetaxel resistance in mCRPC patients with disease progression during or within 6 months after cessation of minimum 6 weeks of docetaxel-based therapy (144). In this phase I/II study, azaciditine and docetaxel were alternately escalated with administration of prednisone. They found there was >50% decline in PSA in 10 out of 19 patients, favorable progression free survival and overall survival, and the common treatment-related adverse event was neutropenia (144). Furthermore, there is a Phase II trial to study effect of azacitidine in modulating PSA in patients continuing treatment with luteinizing hormone-releasing hormone and antiandrogen. This study plans to detect biological activity of azacitidine as well, by measuring fetal hemoglobin and plasma DNA methylation (145).

6 Discussion

Methylation changes in prostate cancer, including hypermethylation of CpG islands, hypomethylation patterns, and 5hmC patterns are reported in cancer transformation and progression. The CIMP subtype in prostate cancer shows decreased expression of tumor suppressor genes and has been associated with distinct clinical features, including higher Gleason score, higher PSA, higher tumor grade, and overall poor outcome. Tumors of this hypermethylation subtype can potentially benefit from FDA-approved demethylating agents, azacitidine and decitabine. There are also distinct patterns of methylation that can help distinguish benign prostate tissue from malignant prostate tumors, as well as the NEPC subtype from castration resistant adenocarcinoma. In addition, it can potentially be used to distinguish mCRPC. Further, the 5hmC landscape of prostate cancer also shows potential to serve as a marker of epigenetic activation throughout disease progression that can also identify distinct oncogenic signaling pathways that define subgroups of advanced prostate cancer and disease states. Prognostic DNA methylation patterns can also be detected in cell-free DNA isolated from plasma of patients with prostate cancer. Since biopsies of prostate cancer metastases can be difficult to obtain in comparison to more readily accessible plasma, analysis of methylation patterns in cfDNA can add to current analyses of cfDNA in advanced cancers to serve as a better liquid biomarker. Studies into methylation patterns in prostate cancer have also been improved with novel methods, such as whole genome bisulfite sequencing and MeDIP sequencing. Inclusion of DNA

methylation data into future multi-omic studies of prostate cancer patient samples of different stages and clinical subtypes will allow better understanding of the molecular heterogeneity of prostate cancer. Delineating the relationship between the driving mutations (e.g. *DNMT*, *IDH*, *TET*, and *BRAF* genes) and aberrant methylation patterns in PCa can underlie the complex mechanism and help predict specific methylation subtypes. Future studies integrating methylation sequencing data with sequencing investigating chromatin structure such as chromatin immunoprecipitation sequencing (ChIP-seq) and chromatin interaction analysis by paired-end tag sequencing (ChIA-PET), could reveal complex 3D epigenetic regulation. Overall, DNA methylation analysis not only could elucidate mechanisms that drive cancer progression but also demonstrate potential for clinical biomarker and novel treatment plan development for prostate cancer.

Author contributions

HS, JH, and HL drafted the article or revised it critically for important intellectual content. All authors contributed to the article and approved the submitted version.

Funding

JH was funded by a Prostate Cancer Foundation Young Investigator Award. HL was supported by the Prostate Cancer Foundation Young Investigator Award.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA Cancer J Clin* (2023) 73:17–48. doi: 10.3322/caac.21763

2. Robinson D, Van Allen EM, Wu Y-M, Schultz N, Lonigro RJ, Mosquera J-M, et al. Integrative clinical genomics of advanced prostate cancer. *Cell* (2015) 161:1215–28. doi: 10.1016/j.cell.2015.05.001

3. Quigley DA, Dang HX, Zhao SG, Lloyd P, Aggarwal R, Alumkal JJ, et al. Genomic hallmarks and structural variation in metastatic prostate cancer. *Cell* (2018) 174:758–769.e9. doi: 10.1016/j.cell.2018.06.039

4. Wang G, Zhao D, Spring DJ, DePinho RA. Genetics and biology of prostate cancer. *Genes Dev* (2018) 32:1105–40. doi: 10.1101/gad.315739.118

5. Beltran H, Demichelis F. Therapy considerations in neuroendocrine prostate cancer: what next? endocr. *Relat Cancer* (2021) 28:T67–78. doi: 10.1530/ERC-21-0140

6. Yamada Y, Beltran H. Clinical and biological features of neuroendocrine prostate cancer. *Curr Oncol Rep* (2021) 23:15. doi: 10.1007/s11912-020-01003-9

7. Lister R, Pelizzola M, Dowen RH, Hawkins RD, Hon G, Tonti-Filippini J, et al. Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature* (2009) 462:315–22. doi: 10.1038/nature08514

8. Domcke S, Bardet AF, Adrian Ginno P, Hartl D, Burger L, Schübeler D. Competition between DNA methylation and transcription factors determines binding of NRF1. *Nature* (2015) 528:575–9. doi: 10.1038/nature16462

9. Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat Genet* (2003) 33:245–54. doi: 10.1038/ng1089

10. Issa J. CpG island methylator phenotype in cancer. *Nature* (2004) 4:988–93. doi: 10.1038/nrc1507

11. Song Y, van den Berg PR, Markoulaki S, Soldner F, Dall'Agnese A, Henninger JE, et al. Dynamic enhancer DNA methylation as basis for transcriptional and cellular heterogeneity of ESCs. *Mol Cell* (2019) 75:905–920.e6. doi: 10.1016/j.molcel.2019.06.045

12. Okano M, Bell DW, Haber DA, Li E. DNA Methyltransferases Dnmt3a and Dnmt3b are essential for *de novo* methylation and mammalian development. *Cell* (1999) 99:247–57. doi: 10.1016/S0092-8674(00)81656-6

13. Rasmussen KD, Helin K. Role of TET enzymes in DNA methylation, development, and cancer. *Genes Dev* (2016) 30:733–50. doi: 10.1101/gad.276568.115

14. Ehrlich M. DNA Hypomethylation in cancer cells. *Epigenomics* (2009) 1:239–59. doi: 10.2217/epi.09.33

15. Esteller M. Epigenetic gene silencing in cancer: the DNA hypermethylome. *Hum Mol Genet* (2007) 16:50–9. doi: 10.1093/hmg/ddm018

16. Miller BF, Sánchez-Vega F, Elnitski L. The emergence of pan-cancer CIMP and its elusive interpretation. *Biomolecules* (2016) 6:1–14. doi: 10.3390/biom6040045

17. Moarii M, Reyal F, Vert J-P. Integrative DNA methylation and gene expression analysis to assess the universality of the CpG island methylator phenotype. *Hum Genomics* (2015) 9:26. doi: 10.1186/s40246-015-0048-9

18. Toyota M, Ahuja N, Ohe-Toyota M, Herman JG, Baylin SB, Issa J-PJ. CpG island methylator phenotype in colorectal cancer. *Proc Natl Acad Sci USA* (1999) 96:8681–6. doi: 10.1073/pnas.96.15.8681

19. Linnekamp JF, Butter R, Spijker R, Medema JP, van Laarhoven HWM. Clinical and biological effects of demethylating agents on solid tumours – a systematic review. *Cancer Treat Rev* (2017) 54:10–23. doi: 10.1016/j.ctrv.2017.01.004

20. Tao Y, Kang B, Petkovich DA, Bhandari YR, In J, Stein-O'Brien G, et al. Aging-like spontaneous epigenetic silencing facilitates wnt activation, stemness, and BrafV600E-induced tumorigenesis. *Cancer Cell* (2019) 35:315–328.e6. doi: 10.1016/j.ccell.2019.01.005

21. Weisenberger DJ, Siegmund KD, Campan M, Young J, Long TI, Faasse MA, et al. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. *Nat Genet* (2006) 38:787–93. doi: 10.1038/ng1834

22. Shen L, Toyota M, Kondo Y, Lin E, Zhang L, Guo Y, et al. Integrated genetic and epigenetic analysis identifies three different subclasses of colon cancer. *Proc Natl Acad Sci USA* (2007) 104:18654–9. doi: 10.1073/pnas.0704652104

23. Samowitz WS, Albertsen H, Herrick J, Levin TR, Sweeney C, Murtaugh MA, et al. Evaluation of a large, population-based sample supports a CpG island methylator phenotype in colon cancer. *Gastroenterology* (2005) 129:837–45. doi: 10.1053/j.gastro.2005.06.020

24. De Sousa E Melo F, Wang X, Jansen M, Fessler E, Trinh A, De Rooij LPMH, et al. Poor-prognosis colon cancer is defined by a molecularly distinct subtype and develops from serrated precursor lesions. *Nat Med* (2013) 19:614–8. doi: 10.1038/nm.3174

25. Cheng YW, Pincas H, Bacolod MD, Schemmann G, Giardina SF, Huang J, et al. CpG island methylator phenotype associates with low-degree chromosomal abnormalities in colorectal cancer. *Clin Cancer Res* (2008) 14:6005–13. doi: 10.1158/1078-0432.CCR-08-0216

26. Baba Y, Nosho K, Shima K, Freed E, Irahara N, Philips J, et al. Relationship of CDX2 loss with molecular features and prognosis in colorectal cancer. *Clin Cancer Res* (2009) 15:4665–73. doi: 10.1158/1078-0432.CCR-09-0401

27. An C, Choi IS, Yao JC, Worah S, Xie K, Mansfield PF, et al. Prognostic significance of CpG island methylator phenotype and microsatellite instability in gastric carcinoma. *Clin Cancer Res* (2005) 11:656–63. doi: 10.1158/1078-0432.656.11.2

28. Kusano M, Toyota M, Suzuki H, Akino K, Aoki F, Fujita M, et al. Genetic, epigenetic, and clinicopathologic features of gastric carcinomas with the CpG island

methylator phenotype and an association with Epstein-Barr virus. *Cancer* (2006) 106:1467–79. doi: 10.1002/cncr.21789

29. Enomoto S, Maekita T, Tsukamoto T, Nakajima T, Nakazawa K, Tatematsu M, et al. Lack of association between CpG island methylator phenotype in human gastric cancers and methylation in their background non-cancerous gastric mucosae. *Cancer Sci* (2007) 98:1853–61. doi: 10.1111/j.1349-7006.2007.00625.x

30. Shigeyasu K, Nagasaka T, Mori Y, Yokomichi N, Kawai T, Fuji T, et al. Clinical significance of MLH1 methylation and CpG island methylator phenotype as prognostic markers in patients with gastric cancer. *PloS One* (2015) 10:e0130409. doi: 10.1371/journal.pone.0130409

31. Oue N, Mitani Y, Motoshita J, Matsumura S, Yoshida K, Kuniyasu H, et al. Accumulation of DNA methylation is associated with tumor stage in gastric cancer. *Cancer* (2006) 106:1250–9. doi: 10.1002/cncr.21754

32. Tahara T, Tahara S, Horiguchi N, Okubo M, Terada T, Yamada H, et al. Molecular subtyping of gastric cancer combining genetic and epigenetic anomalies provides distinct clinicopathological features and prognostic impacts. *Hum Mutat* (2019) 40:347–54. doi: 10.1002/humu.23700

33. Chang MS, Uozaki H, Chong JM, Ushiku T, Sakuma K, Ishikawa S, et al. CpG island methylation status in gastric carcinoma with and without infection of Epstein-Barr virus. *Clin Cancer Res* (2006) 12:2995–3002. doi: 10.1158/1078-0432.CCR-05-1601

34. He D, Zhang Y, Zhang N, Zhou L, Chen J, Jiang Y, et al. Aberrant gene promoter methylation of p16, FHIT, CRBP1, WWOX, and DLC-1 in Epstein–Barr virus-associated gastric carcinomas. *Med Oncol* (2015) 32:92. doi: 10.1007/s12032-015-0525-y

35. Jing F, Yuping W, Yong C, Jie L, Jun L, Xuanbing T, et al. CpG island methylator phenotype of multigene in serum of sporadic breast carcinoma. *Tumor Biol* (2010) 31:321–31. doi: 10.1007/s13277-010-0040-x

36. Fang F, Turcan S, Rimner A, Kaufman A, Giri D, Luc GT, et al. Breast cancer methylomes establish an epigenomic foundation for metastasis. *Sci Transl Med* (2011) 3:75ra25. doi: 10.1126/scitranslmed.3001875

37. Roman-Gomez J, Jimenez-Velasco A, Agirre X, Castillejo JA, Navarro G, Calasanz MJ, et al. CpG island methylator phenotype redefines the prognostic effect of t(12;21) in childhood acute lymphoblastic leukemia. *Clin Cancer Res* (2006) 12:4845–50. doi: 10.1158/1078-0432.CCR-05-2592

38. Kroeger H, Jelinek J, Estecio MRH, He R, Kondo K, Chung W, et al. Aberrant CpG island methylation in acute myeloid leukemia is accentuated at relapse. *Blood* (2008) 112:1366–73. doi: 10.1182/blood-2007-11-126227

39. Sato H, Oka T, Shinnou Y, Kondo T, Washio K, Takano M, et al. Multi-step aberrant CpG island hyper-methylation is associated with the progression of adult T–cell Leukemia/Lymphoma. *Am J Pathol* (2010) 176:402–15. doi: 10.2353/ajpath.2010.090236

40. Kraszewska MD, Dawidowska M, Larmonie NSD, Kosmalska M, Sędek Ł, Szczepaniak M, et al. DNA Methylation pattern is altered in childhood T-cell acute lymphoblastic leukemia patients as compared with normal thymic subsets: insights into CpG island methylator phenotype in T-ALL. *Leukemia* (2012) 26:367–71. doi: 10.1038/leu.2011.208

41. Fu HY, Wu D, Sen, Zhou HR, Shen JZ. CpG island methylator phenotype and its relationship with prognosis in adult acute leukemia patients. *Hematology* (2014) 19:329–37. doi: 10.1179/1607845413Y.0000000137

42. Borssén M, Palmqvist L, Karrman K, Abrahamsson J, Behrendtz M, Heldrup J, et al. Promoter DNA methylation pattern identifies prognostic subgroups in childhood T-cell acute lymphoblastic leukemia. *PloS One* (2013) 8:e65373. doi: 10.1371/journal.pone.0065373

43. Haider Z, Larsson P, Landfors M, Köhn L, Schmiegelow K, Flaegstad T, et al. An integrated transcriptome analysis in T-cell acute lymphoblastic leukemia links DNA methylation subgroups to dysregulated TAL1 and ANTP homeobox gene expression. *Cancer Med* (2019) 8:311–24. doi: 10.1002/cam4.1917

44. Toyota M, Ahuja N, Suzuki H, Itoh F, Ohe-Toyota M, Imai K, et al. Aberrant methylation in gastric cancer associated with the CpG island methylator phenotype. *Cancer Res* (1999) 59:5438–42.

45. Kim H, Kim YH, Kim SE, Kim N-G, Noh SH, Kim H. Concerted promoter hypermethylation of hMLH1,p16INK4A, andE-cadherin in gastric carcinomas with microsatellite instability. *J Pathol* (2003) 200:23–31. doi: 10.1002/path.1325

46. Kondo T, Oka T, Sato H, Shinnou Y, Washio K, Takano M, et al. Accumulation of aberrant CpG hypermethylation by helicobacter pylori infection promotes development and progression of gastric MALT lymphoma. *Int J Oncol* (2009) 35:547–57. doi: 10.3892/ijo_00000366

47. Zong L, Seto Y. CpG island methylator phenotype, helicobacter pylori, Epstein-Barr virus, and microsatellite instability and prognosis in gastric cancer: a systematic review and meta-analysis. *PloS One* (2014) 9:e86097. doi: 10.1371/journal.pone.0086097

48. Oshimo Y, Oue N, Mitani Y, Nakayama H, Kitadai Y, Yoshida K, et al. Frequent epigenetic inactivation of RIZ1 by promoter hypermethylation in human gastric carcinoma. *Int J Cancer* (2004) 110:212–8. doi: 10.1002/ijc.20090

49. Kim JG, Takeshima H, Niwa T, Rehnberg E, Shigematsu Y, Yoda Y, et al. Comprehensive DNA methylation and extensive mutation analyses reveal an association between the CpG island methylator phenotype and oncogenic mutations in gastric cancers. *Cancer Lett* (2013) 330:33–40. doi: 10.1016/j.canlet.2012.11.022

50. Hill VK, Ricketts C, Bieche I, Vacher S, Gentle D, Lewis C, et al. Genome-wide DNA methylation profiling of CpG islands in breast cancer identifies novel genes associated with tumorigenicity. *Cancer Res* (2011) 71:2988–99. doi: 10.1158/0008-5472.CAN-10-4026

51. Li L, Lee K-M, Han W, Choi J-Y, Lee J-Y, Kang GH, et al. Estrogen and progesterone receptor status affect genome-wide DNA methylation profile in breast cancer. *Hum Mol Genet* (2010) 19:4273–7. doi: 10.1093/hmg/ddq351

52. Bae YK, Brown A, Garrett E, Bornman D, Fackler MJ, Sukumar S, et al. Hypermethylation in histologically distinct classes of breast cancer. *Clin Cancer Res* (2004) 10:5998–6005. doi: 10.1158/1078-0432.CCR-04-0667

53. Roessler J, Ammerpohl O, Gutwein J, Steinemann D, Schlegelberger B, Weyer V, et al. The CpG island methylator phenotype in breast cancer is associated with the lobular subtype. *Epigenomics* (2015) 7:187–99. doi: 10.2217/epi.14.74

54. Wang H, Yan W, Zhang S, Gu Y, Wang Y, Wei Y, et al. Survival differences of CIMP subtypes integrated with CNA information in human breast cancer. *Oncotarget* (2017) 8:48807–19. doi: 10.18632/oncotarget.16178

55. Damm F, Markus B, Thol F, Morgan M, Göhring G, Schlegelberger B, et al. TET2 mutations in cytogenetically normal acute myeloid leukemia: clinical implications and evolutionary patterns. *Genes Chromosom Cancer* (2014) 53:824–32. doi: 10.1002/gcc.22191

56. Tian X, Xu Y, Yin J, Tian H, Chen S, Wu D, et al. TET2 gene mutation is unfavorable prognostic factor in cytogenetically normal acute myeloid leukemia patients with NPM1+ and FLT3-ITD– mutations. *Int J Hematol* (2014) 100:96–104. doi: 10.1007/s12185-014-1595-x

57. Kroeze LI, Aslanyan MG, van Rooij A, Koorenhof-Scheele TN, Massop M, Carell T, et al. Characterization of acute myeloid leukemia based on levels of global hydroxymethylation. *Blood* (2014) 124:1110–8. doi: 10.1182/blood-2013-08-518514

58. Figueroa ME, Abdel-Wahab O, Lu C, Ward PS, Patel J, Shih A, et al. Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. *Cancer Cell* (2010) 18:553–67. doi: 10.1016/j.ccr.2010.11.015

59. Kelly AD, Kroeger H, Yamazaki J, Taby R, Neumann F, Yu S, et al. A CpG island methylator phenotype in acute myeloid leukemia independent of IDH mutations and associated with a favorable outcome. *Leukemia* (2017) 31:2011–9. doi: 10.1038/leu.2017.12

60. Martinez R, Schackert G, Esteller M. Hypermethylation of the proapoptotic gene TMS1/ASC: prognostic importance in glioblastoma multiforme. *J Neurooncol* (2007) 82:133–9. doi: 10.1007/s11060-006-9264-4

61. Stone AR, Bobo W, Brat DJ, Devi NS, Van Meir EG, Vertino PM. Aberrant methylation and down-regulation of TMS1/ASC in human glioblastoma. *Am J Pathol* (2004) 165:1151–61. doi: 10.1016/S0002-9440(10)63376-7

62. Kim T-Y, Zhong S, Fields CR, Kim JH, Robertson KD. Epigenomic profiling reveals novel and frequent targets of aberrant DNA methylation-mediated silencing in malignant glioma. *Cancer Res* (2006) 66:7490–501. doi: 10.1158/0008-5472.CAN-05-4552

63. Noushmehr H, Weisenberger DJ, Diefes K, Phillips HS, Pujara K, Berman BP, et al. Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. *Cancer Cell* (2010) 17:510–22. doi: 10.1016/j.ccr.2010.03.017

64. Ceccarelli M, Barthel FP, Malta TM, Sabedot TS, Salama SR, Murray BA, et al. Molecular profiling reveals biologically discrete subsets and pathways of progression in diffuse glioma. *Cell* (2016) 164:550–63. doi: 10.1016/j.cell.2015.12.028

65. Turcan S, Rohle D, Goenka A, Walsh LA, Fang F, Yilmaz E, et al. IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. *Nature* (2012) 483:479–83. doi: 10.1038/nature10866

66. van den Bent MJ, Gravendeel LA, Gorlia T, Kros JM, Lapre L, Wesseling P, et al. A hypermethylated phenotype is a better predictor of survival than MGMT methylation in anaplastic oligodendroglial brain tumors: a report from EORTC study 26951. *Clin Cancer Res* (2011) 17:7148–55. doi: 10.1158/1078-0432.CCR-11-1274

67. Wiestler B, Capper D, Hovestadt V, Sill M, Jones DTW, Hartmann C, et al. Assessing CpG island methylator phenotype, 1p/19q codeletion, and MGMT promoter methylation from epigenome-wide data in the biomarker cohort of the NOA-04 trial. *Neuro Oncol* (2014) 16:1630–8. doi: 10.1093/neuonc/nou138

68. Wiestler B, Capper D, Sill M, Jones DTW, Hovestadt V, Sturm D, et al. Integrated DNA methylation and copy-number profiling identify three clinically and biologically relevant groups of anaplastic glioma. *Acta Neuropathol* (2014) 128:561–71. doi: 10.1007/s00401-014-1315-x

69. Li KK-W, Shi Z-F, Malta TM, Chan AK-Y, Cheng S, Kwan JSH, et al. Identification of subsets of IDH-mutant glioblastomas with distinct epigenetic and copy number alterations and stratified clinical risks. *Neuro-Oncol Adv* (2019) 1:1–11. doi: 10.1093/noajnl/vdz015

70. Aryee MJ, Liu W, Engelmann JC, Nuhn P, Gurel M, Haffner MC, et al. DNA Methylation alterations exhibit intraindividual stability and interindividual heterogeneity in prostate cancer metastases. *Sci Transl Med* (2013) 5:1–14. doi: 10.1126/scitranslmed.3005211

71. Li Y, Tollefsbol TO. DNA Methylation detection: bisulfite genomic sequencing analysis. *Methods Mol Biol* (2011) 791:11–21. doi: 10.1007/978-1-61779-316-5_2

72. Li S, Tollefsbol TO. DNA Methylation methods: global DNA methylation and methylomic analyses. *Methods* (2021) 187:28–43. doi: 10.1016/j.ymeth.2020.10.002

73. Li L, Gao Y, Wu Q, Cheng ASL, Yip KY. New guidelines for DNA methylome studies regarding 5-hydroxymethylcytosine for understanding transcriptional regulation. *Genome Res* (2019) 29:543–53. doi: 10.1101/gr.240036.118

74. Enokida H, Shiina H, Urakami S, Igawa M, Ogishima T, Li LC, et al. Multigene methylation analysis for detection and staging of prostate cancer. *Clin Cancer Res* (2005) 11:6582–8. doi: 10.1158/1078-0432.CCR-05-0658

75. Florl AR, Steinhoff C, Müller M, Seifert HH, Hader C, Engers R, et al. Coordinate hypermethylation at specific genes in prostate carcinoma precedes LINE-1 hypomethylation. *Br J Cancer* (2004) 91:985–94. doi: 10.1038/sj.bjc.6602030

76. Maruyama R, Toyooka S, Toyooka KO, Virmani AK, Zöchbauer-Müller S, Farinas AJ, et al. Aberrant promoter methylation profile of prostate cancers and its relationship to clinicopathological features. *Clin Cancer Res* (2002) 8:514–9.

77. Padar A, Sathyanarayana UG, Suzuki M, Maruyama R, Hsieh JT, Frenkel EP, et al. Inactivation of cyclin D2 gene in prostate cancers by aberrant promoter methylation. *Clin Cancer Res* (2003) 9:4730–4.

78. Kang GH, Lee S, Lee HJ, Hwang KS. Aberrant CpG island hypermethylation of multiple genes in prostate cancer and prostatic intraepithelial neoplasia. *J Pathol* (2004) 202:233–40. doi: 10.1002/path.1503

79. Bhasin JMM, Lee BHH, Matkin L, Taylor MGG, Hu B, Xu Y, et al. Methylome-wide sequencing detects DNA hypermethylation distinguishing indolent from aggressive prostate cancer. *Cell Rep* (2015) 13:2135–46. doi: 10.1016/j.celrep.2015.10.078

80. Yu YP, Ding Y, Chen R, Liao SG, Ren BG, Michalopoulos A, et al. Whole-genome methylation sequencing reveals distinct impact of differential methylations on gene transcription in prostate cancer. *Am J Pathol* (2013) 183:1960–70. doi: 10.1016/j.ajpath.2013.08.018

81. Kim JH, Dhanasekaran SM, Prensner JR, Cao X, Robinson D, Kalyana-Sundaram S, et al. Deep sequencing reveals distinct patterns of DNA methylation in prostate cancer. *Genome Res* (2011) 21:1028–41. doi: 10.1101/gr.119347.110

82. Silva R, Moran B, Baird AM, O'Rourke CJ, Finn SP, McDermott R, et al. Longitudinal analysis of individual cfDNA methylome patterns in metastatic prostate cancer. *Clin Epigenet* (2021) 13:1–10. doi: 10.1186/s13148-021-01155-w

83. Gerhauser C, Favero F, Risch T, Simon R, Feuerbach L, Assenov Y, et al. Molecular evolution of early-onset prostate cancer identifies molecular risk markers and clinical trajectories. *Cancer Cell* (2018) 34:996–1011.e8. doi: 10.1016/j.ccell.2018.10.016

84. Zhao SG, Chen WS, Li H, Foye A, Zhang M, Sjöström M, et al. DNA Methylation landscapes in advanced prostate cancer. *Nat Genet* (2020) 52:778–89. doi: 10.1038/s41588-020-0648-8

85. Beltran H, Prandi D, Mosquera JM, Benelli M, Puca L, Cyrta J, et al. Divergent clonal evolution of castration-resistant neuroendocrine prostate cancer. *Nat Med* (2016) 22:298–305. doi: 10.1038/nm.4045

86. Börno ST, Fischer A, Kerick M, Fälth M, Laible M, Brase JC, et al. Genome-wide DNA methylation events in TMPRSS2-ERG fusion-negative prostate cancers implicate an EZH2-dependent mechanism with miR-26a hypermethylation. *Cancer Discov* (2012) 2:1025–35. doi: 10.1158/2159-8290.CD-12-0041

87. Chen S, Petricca J, Ye W, Guan J, Zeng Y, Cheng N, et al. The cell-free DNA methylome captures distinctions between localized and metastatic prostate tumors. *Nat Commun* (2022) 13:6467. doi: 10.1038/s41467-022-34012-2

88. Sjöström M, Zhao SG, Levy S, Zhang M, Ning Y, Shrestha R, et al. The 5-hydroxymethylcytosine landscape of prostate cancer. *Cancer Res* (2022) 82:3888–902. doi: 10.1158/0008-5472.CAN-22-1123

89. Fang M, Ou J, Hutchinson L, Green MR. The BRAF oncoprotein functions through the transcriptional repressor MAFG to mediate the CpG island methylator phenotype. *Mol Cell* (2014) 55:904–15. doi: 10.1016/j.molcel.2014.08.010

90. Serra RW, Fang M, Park SM, Hutchinson L, Green MR. A KRAS-directed transcriptional silencing pathway that mediates the CpG island methylator phenotype. *Elife* (2014) 3:1–22. doi: 10.7554/eLife.02313

91. Ponciano-Gómez A, Martínez-Tovar A, Vela-Ojeda J, Olarte-Carrillo I, Centeno-Cruz F, Garrido E. Mutations in TET2 and DNMT3A genes are associated with changes in global and gene-specific methylation in acute myeloid leukemia. *Tumor Biol* (2017) 39:101042831773218. doi: 10.1177/1010428317732181

92. Rasmussen KD, Jia G, Johansen JV, Pedersen MT, Rapin N, Bagger FO, et al. Loss of TET2 in hematopoietic cells leads to DNA hypermethylation of active enhancers and induction of leukemogenesis. *Genes Dev* (2015) 29:910–22. doi: 10.1101/gad.260174.115

93. Kamdar S, Isserlin R, Van der Kwast T, Zlotta AR, Bader GD, Fleshner NE, et al. Exploring targets of TET2-mediated methylation reprogramming as potential discriminators of prostate cancer progression. *Clin Epigenet* (2019) 11:54. doi: 10.1186/s13148-019-0651-z

94. Hájková H, Marková J, Haškovec C, Šárová I, Fuchs O, Kostečka A, et al. Decreased DNA methylation in acute myeloid leukemia patients with DNMT3A mutations and prognostic implications of DNA methylation. *Leuk Res* (2012) 36:1128–33. doi: 10.1016/j.leukres.2012.05.012

95. Holz-Schietinger C, Matje DM, Reich NO. Mutations in DNA methyltransferase (DNMT3A) observed in acute myeloid leukemia patients disrupt processive methylation. *J Biol Chem* (2012) 287:30941–51. doi: 10.1074/jbc.M112.366625

96. Jin B, Tao Q, Peng J, Soo HM, Wu W, Ying J, et al. DNA Methyltransferase 3B (DNMT3B) mutations in ICF syndrome lead to altered epigenetic modifications and aberrant expression of genes regulating development, neurogenesis and immune function. *Hum Mol Genet* (2008) 17:690–709. doi: 10.1093/hmg/ddm341

97. Duymich CE, Charlet J, Yang X, Jones PA, Liang G. DNMT3B isoforms without catalytic activity stimulate gene body methylation as accessory proteins in somatic cells. *Nat Commun* (2016) 7:11453. doi: 10.1038/ncomms11453

98. Liao J, Karnik R, Gu H, Ziller MJ, Clement K, Tsankov AM, et al. Targeted disruption of DNMT1, DNMT3A and DNMT3B in human embryonic stem cells. *Nat Genet* (2015) 47:469–78. doi: 10.1038/ng.3258

99. Roll JD, Rivenbark AG, Jones WD, Coleman WB. DNMT3b overexpression contributes to a hypermethylator phenotype in human breast cancer cell lines. *Mol Cancer* (2008) 7:15. doi: 10.1186/1476-4598-7-15

100. Kanai Y, Ushijima S, Kondo Y, Nakanishi Y, Hirohashi S. DNA Methyltransferase expression and DNA methylation of CPG islands and peri-centromeric satellite regions in human colorectal and stomach cancers. *Int J Cancer* (2001) 91:205–12. doi: 10.1002/1097-0215(200002)9999:9999<::AID-IJC1040>3.0.CO;2-2

101. Kobayashi Y, Absher DM, Gulzar ZG, Young SR, McKenney JK, Peehl DM, et al. DNA Methylation profiling reveals novel biomarkers and important roles for DNA methyltransferases in prostate cancer. *Genome Res* (2011) 21:1017–27. doi: 10.1101/gr.119487.110

102. Lamim Lovatel V, de Souza Fernandez C, Ferreira Rodrigues E, de Cassia Tavares R, Sobral da Costa E, Abdelhay E, et al. Expression profiles of DNA methylation and demethylation machinery components in pediatric myelodysplastic syndrome: clinical implications. *Cancer Manage Res* (2020) 12:543–56. doi: 10.2147/CMAR.S219026

103. Glass JL, Hassane D, Wouters BJ, Kunimoto H, Avellino R, Garrett-Bakelman FE, et al. Epigenetic identity in AML depends on disruption of nonpromoter regulatory elements and is affected by antagonistic effects of mutations in epigenetic modifiers. *Cancer Discov* (2017) 7:868–83. doi: 10.1158/2159-8290.CD-16-1032

104. Ahr K, Famulare C, Koche R, Spitzer B, Levine RL, Tallman MS, et al. AML with mutations in IDH1 and DNMT3A exhibits a distinct epigenetic signature with poorer overall survival. *Blood* (2018) 132:1471–1. doi: 10.1182/blood-2018-99-118407

105. Zhang X, Shi J, Zhang J, Yang X, Zhang G, Yang S, et al. Clinical and biological implications of IDH1/2 in acute myeloid leukemia with DNMT3Amut. *Cancer Manage Res* (2018) 10:2457–66. doi: 10.2147/CMAR.S157632

106. Rinaldi L, Datta D, Serrat J, Morey L, Solanas G, Avgustinova A, et al. Dnmt3a and Dnmt3b associate with enhancers to regulate human epidermal stem cell homeostasis. *Cell Stem Cell* (2016) 19:491–501. doi: 10.1016/j.stem.2016.06.020

107. Zhang X, Su J, Jeong M, Ko M, Huang Y-HH, Park HJ, et al. DNMT3A and TET2 compete and cooperate to repress lineage-specific transcription factors in hematopoietic stem cells. *Nat Genet* (2016) 48:1014–23. doi: 10.1038/ng.3610

108. Stanland LJ, Luftig MA. The role of EBV-induced hypermethylation in gastric cancer tumorigenesis. *Viruses* (2020) 12:1222. doi: 10.3390/v12111222

109. Issa JPJ, Ottaviano YL, Celano P, Hamilton SR, Davidson NE, Baylin SB. Methylation of the oestrogen receptor CpG island links ageing and neoplasia in human colon. *Nat Genet* (1994) 7:536–40. doi: 10.1038/ng0894-536

110. Thienpont B, Steinbacher J, Zhao H, D'Anna F, Kuchnio A, Ploumakis A, et al. Tumour hypoxia causes DNA hypermethylation by reducing TET activity. *Nature* (2016) 537:63–8. doi: 10.1038/nature19081

111. Burr S, Caldwell A, Chong M, Beretta M, Metcalf S, Hancock M, et al. Oxygen gradients can determine epigenetic asymmetry and cellular differentiation *via* differential regulation of tet activity in embryonic stem cells. *Nucleic Acids Res* (2018) 46:1210–26. doi: 10.1093/nar/gkx1197

112. O'Hagan HM, Wang W, Sen S, DeStefano Shields C, Lee SS, Zhang YW, et al. Oxidative damage targets complexes containing DNA methyltransferases, SIRT1, and polycomb members to promoter CpG islands. *Cancer Cell* (2011) 20:606–19. doi: 10.1016/j.ccr.2011.09.012

113. Maiuri AR, Peng M, Podicheti R, Sriramkumar S, Kamplain CM, Rusch DB, et al. Mismatch repair proteins initiate epigenetic alterations during inflammation-driven tumorigenesis. *Cancer Res* (2017) 77:3467–78. doi: 10.1158/0008-5472.CAN-17-0056

114. Ding N, Bonham EM, Hannon BE, Amick TR, Baylin SB, O'Hagan HM. Mismatch repair proteins recruit DNA methyltransferase 1 to sites of oxidative DNA damage. *J Mol Cell Biol* (2016) 8:244–54. doi: 10.1093/jmcb/mjv050

115. Ding N, Miller SA, Savant SS, O'Hagan HM. JAK2 regulates mismatch repair protein-mediated epigenetic alterations in response to oxidative damage. *Environ Mol Mutagen* (2019) 60:308–19. doi: 10.1002/em.22269

116. Ricketts CJ, Hill VK, Linehan WM. Tumor-specific hypermethylation of epigenetic biomarkers, including SFRP1, predicts for poorer survival in patients from the TCGA kidney renal clear cell carcinoma (KIRC) project. *PloS One* (2014) 9:e85621. doi: 10.1371/journal.pone.0085621

117. Li G, Xu W, Zhang L, Liu T, Jin G, Song J, et al. Development and validation of a CIMP-associated prognostic model for hepatocellular carcinoma. *EBioMedicine* (2019) 47:128–41. doi: 10.1016/j.ebiom.2019.08.064

118. Van der Auwera I, Yu W, Suo L, Van Neste L, van Dam P, Van Marck EA, et al. Array-based DNA methylation profiling for breast cancer subtype discrimination. *PloS One* (2010) 5:e12616. doi: 10.1371/journal.pone.0012616

119. Barreau O, Assié G, Wilmot-Roussel H, Ragazzon B, Baudry C, Perlemoine K, et al. Identification of a CpG island methylator phenotype in adrenocortical carcinomas. *J Clin Endocrinol Metab* (2013) 98:174–84. doi: 10.1210/jc.2012-2993

120. Park S-Y, Kook MC, Kim YW, Cho N-Y, Jung N, Kwon H-J, et al. CpG island hypermethylator phenotype in gastric carcinoma and its clinicopathological features. *Virchows Arch* (2010) 457:415–22. doi: 10.1007/s00428-010-0962-0

121. Chen HY, Zhu BH, Zhang CH, Yang DJ, Peng JJ, Chen JH, et al. High CpG island methylator phenotype is associated with lymph node metastasis and prognosis in gastric cancer. *Cancer Sci* (2012) 103:73–9. doi: 10.1111/j.1349-7006.2011.02129.x

122. Arai E, Chiku S, Mori T, Gotoh M, Nakagawa T, Fujimoto H, et al. Single-CpG-resolution methylome analysis identifies clinicopathologically aggressive CpG island methylator phenotype clear cell renal cell carcinomas. *Carcinogenesis* (2012) 33:1487–93. doi: 10.1093/carcin/bgs177

123. Cancer Genome Atlas Research Network, Linehan WM, Spellman PT, Rickeets CJ, Creighton CJ, Fei SS, et al. Comprehensive molecular characterization of papillary renal cell carcinoma. *N Engl J Med* (2016) 374:135–45. doi: 10.1056/NEJMoa1505917

124. Assié G, Letouzé E, Fassnacht M, Jouinot A, Luscap W, Barreau O, et al. Integrated genomic characterization of adrenocortical carcinoma. *Nat Genet* (2014) 46:607–12. doi: 10.1038/ng.2953

125. Nishihara R, Wu K, Lochhead P, Morikawa T, Liao X, Qian ZR, et al. Long-term colorectal-cancer incidence and mortality after lower endoscopy. *N Engl J Med* (2013) 369:1095–105. doi: 10.1056/NEJMoa1301969

126. Radpour R, Barekati Z, Kohler C, Lv Q, Bürki N, Diesch C, et al. Hypermethylation of tumor suppressor genes involved in critical regulatory pathways for developing a blood-based test in breast cancer. *PloS One* (2011) 6: e16080. doi: 10.1371/journal.pone.0016080

127. Mur P, Rodríguez de Lope Á, Díaz-Crespo FJ, Hernández-Iglesias T, Ribalta T, Fiaño C, et al. Impact on prognosis of the regional distribution of MGMT methylation with respect to the CpG island methylator phenotype and age in glioma patients. *J Neurooncol* (2015) 122:441–50. doi: 10.1007/s11060-015-1738-9

128. de Souza CF, Sabedot TS, Malta TM, Stetson L, Morozova O, Sokolov A, et al. A distinct DNA methylation shift in a subset of glioma CpG island methylator phenotypes during tumor recurrence. *Cell Rep* (2018) 23:637–51. doi: 10.1016/j.celrep.2018.03.107

129. Zouridis H, Deng N, Ivanova T, Zhu Y, Wong B, Huang D, et al. Methylation subtypes and Large-scale epigenetic alterations in gastric cancer. *Sci Transl Med* (2012) 4:156ra140–156ra140. doi: 10.1126/scitranslmed.3004504

130. Bolouri H, Farrar JE, Triche T, Ries RE, Lim EL, Alonzo TA, et al. The molecular landscape of pediatric acute myeloid leukemia reveals recurrent structural alterations and age-specific mutational interactions. *Nat Med* (2018) 24:103–12. doi: 10.1038/nm.4439

131. Borssén M, Haider Z, Landfors M, Norén-Nyström U, Schmiegelow K, Åsberg AE, et al. DNA Methylation adds prognostic value to minimal residual disease status in pediatric T-cell acute lymphoblastic leukemia. *Pediatr Blood Cancer* (2016) 63:1185–92. doi: 10.1002/pbc.25958

132. Borssén M, Nordlund J, Haider Z, Landfors M, Larsson P, Kanerva J, et al. DNA Methylation holds prognostic information in relapsed precursor b-cell acute lymphoblastic leukemia. *Clin Epigenet* (2018) 10:1–7. doi: 10.1186/s13148-018-0466-3

133. Bediaga NG, Acha-Sagredo A, Guerra I, Viguri A, Albaina C, Ruiz Diaz I, et al. DNA Methylation epigenotypes in breast cancer molecular subtypes. *Breast Cancer Res* (2010) 12:R77. doi: 10.1186/bcr2721

134. Michigami Y, Watari J, Ito C, Nakai K, Yamasaki T, Kondo T, et al. Long-term effects of h. pylori eradication on epigenetic alterations related to gastric carcinogenesis. *Sci Rep* (2018) 8:14369. doi: 10.1038/s41598-018-32717-3

135. Yegnasubramanian S, Kowalski J, Gonzalgo ML, Zahurak M, Piantadosi S, Walsh PC, et al. Hypermethylation of CpG islands in primary and metastatic human prostate cancer. *Cancer Res* (2004) 64:1975–86. doi: 10.1158/0008-5472.CAN-03-3972

136. Berchuck JE, Baca SC, McClure HM, Korthauer K, Tsai HK, Nuzzo PV, et al. Detecting neuroendocrine prostate cancer through tissue-informed cell-free DNA methylation analysis. *Clin Cancer Res* (2022) 28:928–38. doi: 10.1158/1078-0432.CCR-21-3762

137. Dillinger T, Sheibani-Tezerji R, Pulverer W, Stelzer I, Hassler MR, Scheibelreiter J, et al. Identification of tumor tissue-derived DNA methylation biomarkers for the detection and therapy response evaluation of metastatic castration resistant prostate cancer in liquid biopsies. *Mol Cancer* (2022) 21:1–8. doi: 10.1186/s12943-021-01445-0

138. Wu A, Cremaschi P, Wetterskog D, Conteduca V, Franceschini GM, Kleftogiannis D, et al. Genome-wide plasma DNA methylation features of metastatic prostate cancer. *J Clin Invest* (2020) 130:1991–2000. doi: 10.1172/JCI130887

139. Guler GD, Ning Y, Ku C-J, Phillips T, McCarthy E, Ellison CK, et al. Detection of early stage pancreatic cancer using 5-hydroxymethylcytosine signatures in circulating cell free DNA. *Nat Commun* (2020) 11:5270. doi: 10.1038/s41467-020-18965-w

140. Pulliam N, Fang F, Ozes AR, Tang J, Adewuyi A, Keer H, et al. An effective epigenetic-PARP inhibitor combination therapy for breast and ovarian cancers independent of BRCA mutations. *Clin Cancer Res* (2018) 24:3163–75. doi: 10.1158/1078-0432.CCR-18-0204

141. Ateeq B, Unterberger A, Szyf M, Rabbani SA. Pharmacological inhibition of DNA methylation induces proinvasive and prometastatic genes. *In Vitro In Vivo Neoplasia* (2008) 10:266–78. doi: 10.1593/neo.07947

142. Chik F, Szyf M. Effects of specific DNMT gene depletion on cancer cell transformation and breast cancer cell invasion; toward selective DNMT inhibitors. *Carcinogenesis* (2011) 32:224–32. doi: 10.1093/carcin/bgq221

143. Festuccia C, Gravina GL, D'Alessandro AM, Muzi P, Milimaggi D, Dolo V, et al. Azacitidine improves antitumor effects of docetaxel and cisplatin in aggressive prostate cancer models. *Endocr Relat Cancer* (2009) 16:401–13. doi: 10.1677/ERC-08-0130

144. Singal R, Ramachandran K, Gordian E, Quintero C, Zhao W, Reis IM. Phase I/II study of azacitidine, docetaxel, and prednisone in patients with metastatic castration-resistant prostate cancer previously treated with docetaxel-based therapy. *Clin Genitourin Cancer* (2015) 13:22–31. doi: 10.1016/j.clgc.2014.07.008

145. Sonpavde G, Aparicio A, Guttierez I, Boehm KA, Hutson TE, Berry WR, et al. Phase II study of azacitidine to restore responsiveness of prostate cancer to hormonal therapy. *Clin Genitourin Cancer* (2007) 5:457–9. doi: 10.3816/CGC.2007.n.036

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 02 June 2023
DOI 10.3389/fonc.2023.1162820

OPEN ACCESS

EDITED BY
Ran Xu,
Central South University, China

REVIEWED BY
Qi-Dong Xia,
Huazhong University of Science and Technology, China
Pedro Gonzalez-Menendez,
University of Oviedo, Spain

*CORRESPONDENCE
Yozo Mitsui
yozom321@gmail.com

RECEIVED 15 February 2023
ACCEPTED 18 May 2023
PUBLISHED 02 June 2023

CITATION
Mitsui Y, Yamabe F, Hori S, Uetani M, Aoki H, Sakurabayashi K, Okawa M, Kobayashi H, Nagao K and Nakajima K (2023) Combination of C-reactive protein/albumin ratio and time to castration resistance enhances prediction of prognosis for patients with metastatic castration-resistant prostate cancer. *Front. Oncol.* 13:1162820. doi: 10.3389/fonc.2023.1162820

Combination of C-reactive protein/albumin ratio and time to castration resistance enhances prediction of prognosis for patients with metastatic castration-resistant prostate cancer

Yozo Mitsui*, Fumito Yamabe, Shunsuke Hori, Masato Uetani, Hiroshi Aoki, Kei Sakurabayashi, Mizuho Okawa, Hideyuki Kobayashi, Koichi Nagao and Koichi Nakajima

Department of Urology, Toho University Faculty of Medicine, Tokyo, Japan

Objective: This study aimed to identify the prediction accuracy of the combination of C-reactive protein (CRP) albumin ratio (CAR) and time to castration resistance (TTCR) for overall survival (OS) following development of metastatic castration-resistant prostate cancer (mCRPC).

Methods: Clinical data from 98 mCRPC patients treated at our institution from 2009 to 2021 were retrospectively evaluated. Optimal cutoff values for CAR and TTCR to predict lethality were generated by use of a receiver operating curve and Youden's index. The Kaplan–Meier method and Cox proportional hazard regression models for OS were used to analyze the prognostic capabilities of CAR and TTCR. Multiple multivariate Cox models were then constructed based on univariate analysis and their accuracy was validated using the concordance index.

Results: The optimal cutoff values for CAR at the time of mCRPC diagnosis and TTCR were 0.48 and 12 months, respectively. Kaplan–Meier curves indicated that patients with CAR >0.48 or TTCR <12 months had a significantly worse OS (both $p < 0.005$). Univariate analysis also identified age, hemoglobin, CRP, and performance status as candidate prognostic factors. Furthermore, a multivariate analysis model incorporating those factors and excluding CRP showed CAR and TTCR to be independent prognostic factors. This model had better prognostic accuracy as compared with that containing CRP instead of

10.3389/fonc.2023.1162820

CAR. The results showed effective stratification of mCRPC patients in terms of OS based on CAR and TTCR ($p < 0.0001$).

Conclusion: Although further investigation is required, CAR and TTCR used in combination may more accurately predict mCRPC patient prognosis.

KEYWORDS

C-reactive protein albumin ratio, time to castration resistance, metastatic castration-resistant prostate cancer (mCRPC), metastatic hormone sensitive prostate cancer, biomarker

Introduction

Prostate cancer (PC) is the most common type of cancer in men and the second leading cause of cancer-related death worldwide (1). In Japan, PC has the highest prevalence of all male cancers, with 94,748 newly diagnosed cases reported in 2019 (2). Metastatic hormone-sensitive PC (mHSPC) at the initial diagnosis accounts for approximately 4% of all PC cases, with the main systemic therapy commonly given androgen deprivation therapy (ADT), as this cancer type grows in an androgen-dependent manner (3, 4). However, response to ADT by metastatic PC is usually temporary and cancer relapse occurs within 6 months to several years in a large number of patients, leading to metastatic castration-resistant PC (mCRPC).

mCRPC is an advanced condition and with a poor prognosis. When treating affected patients, the ability to predict treatment outcome and life prognosis plays important roles for distinguishing those who may benefit from treatment and avoiding unnecessary adverse effects. Factors, such as the original biological characteristics of the tumor, or genomic alterations in cancer cells and selective survival of highly resistant subclones induced by ADT, have been found to be associated with acquisition of castration resistance in PC cases (5, 6). Nevertheless, the degree of involvement of such factors, type and number of therapeutic drugs available, and necessary treatment period until castration differ among individual cases; thus, mCRPC patients are considered to be a heterogeneous population. It is necessary to comprehensively evaluate factors such as tumor and host environment, and treatment course to accurately predict prognosis.

Serum C-reactive protein (CRP) and albumin levels are representative of chronic inflammation and nutritional status in cancer patients (7, 8). Chronic inflammation is thought to promote tumor progression by influencing the tumor environment, while the tumor itself can also induce inflammation, leading to progression and malignancy (9). In cancer patients, nutritional status deteriorates with progression due to inadequate nutrient intake and tumor overconsumption, resulting in hypoalbuminemia that stimulates various inflammatory cytokines, including interleukin 6, thus promoting CRP production in the liver (10). Therefore, serum CRP and albumin are considered as interrelated serum biomarkers that may reflect host and cancer status, respectively. Indeed, CRP albumin ratio (CAR), consisting of CRP and albumin, has been confirmed as a useful prognostic factor in many cancer types, including gastrointestinal (11–13), lung (14), and urological such as renal cell carcinoma (15). In addition, CAR has potential application for predicting prognosis of mCRPC cases (15–17).

Studies have shown that shorter time to castration resistance (TTCR) is associated with worse overall survival (OS) in PC patients following the initial diagnosis as well as after acquiring castration resistance (17–20). Wenzel et al. (20) speculated that duration of treatment response before PC becomes castration-resistant may be related not only to patient or baseline tumor characteristics, but also to genetic differences or gene mutations occurring in the host or tumor.

Thus, CAR and TTCR reflect prognostic characteristics of mCRPC patients from different aspects, and are speculated to have a mutually complementary relationship. This study investigated whether those in combination could be used to predict prognosis of mCRPC patients with higher accuracy than methods presently available.

Materials and methods

Patients and treatments

The records of 159 PC patients with castration resistance after receiving ADT plus bicalutamide and subsequent first-line treatment at our institution between 1 September 2009 and 31 November 2021 were retrospectively reviewed. After excluding 61 without metastasis at the time of castration resistance acquisition (60 non-meta HSPC cases and 1 mHSPC case at initial PC diagnosis), 98 mCRPC patients were enrolled. As first-line treatment for mCRPC, each received androgen receptor axis-targeted therapy (ARAT) using either enzalutamide or abiraterone, as well as first-generation antiandrogens (AAs) including flutamide and estramustine, docetaxel (DTX), or radium-223 (Ra-223). Therapy was continued until disease progression, occurrence of an unacceptable adverse event, or patient refusal. Since July 2014, ARAT has been available for mCRPC at our institution and 25 of the present patients who started treatment before that time did not have that as a first-line

option, though most had ARAT available for a subsequent treatment course.

For this retrospective study, patient consent was not required, though information was posted on the hospital website indicating how to request exclusion. This study was conducted in accordance with the Declaration of Helsinki after receiving approval from the Ethics Committee of Toho University Omori Medical Center (no. M22168).

Assessments

Patient characteristics at the time of PC diagnosis [serum prostate-specific antigen (PSA) level, Gleason score (GS), and metastatic sites] and start of first-line treatment for mCRPC, including age, body mass index, Eastern Cooperative Oncology Group performance status (PS), chemistry profile, levels of serum hemoglobin, white blood cells, lactate dihydrogen, alkaline phosphatase, total protein, albumin, CRP, and PSA, metastatic sites, and history of treatment with ARAT or DTX, were collected and assessed respectively. CAR was calculated from CRP and albumin values using the following formula: CRP (mg/L)/albumin (g/dl).

mCRPC was defined as serum testosterone level <50 ng/dl and either of the following factors present: (i) PSA value determined at intervals of 4 weeks increased by ≥25% from the lowest value, and with increase ≥2.0 ng/ml; or (ii) radiographic findings showing progression or appearance of new lesions (21). TTCR was defined as duration from beginning ADT treatment in mHSPC patients to first stated date of mCRPC. Serum PSA levels were measured every 4 weeks during treatment. PSA response after first-line treatment for mCRPC was defined as ≥50% reduction from pretreatment baseline. PSA progression was defined as three consecutive increases in that level of ≥50% over the nadir value at a minimum of 4.0 ng/ml.

The primary and secondary endpoints of the study were overall survival (OS) after development of mCRPC and time to PSA progression, respectively. For OS analysis, duration from beginning treatment for mCRPC to patient death during any course was used. Time to PSA progression was calculated from day of mCRPC diagnosis to final day of the study or evidence of progressive disease.

Statistical analyses

Measurement values are expressed as median (interquartile range; IQR), mean ± standard deviation (SD), or number (percent of total). Receiver operating characteristic (ROC) curve and Youden's index values for both CAR and TTCR for predicting lethality were used to determine optimum threshold. The cohort was divided into three groups based on CAR and TTCR risk, then ANOVA or chi-square test results were used to analyze differences in characteristics among them. For evaluation of non-normal distributed continuous variables among the groups, a Kruskal–Wallis test was used. Survival curves were created using the Kaplan–Meier method and differences between them were analyzed with a log-rank test. Univariate analysis for OS was performed using a Cox proportional hazards regression model, followed by construction of two multivariate Cox models for OS based on univariate analysis, with accuracy validated by Harrell's concordance index (C-index). A simple nomogram for predicting mCRPC prognosis was developed using the R "survival" package. *p*-values less than 0.05 were considered to indicate statistical significance. All data were analyzed using the statistical software application EZR (Easy R) (http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmed.html) (22). A flowchart showing determination of patient eligibility, study design, and statistical methods is presented in Figure 1.

Results

Patient characteristics

Clinicopathological characteristics of all 98 mCRPC patients are summarized in Table 1. Median follow-up duration from first mCRPC treatment was 28 months. Mean age at mCRPC diagnosis was 75.3 ± 8.8 years and body mass index was 22.3 ± 83.5 kg/m^2. Forty-three (43.9%) had a PS of 0, and the remaining 55 (56.1%) had a score of 1 or 2 prior to starting first-line treatment. Among blood markers at treatment initiation, mean hemoglobin and albumin levels were 4.1 ± 0.5 and 12.4 ± 1.8 g/dl, respectively; median CRP level was 1.0 mg/L (0–2.0 mg/L) and mean CAR was 0.23 (0–0.59). At the time of mHSPC diagnosis, 19 patients (19.4%) were stage cT4, 48 (49%) had GS 9 or higher, and 74 (75.5%) had high-volume metastatic burden according to the CHAARTED criteria (23). Bone was the most common site of distant metastasis in 88 (89.8%) and visceral metastasis was found in 26 (26.5%). The major sites of visceral metastasis were lung in 12, paraaortic lymph node in 6, and liver in 2 cases. Median TTCR was 13.8 months (8.4 to 23.7 months). Initial therapy for mCRPC was ARAT in 50 (51.0%), first-generation AA in 37 (37.8%), DTX in 9 (9.2%), and Ra-223 in 2 (2.0%). During the study observation period, 90 (91.8%) were treated with ARAT and 42 (42.9%) were treated with DTX in either treatment course.

Evaluations of CAR and TTCR as prognostic factors

Optimal cutoff values of CAR and TTCR for lethality prediction in mCRPC patients were examined. ROC curve analysis using Youden's index revealed an optimal cutoff value of CAR for prediction of lethality of 0.48 (area under the curve 0.637, sensitivity 0.481, and specificity 0.783), while that of TTCR was 12.2 months (area under the curve 0.609, sensitivity 0.577, and specificity 0.630) (Figure 2). Using Cox analysis, these values were compared with the cutoff value defined by the median and the results confirmed that the hazard ratio (HR) for both values was superior as compared to the median value. Using these cutoff levels, patients were divided into low (≤0.48, n = 66) and high (>0.48,

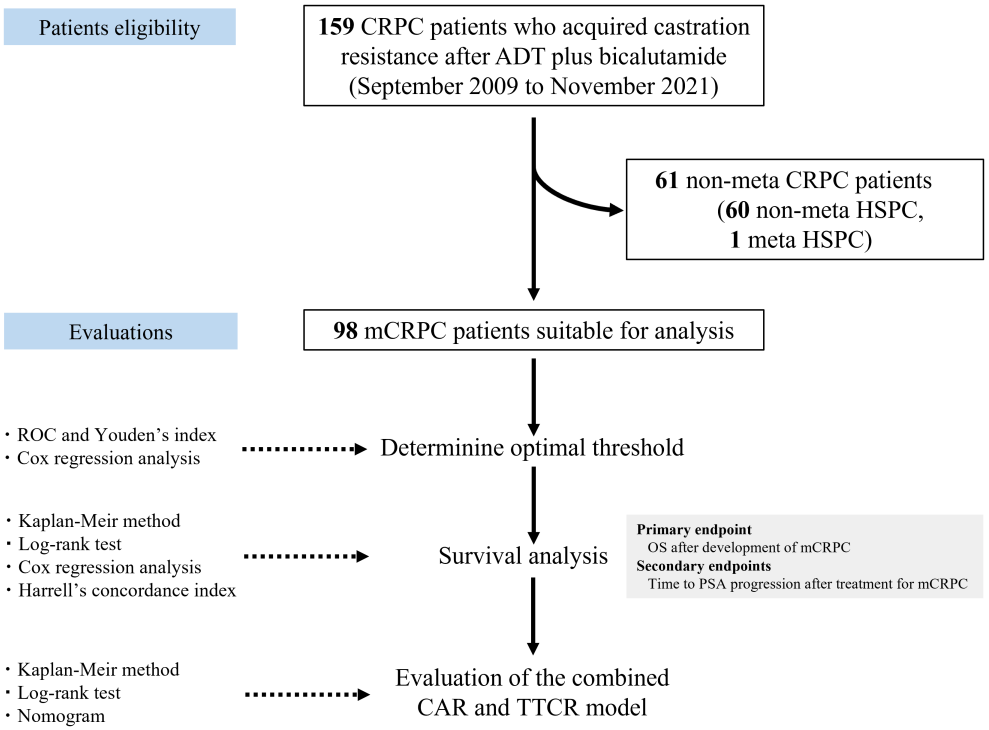


FIGURE 1
Flowchart showing patient eligibility, study design, and statistical methods. CRPC, castration-resistant prostate cancer; mCRPC, metastatic CRPC; ADT, androgen deprivation therapy; HSPC, hormone-sensitive prostate cancer; CAR, C-reactive protein albumin ratio; TTCR, time to castration resistance; ROC, receiver operating curve; OS, overall survival; PSA, prostate-specific antigen.

TABLE 1 Clinicopathological characteristics of 98 mCRPC patients.

Characteristics	
Age at mCRPC diagnosis, years	75.3 ± 8.8
Body mass index, kg/m^2	22.3 ± 3.5
ECOG PS	
0	43 (43.9)
≥1	55 (56.1)
Serum markers at initial PC diagnosis	
PSA levels, ng/ml	188.0 (32.2–523.6)
Serum markers at mCRPC diagnosis	
PSA levels, ng/ml	9.5 (2.5–28.2)
Hemoglobin, g/dl	12.4 ± 1.8
White blood cell, ×10^9/L	6.1 ± 2.0
Lactate dehydrogenase, U/L	222 (198–260)
Alkaline phosphatase, U/L	266 (208–404)
Total protein, g/dl	7.4 ± 0.6
Albumin, g/dl	4.1 ± 0.5
CRP, mg/L	1.0 (0–2.0)
CAR	0.23 (0–0.59)
Clinical T stage	
≤T3	79 (80.6)
T4	19 (19.4)
Gleason score	
≤8	50 (51.0)
≥9	48 (49.0)
Tumor burden at PC diagnosis (CHAARTED)	
High	74 (75.5)
Low	24 (24.5)
Regional lymph node metastasis at mCRPC diagnosis	48 (49.0)
Distant metastasis at mCRPC diagnosis	
Bone (total)	88 (89.8)
Bone (≥4)	67 (68.4)
Any viscera (lung, liver, etc.)	26 (26.5)
Time to castration resistance, months	13.8 (8.4–23.7)
First-line treatment for mCRPC	

(Continued)

TABLE 1 Continued

Characteristics	
ARAT	50 (51.0)
First-generation AAs	37 (37.8)
Docetaxel	9 (9.2)
Radium-223	2 (2.0)
Implementation of ARAT during treatment period	90 (91.8)
Implementation of docetaxel treatment during treatment period	42 (42.9)

Data are presented as median (interquartile range), mean ± standard deviation, or number (percentage). mCRPC, metastatic castration-resistant prostate cancer; ECOG PS, Eastern Cooperative-Oncology Group Performance Status Scale; CRP, C-reactive protein; CAR, CRP/albumin ratio; PSA, prostate-specific antigen; ARAT, androgen receptor axis-targeted therapy; AAs; antiandrogens.

n = 32) CAR groups, and TTCR ≥ 12-month (n = 56) and TTCR < 12-month (n = 42) groups. Kaplan–Meier curve analysis showed that the high CAR group had significantly worse OS than the low CAR group (median 22.2 vs. 30.0 months, $p < 0.0001$) (Figure 3A). Similarly, the TTCR < 12-month group had worse OS than the TTCR ≥ 12-month group (median 20.7 vs. 30.0 months, p = 0.0027). Furthermore, a significantly shorter time to PSA progression was observed in patients with high CAR as compared to those with low CAR, as well as for the TTCR < 12-month as compared with the TTCR ≥ 12-month group (p = 0.0239 and p = 0.0042, respectively) (Figure 3B).

Uni- and multivariate Cox analyses for OS were performed to further evaluate CAR and TTCR prognostic value. Univariate analysis revealed that both CAR (HR 3.147, 95% CI 1.768–5.602, $p < 0.0001$) and TTCR (HR 0.416, 95% CI 0.230–0.750, p = 0.0036) significantly associated with OS (Table 2). Similarly, age (HR 2.135, 95% CI 1.072–4.252, p = 0.0309), ECOG PS (HR 2.318, 95% CI 1.288–4.174, p = 0.0051), hemoglobin level (HR 0.369, 95% CI 0.205–0.663, p = 0.0001), and CRP level (HR 2.459, 95% CI 1.405–4.304, p = 0.0016) were shown as candidate factors for a significant association with OS (Table 2). To avoid the influence of possible multicollinearity between CAR and CRP, two multivariate Cox proportional hazard models based on the same four candidate factors (TTCR, ECOG PS, age, and hemoglobin), which exhibited a significant association in univariate analyses, and CAR or CRP were constructed. The C-index for model I with CAR was 0.757, higher than the value for model II with CRP (0.746) in terms of OS, suggesting that the model incorporating CAR was superior to that incorporating CRP for prediction of lethality in mCRPC patients (Table 3). In addition, using multivariate model I, both CAR (HR 2.815, 95% CI 1.522–5.205, p = 0.0010) and TTCR (HR 0.410, 95% CI 0.215–0.784, p = 0.0070) were consistently found to be independent predictors for OS (Table 3).

Next, whether the combination of CAR and TTCR could be used to predict mCRPC patient prognosis with greater accuracy was assessed. The cohort was divided into three groups (0, 1, and 2 factors) based on the presence of CAR (>0.48) and/or TTCR (<12 months) (Table 4). Significant differences among the groups were found for several blood factors, including hemoglobin, white blood cells, CRP, and albumin. The presence of regional lymph node metastasis, visceral metastasis, and high tumor burden was also significantly correlated with number of factors present, while GS was found to have an inverse association. Further stratification using the combination of CAR and TTCR identified a stepwise reduction in both OS and PSA progression-free survival probabilities, with the shortest period found in the high CAR

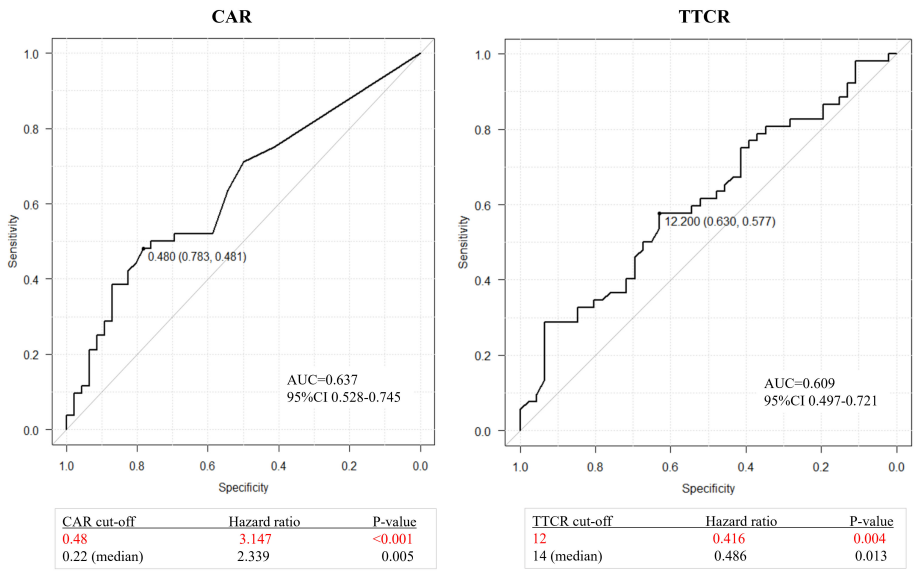


FIGURE 2
ROC curves for overall survival after castration resistance shown by CAR (CRP/Alb ratio) or TTCR (time to castration resistance). Optimal cutoff values for CAR and TTCR were determined to be 0.48 (area under the curve 0.637, sensitivity 0.481, and specificity 0.783) and 12.2 months (area under the curve 0.609, sensitivity 0.577, and specificity 0.630), respectively. Comparisons of these values with the cutoff value defined by the median confirmed the superiority of values determined with use of the Youden index.

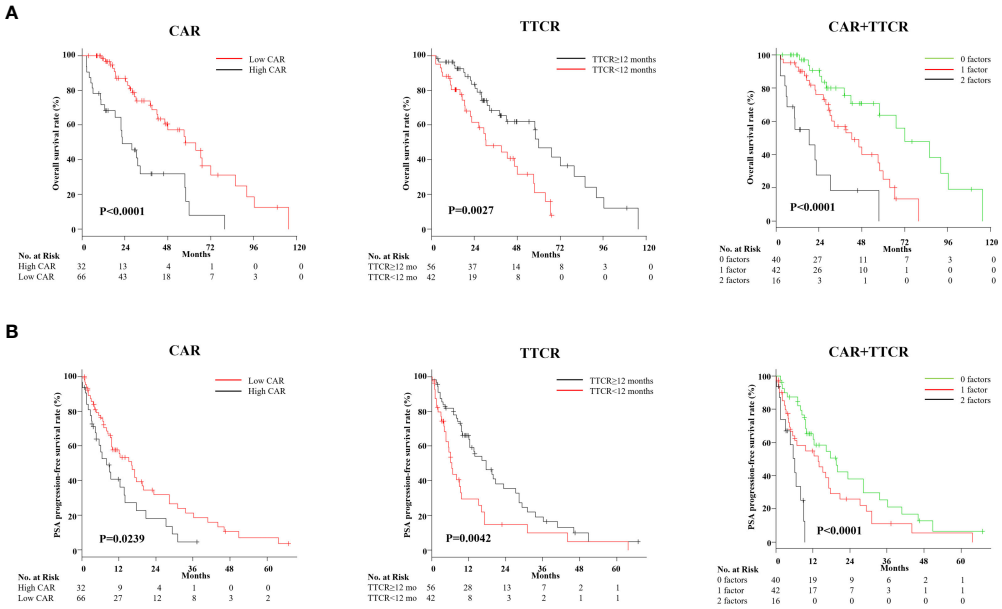


FIGURE 3
Kaplan–Meier analysis of overall survival after castration resistance, and PSA progression-free survival following first-line treatment for mCRPC based on CAR (CRP/Alb ratio) and TTCR (time to castration resistance). **(A)** Kaplan–Meier curves for OS based on CAR and TTCR, and those in combination. OS for the high CAR and TTCR <12-month groups was significantly worse than for the low CAR and TTCR ≥12-month groups, respectively. Risk stratification according to values for CAR and TTCR effectively stratified the prognosis of mCRPC patients. **(B)** Kaplan–Meier curves for PSA progression-free rate after first-line treatment against mCRPC based on CAR and TTCR, and those in combination. Similar to the results seen in the OS analysis, CAR, TTCR, and the combination of both factors provided correct risk classification regarding the duration of first-line treatment response.

group with TTCR <12 months (2 factors), while the low CAR group with TTCR ≥12 months (0 factors) had the longest period (Figures 3A, B).

In addition, an OS prediction nomogram incorporating CAR, TTCR, age, ECOG PS, and hemoglobin level, shown to be candidate factors in univariate analysis, was developed. This nomogram composed of five factors also had good OS predictive ability. However, its use did not improve prognostic predictive power as compared to models that used only CAR and TTCR. Details regarding this nomogram are provided as Supplementary Figure 1.

Effects and prognosis for each first-line treatment method

Finally, first-line treatment effects and prognosis of 96 mCRPC patients, after excluding two treated with Ra-223, were evaluated. PSA response was achieved in 62.2% overall, with ARAT having the highest rate of 82.0% among the three treatments (Figure 4A). Furthermore, patients who received ARAT as first-line therapy had a significantly longer time to PSA progression than those treated with DTX or AA (Figure 4B, p = 0.0011), while OS was not significantly different among the treatments (Figure 4B, p = 0.7220). Analysis of Kaplan–Meier curves showed risk stratification according to CAR and TTCR number useful for classifying PSA recurrence-free survival probability associated with each treatment, though statistical significance was not reached for patients treated with AA (Figure 4C). Furthermore, this risk stratification model was found to effectively stratify mCRPC patients treated with each treatment in terms of OS (Figure 4D).

Discussion

We speculated that CAR and TTCR reflect mCRPC patient prognosis, and their use in combination could be useful for prognostic prediction. A retrospective investigation of mCRPC patients treated at our institution was performed with noteworthy findings obtained, as detailed in the following.

mCRPC patients with CAR greater than 0.48 had significantly shorter survival and duration of PSA response after initial treatment as compared with those with lower CAR. Notably, CAR remained an important prognostic factor for OS even in multivariate analysis that incorporated various patient and tumor factors. These findings are consistent with previous studies of castration-resistant PC patients (16, 17), especially that presented by Uchimoto et al. (17), which noted an optimal CAR cutoff value of 0.50, nearly the same as in the present study.

Chronic inflammation is closely related to cancer progression; thus, attention has focused on the relationship between elevated CRP and prognosis in cancer patients including PC. A prospective population-based cohort study conducted by Stikbakke et al. showed that elevated serum CRP levels had adverse effects on PC risk and prognosis (24). Also, two studies that employed meta-analyses of data obtained from previous reports confirmed CRP as

10.3389/fonc.2023.1162820

TABLE 2 Univariate Cox proportional hazards analysis findings for overall survival rate after castration resistance.

Covariates	HR (95% CI)	*p*-value
Age at mCRPC diagnosis (≥80 years)	2.135 (1.072–4.252)	0.0309
Body mass index (≥22.3 kg/m^2)	0.786 (0.438–1.412)	0.4213
ECOG PS (≥1)	2.318 (1.288–4.174)	0.0051
Hemoglobin (≥12.4 g/dl)	0.369 (0.205–0.663)	0.0001
White blood cell (≥6,100×10^9/L)	1.529 (0.880–2.655)	0.1316
Lactate dehydrogenase (>222 U/L)	1.315 (0.756–2.290)	0.3324
Alkaline phosphatase (>266 U/L)	1.733 (0.986–3.044)	0.0588
Total protein (>7.4 g/dl)	1.138 (0.656–1.977)	0.6451
Albumin (>4.1 g/dl)	0.588 (0.331–1.045)	0.0701
CRP (>1.0 mg/L)	2.459 (1.405–4.304)	0.0016
CAR (>0.48)	3.147 (1.768–5.602)	<0.0001
PSA levels at PC diagnosis (>188.0 ng/ml)	0.653 (0.372–1.147)	0.1378
PSA levels at mCRPC diagnosis (>9.5 ng/ml)	1.409 (0.801–2.480)	0.2338
Clinical T stage (T4)	0.879 (0.467–1.655)	0.6890
Gleason score (≥9)	1.439 (0.823–2.516)	0.2017
Bone metastasis (≥4)	1.649 (0.860–3.164)	0.1323
Regional lymph node metastasis	1.271 (0.729–2.216)	0.3973
Visceral metastasis	1.197 (0.646–2.216)	0.5677
Time to castration resistance (≥12 months)	0.416 (0.230–0.750)	0.0036
First-line treatment for mCRPC (ARAT)	1.028 (0.552–1.914)	0.9318
Implementation of ARAT during treatment period (yes)	0.671 (0.264–1.706)	0.4020
Implementation of docetaxel treatment during treatment period (yes)	1.141 (0.646–2.016)	0.6483

HR, hazard ratio; CRPC, castration-resistant prostate cancer; ECOG PS, Eastern-Cooperative Oncology-Group Performance-Status Scale; CRP; C-reactive protein; CAR, CRP/albumin ratio; PSA, prostate-specific antigen; ARAT, androgen receptor axis-targeted therapy.

TABLE 3 Differences in C-index between two models containing CAR (CRP/Alb ratio) or CRP using multivariate Cox proportional hazards model.

Variables	HR (95% CI)	*p*-value	C-index
Model I			0.757
CAR (>0.48)	2.815 (1.522–5.205)	0.0010	
Time to castration resistance (≥12 months)	0.410 (0.215–0.784)	0.0070	
ECOG PS (≥1)	1.895 (0.989–3.629)	0.0539	
Age at mCRPC diagnosis (≥80 years)	1.552 (0.713–3.377)	0.2682	
Hemoglobin (≥12.4 g/dl)	0.595 (0.311–1.137)	0.1158	
Model II			0.746
CRP (>1.0 mg/L)	2.315 (1.297–4.134)	0.0045	
Time to castration resistance (≥12 months)	0.467 (0.247–0.882)	0.0190	
ECOG PS (≥1)	1.985 (1.032–3.817)	0.0400	
Age at mCRPC diagnosis (≥80 years)	1.530 (0.699–3.348)	0.2875	
Hemoglobin (≥12.4 g/dl)	0.475 (0.254–0.889)	0.0199	

HR, hazard ratio; C-index, concordance index; CRP, C-reactive protein; CAR, CRP/albumin ratio; ECOG PS, Eastern Cooperative Oncology Group Performance-Status Scale; mCRPC, metastatic castration-resistant prostate cancer.

10.3389/fonc.2023.1162820

TABLE 4 Clinicopathologic features of patients divided into three groups using CAR (CRP/Alb ratio) and TTCR (time to castration resistance) risk numbers.

Characteristics	0 factors	1 factor	2 factors	*p*-value
	N = 40	*N* = 42	*N* = 16	
Age at mCRPC diagnosis, years	74.9 ± 9.2	76.4 ± 6.9	73.2 ± 12.0	0.2235
Body mass index, kg/m^2	21.8 ± 3.1	22.7 ± 3.8	22.6 ± 3.8	0.5043
ECOG PS				0.0842
0	20 (50.0)	20 (47.6)	3 (18.8)	
≥1	20 (50.0)	22 (52.4)	13 (81.2)	
Serum markers at initial PC diagnosis				
PSA levels, ng/ml	203.1 (31.7–755.3)	104.1 (23.9–425.3)	268.0 (56.2–485.5)	0.543
Serum markers at mCRPC diagnosis				
PSA levels, ng/ml	8.0 (2.1–17.4)	11.7 (2.8–26.8)	23.0 (3.7–51.5)	0.1144
Hemoglobin, g/dl	13.2 ± 1.3	12.3 ± 1.8	10.9 ± 1.7	<0.0001
White blood cell, ×10^9/L	5.6 ± 1.6	6.1 ± 1.7	7.2 ± 2.8	0.0209
Lactate dehydrogenase, U/L	222 (192–266)	212 (198–252)	243 (208–289)	0.1462
Alkaline phosphatase, U/L	239 (204–333)	264 (202–405)	409 (227–574)	0.1348
Total protein, g/dl	7.4 ± 0.4	7.4 ± 0.7	7.3 ± 0.6	0.8166
Albumin, g/dl	4.2 ± 0.4	4.1 ± 0.5	3.6 ± 0.7	0.0004
CRP, mg/L	0 (0–1.0)	1.0 (0–4.5)	9.0 (4.0–19.0)	<0.0001
CAR	0 (0–0.23)	0.26 (0.2–1.2)	2.1 (0.9–5.9)	<0.0001
Clinical T stage				0.5998
≤T3	34 (85.0)	32 (76.2)	13 (81.2)	
T4	6 (15.0)	10 (23.8)	3 (18.8)	
Gleason score				0.0093
≤8	12 (30.0)	24 (57.1)	11 (68.8)	
≥9	28 (70.0)	18 (42.9)	5 (31.2)	
Regional lymph node metastasis	13 (32.5)	23 (54.8)	12 (75.0)	0.0098
Distant metastatic site				
Bone (total)	36 (90.0)	36 (85.7)	16 (100)	0.2748
Bone (≥4)	24 (60.0)	29 (69.0)	14 (87.5)	0.1345
ny viscera (lung, liver, muscle)	8 (20.0)	9 (21.4)	8 (50.0)	0.0484
Tumor burden at PC diagnosis (CHAARTED)				0.0272
High	25 (62.5)	34 (81.0)	15 (93.8)	
Low	15 (37.5)	8 (19.0)	1 (6.2)	
Time to castration resistance				<0.0001
<12 months	0 (0)	26 (61.9)	16 (100)	
≥12 months	40 (100)	16 (381)	0 (0)	
First-line treatment for mCRPC				0.6317
ARAT	23 (57.5)	20 (47.6)	7 (43.8)	
First-generation AA	14 (35.0)	17 (40.5)	6 (37.5)	

(Continued)

TABLE 4 Continued

Characteristics	0 factors	1 factor	2 factors	*p*-value
	N = 40	*N* = 42	*N* = 16	
Docetaxel	2 (5.0)	5 (11.9)	2 (12.5)	
Radium-223	1 (2.5)	0 (0)	1 (6.2)	
Implementation of ARAT during treatment period	38 (95.0)	38 (90.5)	14 (87.5)	0.5948
Implementation of docetaxel during treatment period	13 (32.5)	22 (52.4)	7 (43.8)	0.1908

Data are presented as median (interquartile range), mean ± standard deviation, or number (percentage). mCRPC, metastatic castration-resistant prostate cancer; ECOG PS, Eastern Cooperative Oncology Group Performance Status Scale; CRP, C-reactive protein; CAR, CRP/albumin ratio; PSA, prostate-specific antigen; ARAT, androgen receptor axis-targeted treatment; AA; antiandrogens.

an effective predictor of poor outcome in PC cases including mCRPC (25, 26). Interestingly, one of these (26) showed that low albumin was also a significant factor associated with poor prognosis in mCRPC patients. Decreased albumin leads to increased CRP through release of various cytokines, indicating a negative correlation between these factors (10). Furthermore, changes in CAR, composed of CRP and albumin, may be more sensitive to patient and/or cancer conditions than CRP or albumin alone. Indeed, the present findings showed that multivariate models incorporating CAR more accurately predicted OS in patients with mCRPC than models incorporating CRP. This superiority of CAR over CRP or albumin for predicting mCRPC patient prognosis was also confirmed by Uchimoto et al. (17).

TTCR was also confirmed as an independent predictor of OS after mCRPC development. Patients with a TTCR of ≥12 months had a median OS of 30 months, whereas those with a TTCR of <12 months was significantly shorter (20.7 months). This trend was also found for the period until PSA progression. Although some studies failed to identify OS differences between TTCR subgroups after castration resistance was acquired (18, 20), these findings show a clear prognostic difference based on TTCR classification, as previously reported (17, 19, 27). Importantly, use of 12 months for prognostic definition by TTCR was also adopted in studies of PC patients in Japan treated with ADT who acquired castration resistance, while Miyake et al. further classified TTCR and reported that those with ≤6 months had the worst prognosis (17–19, 27). A study that divided mHSPC patients into those who received ADT+ARAT or DTX also showed that TTCR <12 months strongly associated with poor prognosis (20). Therefore, TTCR <12 months seems accurate for predicting worse OS even in this combination therapy era.

Recently, studies have analyzed changes induced in mHSPC by hormone therapy at the genetic level, with interesting results obtained. Zurita et al. showed that amplification of *AR* and *MYC*, or loss of *TP53* and *RB1*, known as poor prognostic factors, was enhanced after hormone therapy resistance (28). Also, genome-wide loss-of-heterozygosity (gLOH), a genomic instability marker, was increased with emerging resistance to hormonal therapy, while higher gLOH was closely associated with the presence of altered homologous recombination-repair (HRR) genes (*BRCA2*, *PALB2*, and *FANCA*). Kimura et al. reported that mHSPC patients with germline HRR mutations including *BRCA2* and *PALB2* had significantly shorter TTCR (29). Thus, it is considered that shorter TTCR reflects, at least in part, genetic differences or mutations in the host or tumors.

Finally, prediction of OS and time to PSA progression was confirmed possible by dividing mCRPC patients into three groups according to values for CAR (>0.48) and TTCR (<12 months), identified as poor prognostic factors in this study. Furthermore, the combined classification of CAR and TTCR was able to predict duration of response and prognosis associated with each first-line mCRPC treatment. These observations are not surprising, as use of these factors combined involves differences in a variety of host- and tumor-side poor prognostic factors, such as low PS, anemia, high tumor stage, and metastasis. Previous results indicating CAR or TTCR ability to predict treatment outcome in mCRPC patients also support our findings. Specifically, Uchimoto et al. reported that prognosis of patients with high CAR was poor regardless of ARAT, AA, or DTX treatment (17). Gültürk et al. showed that DTX-treated mCRPC patients with TTCR <12 months had significantly shorter durations of response and OS than those with TTCR >12 months (30). Thus, we concluded that classification of mCRPC patients based on both CAR and TTCR enables accurate predictions of patient prognosis as well as efficacy of each therapy.

This study has several limitations, including retrospective design and low number of mCRPC patients treated at a single hospital. Owing to the small sample size, the CAR and TTCR cutoff thresholds used may not be adequate to reflect prognosis in other cohorts. However, several previous studies have used prognostic cutoff values close to those defined in the present study for both CAR and TTCR. Furthermore, patients who started initial treatment for mCRPC before ARAT was introduced in Japan were also included. Selection bias may exist regarding treatment options, since therapy choice for individual patients might have been based on disease severity. Also, patients who received combination therapy in a castration-sensitive stage or did not have distant metastasis at the time of castration resistance did not receive focus. Finally, exclusion of other candidate blood biomarkers, including neutrophil–lymphocyte ratio and inflammatory line interleukin, is another limitation. For example, it has been pointed out that pivotal inflammatory cytokines that are members of the interleukin-1 family may serve as important

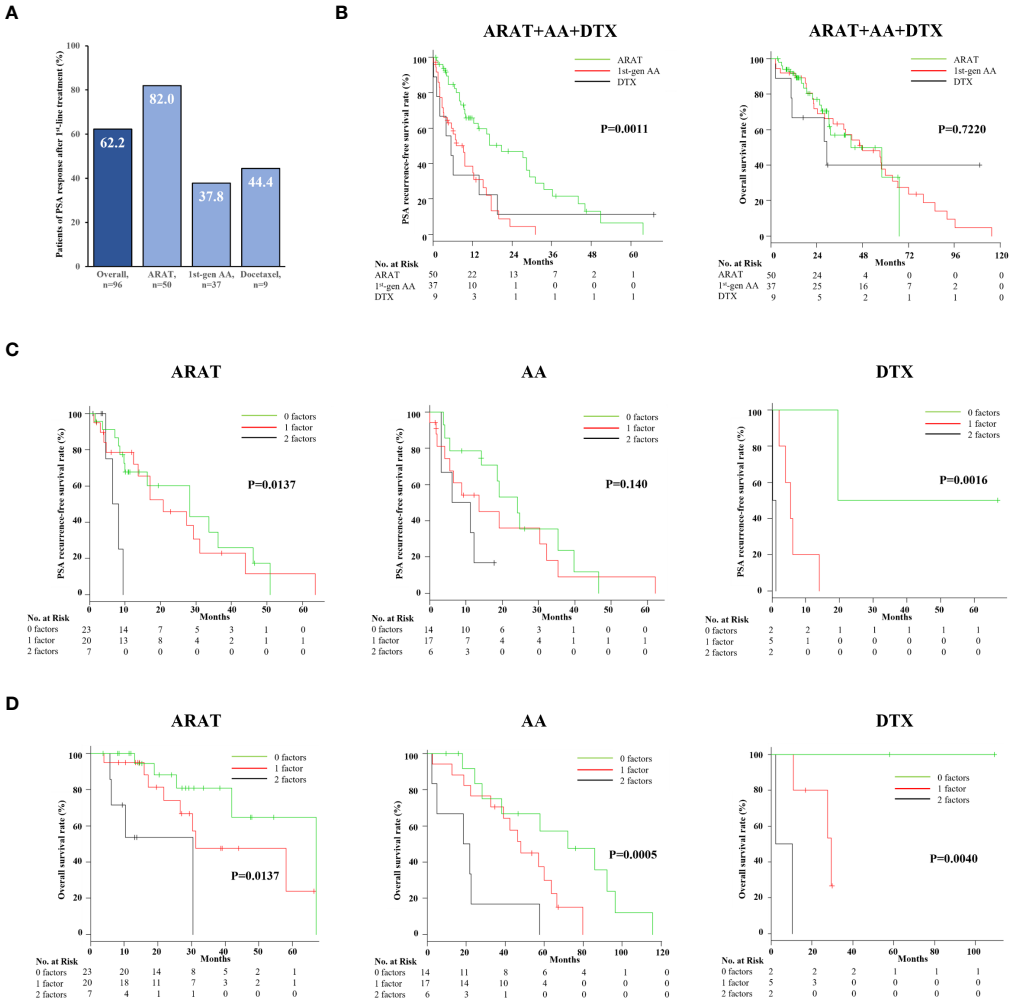


FIGURE 4
Efficacy and impact on overall survival and PSA progression-free survival of different first-line agents for mCRPC. **(A)** PSA responses for patients with ARAT, AA, and DTX treatment were 82.0%, 37.8%, and 34%, respectively. **(B)** The PSA progression-free survival rate was significantly better in ARAT patients, whereas OS was not significantly different among the three treatments. **(C)** Kaplan–Meier curve showing PSA progression-free rate after first-line treatment for mCRPC with the three treatments. The duration of PSA response in ARAT- and DTX-treated patients was significantly different among the three groups classified by CAR and TTCR. **(D)** Kaplan–Meier curve showing OS after first-line treatment for mCRPC with the three treatments. CAR- and CRP-based risk categorization effectively stratified the respective OS of mCRPC patients treated with the three different agents.

biomarkers for predicting clinical stage and prognosis in patients with PC (31). Prospective studies with larger populations that overcome these limitations are required to validate and confirm our findings.

Conclusion

CAR and TTCR were found to be independent predictors of prognosis and treatment response in mCRPC patients. In addition, prognosis after mCRPC development and therapeutic efficacy of treatment options may be predicted more accurately by combining CAR and TTCR. It is considered that this method can accurately identify patients who may benefit from treatment and also provide useful information regarding optimal treatment. Future large-scale prospective studies will be necessary to confirm the present preliminary findings and may lead to development of effective risk models.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of Toho University Omori Medical Center. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

YM, KNag, and KNak contributed to conception and design of the study. FY, SH, MU, and HA collected patient data. YM wrote the first draft of the manuscript. KS performed the statistical analysis. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1162820/full#supplementary-material

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin* (2022) 72:7–33. doi: 10.3322/caac.21708

2. Cancer Information Service, National Cancer Center. Japan (Ministry of health, labour and welfare, national cancer registry). In: *Cancer registry and statistics*, Japan: National Cancer Center (2019). Available at: https://ganjoho.jp/reg_stat/stati stics/ dl/index.html.

3. Weiner AB, Matulewicz RS, Eggener SE, Schaeffer EM. Increasing incidence of metastatic prostate cancer in the united states (2004-2013). *Prostate Cancer Prostatic Dis* (2016) 19:395–7. doi: 10.1038/pcan.2016.30

4. Mosillo C, Iacovelli R, Ciccarese C, Fantinel E, Bimbatti D, Brunelli M, et al. *De novo* metastatic castration sensitive prostate cancer: state of art and future perspectives. *Cancer Treat Rev* (2018) 70:67–74. doi: 10.1016/j.ctrv.2018.08.005

5. Annala M, Taavitsainen S, Khalaf DJ, Vandekerkhove G, Beja K, Sipola J, et al. Evolution of castration-resistant prostate cancer in ctDNA during sequential androgen receptor pathway inhibition. *Clin Cancer Res* (2021) 27:4610–23. doi: 10.1158/1078-0432.CCR-21-1625

6. Sumanasuriya S, Seed G, Parr H, Christova R, Pope L, Bertan C, et al. Elucidating prostate cancer behaviour during treatment *via* low-pass whole-genome sequencing of circulating tumour DNA. *Eur Urol* (2021) 80:243–53. doi: 10.1016/j.eururo.2021.05.030

7. McMillan DC. Systemic inflammation, nutritional status and survival in patients with cancer. *Curr Opin Clin Nutr Metab Care* (2009) 12:223–6. doi: 10.1097/MCO.0b013e32832a7902

8. Coussens LM, Werb Z. Inflammation and cancer. *Nature* (2002) 420:860–7. doi: 10.1038/nature01322

9. Diakos CI, Charles KA, McMillan DC, Clarke SJ. Cancer-related inflammation and treatment effectiveness. *Lancet Oncol* (2014) 15:e493–503. doi: 10.1016/S1470-2045(14)70263-3

10. Almasaudi AS, Dolan RD, Edwards CA, McMillan DC. Hypoalbuminemia reflects nutritional risk, body composition and systemic inflammation and is independently associated with survival in patients with colorectal cancer. *Cancers (Basel)* (2020) 12:1986. doi: 10.3390/cancers12071986

11. Yu Q, Li KZ, Fu YJ, Tang Y, Liang XQ, Liang ZQ, et al. Clinical significance and prognostic value of c-reactive protein/albumin ratio in gastric cancer. *Ann Surg Treat Res* (2021) 100:338–46. doi: 10.4174/astr.2021.100.6.338

12. Tamai K, Okamura S, Makino S, Yamamura N, Fukuchi N, Ebisui C, et al. C-reactive protein/albumin ratio predicts survival after curative surgery in elderly patients with colorectal cancer. *Updates Surg* (2022) 74:153–62. doi: 10.1007/s13304-021-01011-9

13. Bao Y, Yang J, Duan Y, Chen Y, Chen W, Sun D. The c-reactive protein to albumin ratio is an excellent prognostic predictor for gallbladder cancer. *Biosci Trends* (2021) 14:428–35. doi: 10.5582/bst.2020.03326

14. Araki T, Tateishi K, Sonehara K, Hirota S, Komatsu M, Yamamoto M, et al. Clinical utility of the c-reactive protein:albumin ratio in non-small cell lung cancer patients treated with nivolumab. *Thorac Cancer* (2021) 12:603–5. doi: 10.1111/1759-7714.13788

15. Wu M, Zhou Y, Chen Q, Yu Z, Gu H, Lin P, et al. Prognostic role of pretreatment c-reactive protein to albumin ratio in urological cancers: a systematic review and meta-analysis. *Front Oncol* (2022) 12:879803. doi: 10.3389/fonc.2022.879803

16. Yamashita S, Kohjimoto Y, Iguchi T, Koike H, Kusumoto H, Iba A, et al. Prognostic factors and risk stratification in patients with castration-resistant prostate cancer receiving docetaxel-based chemotherapy. *BMC Urol* (2016) 16:13. doi: 10.1186/s12894-016-0133-y

17. Uchimoto T, Komura K, Fujiwara Y, Saito K, Tanda N, Matsunaga T, et al. Prognostic impact of c-reactive protein-albumin ratio for the lethality in castration-resistant prostate cancer. *Med Oncol* (2019) 37:9. doi: 10.1007/s12032-019-1332-7

18. Miyake H, Matsushita Y, Watanabe H, Tamura K, Motoyama D, Ito T, et al. Prognostic significance of time to castration resistance in patients with metastatic castration-sensitive prostate cancer. *Anticancer Res* (2019) 39:1391–6. doi: 10.21873/anticanres.13253

19. Okamoto T, Hatakeyama S, Takahashi M, Marita S, Ishida M, Hoshi S, et al. The impact of time-to-castration resistance on survival in patients with metastatic hormone-naïve prostate cancer: a multicenter retrospective study. *J Clin Oncol* (2020) 38(suppl 6):213. doi: 10.1200/JCO.2020.38.6_suppl.213

20. Wenzel M, Preisser F, Hoeh B, Schroeder M, Würnschimmel C, Steuber T, et al. Impact of time to castration resistance on survival in metastatic hormone sensitive prostate cancer patients in the era of combination therapies. *Front Oncol* (2021) 11:659135. doi: 10.3389/fonc.2021.659135

21. Scher HI, Halabi S, Tannock I, Morris M, Sternberg CN, Carducci MA, et al. Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the prostate cancer clinical trials working group. *J Clin Oncol* (2008) 26:1148–59. doi: 10.1200/JCO.2007.12.4487

22. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant* (2013) 48:452–8. doi: 10.1038/bmt.2012.244

23. Sweeney CJ, Chen YH, Carducci M, Liu G, Jarrard DF, Eisenberger M, et al. Chemohormonal therapy in metastatic hormone-sensitive prostate cancer. *N Engl J Med* (2015) 373:737–46. doi: 10.1056/NEJMoa1503747

24. Stikbakke E, Richardsen E, Knutsen T, Wilsgaard T, Giovannucci EL, McTiernan A, et al. Inflammatory serum markers and risk and severity of prostate cancer: the PROCA-life study. *Int J Cancer* (2020) 147:84–92. doi: 10.1002/ijc.32718

25. Liao DW, Hu X, Wang Y, Yang ZQ, Li X. C-reactive protein is a predictor of prognosis of prostate cancer: a systematic review and meta-analysis. *Ann Clin Lab Sci* (2020) 50:161–71.

26. Yanagisawa T, Kawada T, Rajwa P, Mostafaei H, Motlagh RS, Quhal F, et al. Sequencing impact and prognostic factors in metastatic castration-resistant prostate cancer patients treated with cabazitaxel: a systematic review and meta-analysis. *Urol Oncol* (2022) S1078-1439(22):00238–1. doi: 10.1016/j.urolonc.2022.06.01

27. Hakozaki Y, Yamada Y, Kawai T, Nakamura M, Takeshima Y, Iwaki T, et al. Time to castration resistance is a novel prognostic factor of cancer-specific survival in patients with nonmetastatic castration-resistant prostate cancer. *Sci Rep* (2022) 12:16202. doi: 10.1038/s41598-022-20319-z

28. Zurita AJ, Graf RP, Villacampa G, Raskina K, Sokol E, Jin D, et al. Genomic biomarkers and genome-wide loss-of-heterozygosity scores in metastatic prostate cancer following progression on androgen-targeting therapies. *JCO Precis Oncol* (2022) . 6:e2200195. doi: 10.1200/PO.22.00195

29. Kimura H, Mizuno K, Shiota M, Narita S, Terada N, Fujimoto N, et al. Prognostic significance of pathogenic variants in BRCA1, BRCA2, ATM and PALB2 genes in men undergoing hormonal therapy for advanced prostate cancer. *Br J Cancer* (2022) 127:1680–90. doi: 10.1038/s41416-022-01915-2

30. Gültürk İ, Erdal GS, Sönmezoz GB, Yılmaz M, Tacar SY, Özmen A, et al. Time to castration resistance as a predictor of response to docetaxel in metastatic castration resistance in prostate cancer. *İstanbul Med J* (2022) 23:269–74. doi: 10.4274/imj.galenos.2022.56804

31. Tong Y, Cao Y, Jin T, Huang Z, He Q, Mao M. Role of interleukin-1 family in bone metastasis of prostate cancer. *Front Oncol* (2022) 12:951167. doi: 10.3389/fonc.2022.951167

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 09 June 2023
DOI 10.3389/fonc.2023.1165188

OPEN ACCESS

EDITED BY
Sifeng Qu,
Shandong University, China

REVIEWED BY
Che-Kai Tsao,
Icahn School of Medicine at Mount Sinai, United States
Ahmed El-Zawahry,
University of Toledo, United States

*CORRESPONDENCE
Shihua Wang
Shihua.Wang@osumc.edu
Steven K. Clinton
Steven.clinton@osumc.edu

RECEIVED 13 February 2023
ACCEPTED 18 May 2023
PUBLISHED 09 June 2023

CITATION
Wang S, Yin M, Wang P, Folefac E, Monk JP, Tabung FK and Clinton SK (2023) Chemotherapy for the initial treatment of metastatic prostate adenocarcinoma and neuroendocrine carcinoma at diagnosis: real world application and impact in the SEER database (2004 –2018). *Front. Oncol.* 13:1165188. doi: 10.3389/fonc.2023.1165188

Chemotherapy for the initial treatment of metastatic prostate adenocarcinoma and neuroendocrine carcinoma at diagnosis: real world application and impact in the SEER database (2004 –2018)

Shihua Wang[1]*, Ming Yin[1,2], Peng Wang[1,2], Edmund Folefac[1,2], J. Paul Monk[1,2], Fred K. Tabung[1,2] and Steven K. Clinton[1,2]*

[1]The Ohio State University Comprehensive Cancer Center and Arthur G. James Cancer Hospital, Columbus, OH, United States, [2]Division of Medical Oncology, Department of Internal Medicine, The Ohio State University College of Medicine, The Ohio State University, Columbus, OH, United States

Background: Randomized controlled phase III trials have reported significant improvements in disease response and survival with the addition of chemotherapy to androgen deprivation therapy for men presenting with metastatic prostate cancer. We examined the implementation of such knowledge and its impact within the Surveillance, Epidemiology, and End Results (SEER) database.

Method: The administration of chemotherapy for men with an initial presentation of metastatic prostate cancer from 2004 to 2018 in the SEER database and its association with survival outcomes was examined. Kaplan–Meier estimates were applied to compare survival curves. Cox proportion hazard survival models were used to analyze the association of chemotherapy and other variables with both cancer- specific and overall survival.

Result: A total of 727,804 patients were identified with 99.9% presenting with adenocarcinoma and 0.1% with neuroendocrine histopathology. Chemotherapy as initial treatment for men with *de novo* distant metastatic adenocarcinoma increased from 5.8% during 2004–2013 to 21.4% during 2014–2018. Chemotherapy was associated with a poorer prognosis during 2004–2013 but was associated with improved cancer-specific (hazard ratio (HR) = 0.85, 95% confidence interval (CI): 0.78–0.93, p=0.0004) and overall survival (HR= 0.78, 95% CI: 0.71–0.85, p < 0.0001) during 2014–2018. The improved prognosis during 2014–2018 was observed in patients with visceral or bone metastasis and most impactful for patients aged 71–80 years. These findings were confirmed by subsequent propensity score matching analyses. Furthermore, chemotherapy was consistently provided to 54% of patients with neuroendocrine carcinoma at diagnosis from 2004 to 2018. Treatment was associated with improved cancer-specific survival (HR= 0.62, 95% CI: 0.45–0.87, p=0.0055) and overall survival (HR= 0.69, 95% CI: 0.51–0. 94, p=0.0176) during 2014–2018 but not significant in earlier years.

Conclusion: Chemotherapy at initial diagnosis was increasingly employed in men with metastatic adenocarcinoma after 2014 and consistent with the evolution of National Comprehensive Cancer Network (NCCN) guidelines. Benefits for chemotherapy are suggested after 2014 in the treatment of men with metastatic adenocarcinoma. The use of chemotherapy for neuroendocrine carcinoma at diagnosis has remained stable, and outcomes have improved in more recent years. Further development and optimization of chemotherapy continues to evolve for men with *de novo* diagnosis of metastatic prostate cancer.

KEYWORDS

prostate cancer, chemotherapy, SEER, adenocarcinoma, neuroendocrine

Introduction

The burden of metastatic prostate cancer to society is enormous, both in terms of health care resources and human suffering; thus, the implementation of knowledge derived from quality clinical trials to community practice is imperative (1). Prostate cancer continues to be the most frequently diagnosed non-cutaneous cancer in American men and the second leading cause of cancer-related death (1), suggesting a critical need for improved screening and early diagnosis at a curable stage, and enhanced efficacy of therapy for advanced metastatic disease. Following the US Preventive Services Task Force (USPSTF) report in 2012 (2), there was a significantly reduced utilization of prostate cancer screening with prostate-specific antigen (PSA) testing, resulting in a lower overall detection of prostate cancer, but an unfortunate increase has emerged in the proportion of men presenting at advanced stages (1, 3–5). For example, recent data show a significant 41% increase in metastatic prostate cancer from 2010 to 2018 in men aged 45 –75 (3). This report focuses upon the treatments provided to the subgroup of men presenting with *de novo* metastatic disease in the real-world setting.

For decades, suppression of testosterone by castration or androgen deprivation therapy (ADT) has been the cornerstone of life-prolonging therapy for metastatic prostate cancer (6) and continues to improve with newer agents targeting specific components of the androgen signaling pathway (7–9). Yet, metastatic disease is essentially incurable, and mortality is nearly 70% by 5 years after diagnosis (10–13). Sadly, the median survival for men with castrate-resistant prostate cancer (CRPC) ranges from 18 to 24 months in most studies (12, 14, 15). Cytotoxic chemotherapy emerged as a beneficial treatment modality for metastatic CRPC, initially in the management of pain with mitoxantrone (16) and subsequently with docetaxel prolonging survival in landmark phase III trials by 2004 (17, 18) and supported by subsequent studies (19, 20). Soon thereafter, cabazitaxel, a second-generation taxane, showed a survival benefit in docetaxel refractory CRPC (21). With success in CRPC in the metastatic setting, the potential of adding taxane chemotherapy to androgen deprivation therapy (ADT) for men who present at initial diagnosis with treatment-naive metastatic disease was investigated in studies demonstrating improved overall survival and improved secondary endpoints such as prostate–specific antigen (PSA) failure and time to recurrence, particularly for those with higher volume disease (10, 11, 22, 23). National Comprehensive Cancer Network (NCCN) guidelines recommend ADT with docetaxel for six cycles as one of several options for the initial treatment of castration-naive metastatic prostate adenocarcinoma and was first included in the 2014 update (10, 24).

Our objective is to assess how the studies of chemotherapy combined with hormone therapy over recent decades have translated into real-world clinical practice for men with a new diagnosis of metastatic prostate cancer. The present study provides a comprehensive and contemporary (2004 –2018) summary of the large Surveillance, Epidemiology, and End Results (SEER) database. We also report the impact of initial chemotherapy on survival based upon the histopathological subtype and a number of relevant clinical and demographic factors.

Methods

Data source

We employed the population-based SEER Research Plus Data, 18 registries (2000–2018) using the SEERStat 8.3.9 software to identify patients 18 and older with an initial diagnosis of prostate cancer. We included those diagnosed during 2004–2018 because SEER collected PSA information since 2004 and the modern chemotherapy regimens (e.g., docetaxel) for metastatic disease were supported by clinical trial results in 2004. Those with stage Tis or T0 (no indication of cancer), with unknown T, N, and M stages and unknown survival time were excluded from the study. The primary endpoints were prostate-cancer-specific survival and overall survival. Based on the International Classification of Diseases for Oncology, Third Edition (ICD-O-3), we only included patients with prostate adenocarcinoma (8,140) or

neuroendocrine carcinoma (8,012, 8,013, 8,041, 8,042, 8,045, 8,240, 8,241, 8,246, and 8,574) (25, 26). The SEER registries collect information on the first course of treatment. Chemotherapy data are categorized as either "yes— patient had chemotherapy" or "no/unknown— no evidence of chemotherapy was found in the medical records examined." Patients with *de novo* distant metastatic disease were defined by M stage as 1. M stage was further grouped into M1a, M1b, M1c, and M1x. The following demographic and clinicopathological variables were included: age at diagnosis; PSA concentration; ethnicity (White, Black, Asian or Pacific Islander, and other); marital status; region of the US; Gleason score; T, N, and M stage; and treatments including surgery, radiotherapy, and chemotherapy. As the data are de-identified, institutional review board approval was not necessary for this project.

Statistical analysis

Continuous data were evaluated by T-test. Square root or log transformation of the original data was applied to satisfy the assumption of equal variances. Categorical data were compared using the Pearson's chi-square test. The trend for the proportion of patients receiving chemotherapy was examined by the Cochran–Armitage test. Survival curves were defined by Kaplan–Meier methodology and compared through log rank testing. Univariate and multivariate Cox proportional hazards regression analyses were utilized to examine the impact of chemotherapy and predictors on cancer- specific and overall survival. The multivariable model was constructed with a backward selection strategy with an entry level of 0.05 at every step. Only variables with a p-value < 0.10 in the univariate analyses were included, except that chemotherapy was always included in the multivariate analysis. To address potential disparities between patients treated with or without chemotherapy, impacts of chemotherapy on prognosis were determined in propensity score matching analyses. Matching variables included age; PSA; Gleason score; T, N, and M stages; race; marital status; region; and local treatment. All statistical tests were two-sided with $p< 0.05$ to be significant. Data analyses were performed using SAS 9.4 (Raleigh, NC).

Results

Patients with prostate adenocarcinoma or neuroendocrine carcinoma diagnosed during 2004–2018

A total of 727,804 patients diagnosed with prostate cancer during 2004–2018 (Supplementary Table S1) were identified with 727,133 (99.9%) having adenocarcinoma and 671 (0.1%) with neuroendocrine histopathology. Those with neuroendocrine cancer at presentation were older and exhibited higher PSA and greater Gleason grade. The proportion of men with metastatic adenocarcinoma at diagnosis was 3%, which was much lower than 57% of those with neuroendocrine histology (Supplementary Table S1).

Time trends for chemotherapy administration for metastatic prostate cancer at diagnosis during 2004–2018

As expected, the proportion of men with non-metastatic adenocarcinoma receiving chemotherapy was between 0.2% and 0.5% over time (p trend <0.0001) (Figure 1A). For men with metastatic adenocarcinoma at presentation, chemotherapy was provided to 5.8% during years 2004–2013 and increased to 21.4% during the years of 2014–2018 (p trend < 0.0001) (Figure 1A). In this population, the administration of chemotherapy was strongly age dependent after 2013 (all p trends < 0.0001) (Figure 1B; Table 1), with younger men more likely to receive chemotherapy. A much high proportion of men presenting with neuroendocrine cancer received chemotherapy (54%), and the proportion remained steady between 2004 and 2018, with year-to-year variation due to the overall smaller number of cases compared to adenocarcinoma (p trend=0.1349) (Figure 1C).

Characteristics of patients with metastatic prostate adenocarcinoma at diagnosis and initially treated with or without chemotherapy during 2004–2013 and 2014–2018

Table 1 outlines factors contributing to the selection of chemotherapy for initial treatment of men presenting with metastatic prostate adenocarcinoma at primary diagnosis for the intervals of 2004–2013 (5.8% receiving chemotherapy) and 2014–2018 (21.4% receiving chemotherapy). Younger age at diagnosis was strongly associated with selection of initial chemotherapy particularly after 2013 (Table 1). Patients with cancers characterized by higher Gleason score (9, 10), more advanced T stage, positive lymph node metastasis, and more advanced M stage (M1c) were significantly more likely to receive chemotherapy in both periods. A higher PSA (> 90 ng/ml) emerged as a modest predictor for chemotherapy treatment during 2014–2018.

Impact of chemotherapy on cancer-specific and overall survival in patients presenting with metastatic prostate adenocarcinoma during 2004–2013 and 2014–2018

During 2004–2013, there were significantly higher proportions of both cancer-specific and overall deaths in metastatic patients receiving chemotherapy compared with those receiving no chemotherapy (Supplementary Table S2; Figure 2). In contrast, during 2014–2018, the proportion of overall death in patients receiving chemotherapy was significantly less than in those without chemotherapy, while cancer-specific death was not significantly impacted by chemotherapy selection (Supplementary Table S2). Survival curves illustrate that chemotherapy was

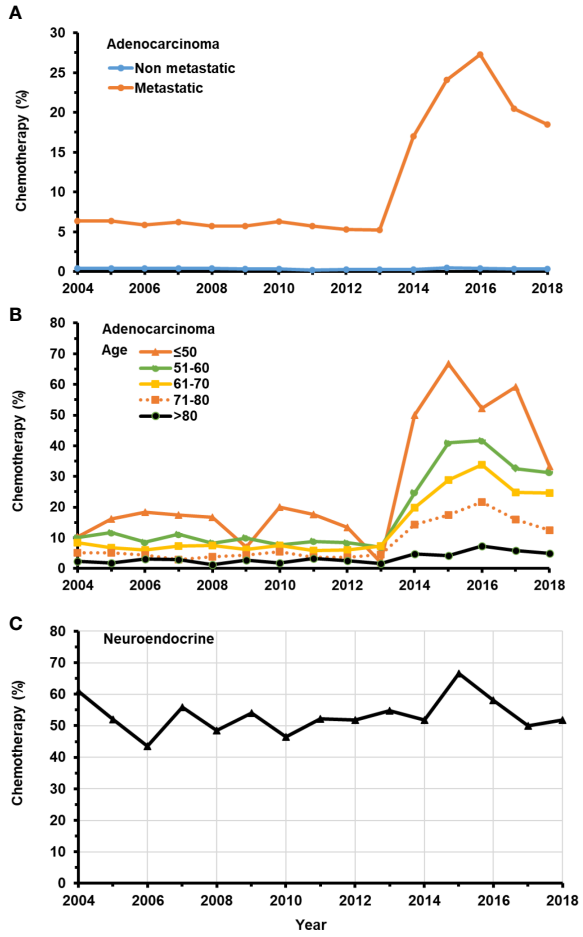


FIGURE 1
Temporal change in the percentage of patients with *de novo* metastatic prostate adenocarcinoma receiving chemotherapy from 2004–2018. **(A)** Patients with or without *de novo* metastatic prostate adenocarcinoma. **(B)** Patients in different age groups with *de novo* metastatic prostate adenocarcinoma. **(C)** All patients with prostate neuroendocrine carcinoma.

associated with significantly worse cancer-specific and overall survival in patients with metastatic prostate adenocarcinoma carcinoma during 2004–2013 (Figures 2A, B) but was associated with significantly improved prognoses during 2014–2018 (Figures 2C, D).

Table 2 presents multivariate survival analyses showing greater depth of insight with reduced bias. Chemotherapy was associated with significantly improved cancer-specific survival (HR= 0.85, 95% CI: 0.78 –0.93, p=0.0004) and overall survival (HR= 0.78, 95% CI: 0.71 –0.85, p<0.0001) in patients with metastatic prostate adenocarcinoma diagnosed during 2014–2018. In comparison, during the period of 2004–2013, chemotherapy was associated with significantly worse cancer-specific survival (HR =1.48, 95% CI: 1.37 –1.61, p<0.0001) and overall survival (HR =1.39, 95% CI: 1.28 –1.50, p<0.0001) (Table 2). Other factors significantly predicting poor outcomes in both time intervals were greater age, higher PSA, T4 stage, extensive metastasis beyond M1a, and higher Gleason score. The inclusion of radiotherapy or surgery to the initial treatment plan was not associated with a change in cancer-specific or overall survival during either time period. However, combined radiotherapy and surgery was associated with significantly worse survival during the earlier time frame of 2004–2013 (Table 2). Men who were married, as an indicator of support systems, fared significantly better than those who were not by 18% – 25%. Men with metastatic prostate cancer living in the South fared significantly worse than in other regions regardless of treatment and time interval.

Age at diagnosis, which clearly impacted selection of chemotherapy, appears to be associated with survival response to chemotherapy. We observed no significant improvement in overall or cancer-specific survival for younger men <70 years or for those over 80. In contrast, improved cancer- specific and overall survival were seen among men aged 71–75 and 76–80 years (Table 3).

The benefits of chemotherapy are related to metastatic disease burden at diagnosis. Our data showed that chemotherapy was associated with significantly improved cancer- specific and overall survival in patients with metastasis to either visceral (liver, lung, or brain), or bone alone. In contrast, chemotherapy was not associated with improvements in cancer-specific survival or overall survival in patients presenting with only distant lymph node metastasis (Table 4).

Propensity score matching analyses of impact of chemotherapy on prognosis in patients presenting with metastatic prostate adenocarcinoma during 2004–2013 and 2014–2018

After the propensity score matching, equal numbers of patients with comparable features treated with or without chemotherapy were selected in those having metastatic adenocarcinoma during 2004–2013 and 2014–2018 (Supplementary Table S3). We observed similar and consistent patterns of death and survival curves with propensity score matching. (Supplementary Table S4; Figures S1A–D). Most importantly, we confirmed that in patients with metastatic prostate adenocarcinoma, chemotherapy was associated with worse prognoses during 2004–2013 but improved cancer-specific and overall survival during 2014–2018 (Table 5).

Baseline characteristics of patients with prostate neuroendocrine carcinoma with or without chemotherapy during 2004–2013 and 2014–2018

Younger patients with neuroendocrine carcinoma with lower serum PSA levels, more advanced T stage, lymph node and distant metastasis, and radiotherapy were more likely to receive chemotherapy during both periods. During 2004–2013, patents of 51–60 years old compared to older individuals were more likely to receive chemotherapy. In addition, during 2014–2018, patients of 61–70 years and from West and Northeast regions were more likely to receive chemotherapy (Table 6). Detailed treatments provided to these patients is presented in Supplementary Table S5.

TABLE 1 Descriptive characteristics of patients with a *de novo* diagnosis of metastatic prostate adenocarcinoma who were initially treated with or without chemotherapy during 2004–2013 and 2014–2018.

Variable	2003–2014 n (%)			2014–2018 n (%)		
	No Chemotherapy 12,451 (94.2)	Chemotherapy 772 (5.8)	*p*-value	No Chemotherapy 8,471 (78.6)	Chemotherapy 2,310 (21.4)	*p*-value
Age (years)						
Mean ± SD	70.3 ± 10.8	64.9 ± 10.1	<0.0001	71.7 ± 10.1	65.1 ± 8.9	<0.0001
Median (range)	70 (35–100)	64 (38–98)		72 (39–100)	65 (34–95)	
Distribution			<0.0001			<0.0001
≤50	368 (3).	60 (8)		119 (1)	134 (6)	
51–60	2,132 (17)	211 (27)		1,088 (13)	564 (24)	
61–70	3,788 (30)	278 (36)		2,754 (33)	990 (43)	
71–80	3,650 (29)	164 (21)		2,645 (31)	515 (22)	
>80	2,513 (20)	59 (8)		1,865 (22)	107 (5)	
PSA (ng/ml)						
Mean ± SD	62.7 ± 38.1	62.2 ± 39.6	0.6884	61.3 ± 38.0	66.2 ± 37.0	<0.0001
Median (range)	83.7 (0.1–99.8)	88 (0.1–99.8)		73.2 (0.1–99.8)	98 (0.1–99.8)	
Distribution			0.0044			<0.0001
<20.0	2,761 (22)	205 (27)		2,008 (24)	448 (19)	
20–90.0	3,239 (26)	164 (21)		2,347 (28)	628 (27)	
>90	5,691 (46)	360 (47)		3,740 (44)	1,178 (51)	
Unknown	760 (6)	43 (6)		376 (4)	56 (2)	
Gleason score			<0.0001			<0.0001
≤6	560 (5)	22 (2.9)		129 (2)	23 (1)	
7	2,117 (17)	101 (13)		1,072 (13)	172 (8)	
8	2,629 (21)	132 (17)		1,819 (21)	415 (18)	
9–10	5,355 (43)	403 (52)		4,185 (49)	1,425 (62)	
Unknown	1,790 (14)	114 (15)		1,266 (15)	275 (12)	
T stage			<0.0001			0.0066
T1	3,977 (32)	221 (29)		2,786 (33)	728 (32)	
T2	5,056 (41)	273 (35)		2,934 (35)	779 (34)	
T3	1,512 (12)	114 (15)		1,433 (17)	374 (16)	
T4	1,906 (15)	164 (21)		1,318 (16)	429 (19)	
N stage			<0.0001			<0.0001
N0	9,035 (73)	487 (63)		5,316 (63)	1,145 (50)	
N1	3,416 (27)	285 (37)		3155 (37)	1,165 (50)	
M stage			<0.0001			<0.0001
M1a	771 (6)	53 (7)		739 (9)	126 (5)	
M1b	8,914 (72)	480 (62)		6,013 (71)	1,607 (70)	
M1c	2,403 (19)	202 (26)		1,080 (13)	425 (18)	
M1x	363 (3)	37 (5)		639 (7)	152 (7)	

(Continued)

TABLE 1 Continued

Variable	2003–2014 n (%)			2014–2018 n (%)		
	No Chemotherapy 12,451 (94.2)	Chemotherapy 772 (5.8)	*p*-value	No Chemotherapy 8,471 (78.6)	Chemotherapy 2,310 (21.4)	*p*-value
Marital status			<0.0001			0.0002
Married	7,188 (58)	517 (67)		4,885 (58)	1,442 (62)	
Unmarried#	4,390 (35)	208 (27)		3,030 (36)	732 (32)	
Unknown	873 (7)	47 (6)		556 (7)	136 (6)	
Race			0.0339			0.1817
White	9,185 (74)	599 (78)		6,368 (75)	1,769 (77)	
Black	2,473 (20)	138 (18)		1,459 (17)	387 (17)	
Other	739 (6)	35 (5)		567 (7)	142 (6)	
Unknown	54 (0.4)	0 (0)		77 (1)	12 (1)	
Region			0.1650			0.5063
West	6,140 (49)	352 (45.6)		4,446 (53)	1,188 (51)	
South	3,007 (24)	191 (24.7)		2,037 (24)	558 (24)	
Midwest	1,415 (11)	102 (13.2)		859 (10)	229 (10)	
Northeast	1,889 (15)	127 (16.5)		1,129 (13)	335 (15)	
Local treatment			<0.0001			<0.0001
No local treatment	8,144 (65)	411 (53)		5,359 (63)	1,613 (70)	
Radiotherapy only	2,578 (21)	250 (32)		1,746 (21)	468 (20)	
Surgery only	1,435 (12)	73 (10)		1,107 (13)	178 (8)	
Radiotherapy and surgery	294 (2)	38 (5)		259 (3)	51 (2)	

Unmarried including divorced, separated, single (never married), unmarried or domestic Partner, widowed.

Impact of chemotherapy on survival in patients with neuroendocrine carcinoma

Patients with prostate neuroendocrine carcinoma receiving chemotherapy had significantly higher proportions of cancer-specific death and overall death compared to those without chemotherapy during 2004–2013. During 2014–2018, the proportions of cancer- specific and overall deaths were comparable between chemotherapy and no-chemotherapy groups (Supplementary Table S6). Survival curves showed that chemotherapy was associated with slightly worse cancer- specific survival (p=0.0225) but not overall survival (p=0.5559) in patients with neuroendocrine carcinoma during 2004–2013 (Figures 3A, B). Chemotherapy was not associated with cancer- specific (p=0.1060) and overall (p=0.1011) survival during 2014–2018 (Figures 3C, D). Multivariate survival analyses showed that chemotherapy was associated with improved cancer- specific survival (HR= 0.62, 95% CI: 0.45–0.87, p=0.0055) and overall survival (HR=0.69, 95% CI: 0.51–0.94, p = 0.0176) in patients with neuroendocrine carcinoma during 2014–2018. Conversely, chemotherapy was not significantly associated with cancer- specific survival (HR = 0.99, 95% CI: 0.76–1.29, p = 0.9138) and survival (HR= 0.89, 95% CI: 0.70–1.14, p=0.3540) during 2004–2013 (Table 7).

Discussion

The SEER 18 database, capturing approximately 28% of the total United States population, provides a valuable resource to assess patterns of care for prostate cancer. We specifically examined the utilization of systemic cytotoxic chemotherapy for men with metastatic disease at an initial diagnosis from 2004 to 2018, a period when results of clinical trials suggested new strategies for care. As expected, few men not showing metastatic disease received initial chemotherapy throughout the period. During 2004–2013, the proportion of patients with *de novo* metastatic adenocarcinoma receiving chemotherapy was low (5.8%) but significantly increased to an average of 21.4% during 2014 –2018. The pattern observed likely represents a shared decision- making process (27) between the patient and provider throughout the interval (2004–2018) with utilization of chemotherapy increasingly being offered as an option following publication of

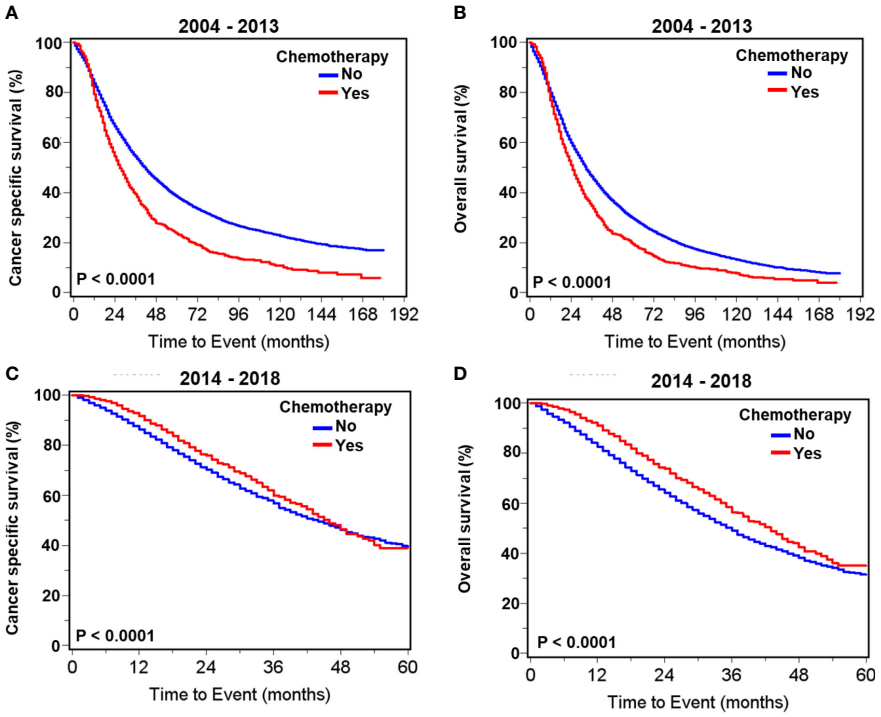


FIGURE 2
Kaplan–Meier survival curves for cancer- specific and overall survival in all patients with *de novo* metastatic prostate adenocarcinoma with or without chemotherapy. For patients diagnosed during 2004–2013, curves of cancer- specific survival **(A)** and overall survival **(B)**. For patients diagnosed during 2014–2018, curves of cancer- specific survival **(C)** and overall survival **(D)**.

new clinical trial results approximately 2014 (10, 11, 22, 23). By 2014, National Comprehensive Cancer Network (NCCN) guidelines recommended ADT plus docetaxel for six cycles as one of several options for the initial treatment of castration- naive metastatic prostate adenocarcinoma (24). Multivariate survival analysis of data from 2004 to 2013 showed that chemotherapy in men with distant metastasis was associated with worse cancer-specific and overall survival; by contrast, it was associated with improved prognosis during 2014–2018.

This is most likely related to patient selection, with chemotherapy being used for men with the most ominous presentation. In contrast to adenocarcinoma, men with neuroendocrine prostate carcinoma (0.1% of all prostate cancer) show an average of 54% of patients receiving chemotherapy with no clear directional change over the entire period. Interestingly, chemotherapy was associated with improved cancer-specific and overall survival in neuroendocrine carcinoma patients during 2014–2018 but not during 2004–2013, perhaps due to improvements in supportive care and patient selection.

For decades, studies of cytotoxic chemotherapy failed to demonstrate benefits for men with metastatic prostate cancer due to challenges in objectively measuring response for a disease dominated by nodal and bone metastasis coupled with a lack of efficacy (28–30). The approval of mitoxantrone for pain control established chemotherapy as an option for men with advanced metastatic disease in 1999 (16, 31). By 2004, a landmark series of studies showed that docetaxel-based therapy for the first time demonstrated improved survival for men with metastatic-castration-resistant prostate adenocarcinoma (17, 18, 31). Newer studies refined our knowledge and documented benefits dependent upon dose intensity (32) and that taxane analogues may prolong benefits (21). Such progress led investigators to consider moving docetaxel chemotherapy into initial treatment strategies for newly diagnosed hormone-sensitive metastatic prostate cancer. Trials showing improved biochemical progression-free survival with the addition of docetaxel to ADT in metastatic hormone-naive prostate cancer patients were emerging by 2013 (23), and by 2015, studies were showing that upfront docetaxel chemotherapy improved overall survival, failure-free survival, and progression-free survival (10, 22, 23). Our study examines how this knowledge impacted therapy for prostate cancer patients in the non-protocol standard practice over the time frame that these results became available (33).

As expected, our study revealed that chemotherapy for those with metastatic adenocarcinoma at diagnosis was sparingly employed at 5.8% prior to 2014 followed by a dramatic rise to nearly 30% from 2014 to 2016. The rapid change was certainly driven by the enthusiasm derived from the new studies and guidelines (23). Although it is possible that the wider insurance coverage becoming available at this time resulting from the American Afford Care Act (34) contributed somewhat, we suspect that the main driving force of adoption was the published research and NCCN guidelines. Yet, it may be surprising to some that only 20%–30% are receiving upfront chemotherapy after 2013–2014. We suspect that both providers and patients contribute to these

TABLE 2 Multivariate survival analyses of variables associated with survival in patients with metastatic prostate adenocarcinoma diagnosed during 2004–2013 and 2014–2018.

Variable	Cancer-specific survival				Overall survival			
	2004–2013		2014–2018		2004–2013		2014–2018	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Age (years)								
≤50	1		1		1		1	
51–60	0.96 (0.85–1.08)	0.5143	0.83 (0.65–1.06)	0.1337	1.00 (0.89–1.12)	0.9889	0.82 (0.65–1.03)	0.0806
61–70	0.96 (0.86–1.08)	0.5232	0.91 (0.72–1.15)	0.4317	1.08 (0.96–1.21)	0.1879	0.96 (0.77–1.19)	0.6824
71–80	1.13 (1.01–1.28)	0.0387	1.15 (0.91–1.45)	0.2492	1.37 (1.23–1.53)	<0.0001	1.22 (0.98–1.52)	0.0755
>80	1.52 (1.35–1.72)	<0.0001	1.56 (1.23–1.98)	0.0003	2.03 (1.81–2.27)	<0.0001	1.74 (1.39–2.17)	<0.0001
PSA (ng/ml)								
<20.0	1		1		1		1	
20–90.0	1.28 (1.2–1.36)	<0.0001	1.23 (1.1–1.37)	0.0002	1.22 (1.16–1.29)	<0.0001	1.22 (1.11–1.35)	<0.0001
>90	1.58 (1.49–1.68)	<0.0001	1.51 (1.36–1.66)	<0.0001	1.49 (1.41–1.57)	<0.0001	1.46 (1.34–1.6)	<0.0001
Unknown	1.37 (1.24–1.51)	<0.0001	1.68 (1.39–2.04)	<0.0001	1.37 (1.26–1.50)	<0.0001	1.70 (1.43–2.01)	<0.0001
T stage								
T1	1.06 (0.98–1.14)	0.1547	1.34 (1.19–1.51)	<.0001	1.07 (1.00–1.14)	0.0561	1.34 (1.21–1.50)	<0.0001
T2	1.06 (0.98–1.13)	0.1283	1.31 (1.16–1.47)	<.0001	1.07 (1.01–1.14)	0.0248	1.28 (1.15–1.42)	<0.0001
T3	1		1		1		1	
T4	1.43 (1.32–1.55)	<0.0001	1.77 (1.56–2.01)	<0.0001	1.39 (1.30–1.50)	<0.0001	1.71 (1.52–1.91)	<0.0001
N stage								
N0	1				1			
N1	1.10 (1.05–1.16)	0.0002			1.07 (1.02–1.11)	0.0060		
M stage								
M1a	1		1		1		1	
M1b	1.60 (1.45–1.77)	<0.0001	1.73 (1.47–2.04)	<.0001	1.38 (1.27–1.50)	<0.0001	1.66 (1.43–1.91)	<0.0001
M1c	1.95 (1.75–2.17)	<0.0001	2.30 (1.92–2.75)	<.0001	1.64 (1.50–1.80)	<0.0001	2.11 (1.80–2.47)	<0.0001
M1x	1.62 (1.38–1.89)	<0.0001	1.81 (1.47–2.23)	<.0001	1.44 (1.26–1.64)	<0.0001	1.83 (1.52–2.2)	<0.0001
Gleason score								
≤6	0.68 (0.59–0.79)	<0.0001	0.78 (0.52–1.19)	0.2477	0.80 (0.71–0.89)	<.0001	0.79 (0.55–1.13)	0.2028
7	1				1		1	
8	1.23 (1.14–1.32)	<0.0001	1.14 (0.97–1.33)	0.1089	1.13 (1.06–1.20)	0.0002	1.13 (0.98–1.30)	0.0842
9–10	1.79 (1.68–1.91)	<0.0001	1.98 (1.72–2.27)	<0.0001	1.60 (1.51–1.69)	<0.0001	1.84 (1.63–2.07)	<0.0001
Unknown	1.63 (1.50–1.77)	<0.0001	2.32 (1.98–2.72)	<0.0001	1.47 (1.37–1.57)	<0.0001	2.16 (1.88–2.48)	<0.0001
Local treatment								
No treatment			1					
Radiotherapy only			1.04 (0.96–1.14)	0.3435				
Surgery only			1.02 (0.91–1.14)	0.7578				
Radiotherapy and surgery			1.33 (1.08–1.63)	0.0066				

(Continued)

TABLE 2 Continued

Variable	Cancer-specific survival				Overall survival			
	2004–2013		2014–2018		2004–2013		2014–2018	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Chemotherapy								
No	1		1		1		1	
Yes	1.48 (1.37–1.61)	<0.0001	0.85 (0.78–0.93)	0.0004	1.39 (1.28–1.50)	<0.0001	0.78 (0.71–0.85)	<0.0001
Marital status								
Married	1		1		1		1	
Unmarried#	1.18 (1.13–1.24)	<0.0001	1.18 (1.10–1.28)	<0.0001	1.22 (1.17–1.27)	<0.0001	1.25 (1.17–1.34)	<0.0001
Unknown	0.95 (0.87–1.04)	0.2353	0.88 (0.75–1.03)	0.1073	0.96 (0.89–1.04)	0.3059	0.92 (0.80–1.06)	0.2490
Race								
White	1		1		1		1	
Black	0.98 (0.92–1.03)	0.3815	0.94 (0.85–1.04)	0.236	0.98 (0.94–1.04)	0.5274	0.94 (0.86–1.03)	0.1606
Other	0.74 (0.67–0.82)	<0.0001	0.64 (0.54–0.76)	<0.0001	0.79 (0.72–0.86)	<0.0001	0.69 (0.59–0.80)	<0.0001
Unknown	0.26 (0.14–0.47)	<0.0001	0.19 (0.07–0.50)	0.0009	0.43 (0.29–0.64)	<0.0001	0.23 (0.10–0.50)	0.0003
Region								
West	1		1		1		1	
South	1.16 (1.10–1.23)	<0.0001	1.14 (1.05–1.25)	0.0032	1.18 (1.12–1.24)	<.0001	1.21 (1.12–1.31)	<0.0001
Midwest	1.06 (0.99–1.14)	0.1171	1.11 (0.99–1.25)	0.0833	1.10 (1.03–1.17)	0.0029	1.17 (1.05–1.30)	0.0038
Northeast	1.00 (0.94–1.07)	0.9967	0.94 (0.84–1.05)	0.2603	1.07 (1.01–1.13)	0.0162	1.02 (0.92–1.12)	0.7366

#Unmarried including divorced, separated, single (never married), unmarried or domestic partner, and widowed.

findings. Our analysis indicates that practitioners are using a range of criteria in patient selection for early chemotherapy, which of course is then modulated by patient preferences after considering risks and benefits. Men receiving initial chemotherapy are younger, typically married (perhaps a marker of support systems), with more advanced T stage, higher Gleason score, positive lymph node metastasis, and more advanced M staging. Thus, it is likely that patients perceived to have a more aggressive phenotype based upon established risk factors are more likely to be offered chemotherapy by practitioners. Of course, we do not have data regarding the initial discussion of options for these men and what percentage were offered chemotherapy and declined. One additional factor may be

TABLE 3 Multivariate survival analyses of impacts of chemotherapy on survival in patients with *de novo* metastatic prostate adenocarcinoma among different age groups during 2014–2018.

Variable	Cancer- specific survival		Overall survival	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Age groups				
≤ 70 years				
Chemotherapy (yes vs no)	1.00 (0.89–1.12)	0.9970	0.93 (0.84–1.03)	0.1712
71–75 years				
Chemotherapy (yes vs no)	0.75 (0.6–0.94)	0.0134	0.68 (0.55–0.85)	0.0005
76–80 years				
Chemotherapy (yes vs no)	0.65 (0.48–0.88)	0.0060	0.60 (0.45–0.79)	0.0004
> 80 years				
Chemotherapy (yes vs no)	1.01 (0.74–1.37)	0.9734	0.85 (0.64–1.13)	0.2656

TABLE 4 Multivariate survival analyses of impacts chemotherapy on survival in patients with *de novo* metastatic prostate adenocarcinoma at varied metastatic sites during 2014–2018.

Metastatic site	Cancer- specific survival		Overall survival	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Distant lymph node metastasis only				
Chemotherapy (yes vs no)	1.01 (0.61–1.66)	0.9741	0.79 (0.49–1.28)	0.3376
Bone metastasis only with or without lymph node metastasis				
Chemotherapy (yes vs no)	0.87 (0.78–0.96)	0.0059	0.59 (0.48–0.73)	<0.0001
Visceral metastasis (lung, liver or brain (with or without bone or lymph node metastasis)				
Chemotherapy (yes vs no)	0.63 (0.50–0.79)	<0.0001	0.58 (0.47–0.72)	<0.0001

TABLE 5 Multivariate analyses of risk factors correlated with survival in propensity score matched patients with metastatic prostate adenocarcinoma diagnosed during 2004–2013 and 2014–2018.

	Cancer- specific survival				Overall survival			
	2004–2013		2014–2018		2004–2013		2014–2018	
Variable	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Age (years)								
≤50	1		1		1		1	
51–60	0.98 (0.78–1.24)	0.8659	0.84 (0.65–1.08)	0.1635	1.02 (0.82–1.28)	0.8346	0.81 (0.64–1.03)	0.0889
61–70	0.98 (0.78–1.23)	0.8723	0.94 (0.74–1.19)	0.5914	1.07 (0.86–1.33)	0.5353	1.00 (0.80–1.26)	0.9904
71–80	1.18 (0.93–1.50)	0.186	1.02 (0.79–1.31)	0.8895	1.36 (1.08–1.71)	0.0085	1.10 (0.87–1.40)	0.4122
>80	1.68 (1.25–2.27)	0.0006	1.52 (1.11–2.08)	0.0088	2.07 (1.57–2.71)	<0.0001	1.55 (1.15–2.08)	0.0036
PSA (ng/ml)								
<20.0	1		1		1		1	
20–90.0	1.19 (1.00–1.42)	0.0508	1.13 (0.96–1.34)	0.1468	1.12 (0.95–1.32)	0.1685	1.15 (0.98–1.34)	0.0931
>90	1.44 (1.24–1.67)	<0.0001	1.33 (1.14–1.55)	0.0003	1.36 (1.19–1.56)	<0.0001	1.36 (1.17–1.57)	<0.0001
Unknown	0.93 (0.70–1.26)	0.6520	1.38 (0.93–2.06)	0.1094	0.99 (0.76–1.29)	0.9427	1.57 (1.11–2.23)	0.0108
T stage								
T1	1		1		1		1	
T2	0.90 (0.77–1.04)	0.1576	0.99 (0.86–1.13)	0.8491	0.94 (0.82–1.08)	0.3940	1.00 (0.88–1.13)	0.9927
T3	0.85 (0.70–1.03)	0.1009	0.77 (0.65–0.93)	0.0054	0.86 (0.72–1.04)	0.1121	0.80 (0.68–0.94)	0.0082
T4	1.35 (1.14–1.60)	0.0005	1.23 (1.05–1.43)	0.0094	1.32 (1.13–1.55)	0.0006	1.25 (1.08–1.44)	0.0025
M stage								
M1a	1		1		1		1	
M1b	1.66 (1.28–2.16)	0.0001	1.75 (1.31–2.36)	0.0002	1.58 (1.25–2.00)	0.0002	1.69 (1.29–2.22)	0.0001
M1c	2.02 (1.53–2.65)	<0.0001	2.39 (1.75–3.26)	<0.0001	1.90 (1.48–2.44)	<0.0001	2.27 (1.71–3.02)	<0.0001
M1x	1.32 (0.89–1.94)	0.1648	1.83 (1.26–2.66)	0.0015	1.41 (1.00–1.98)	0.0534	1.98 (1.41–2.77)	<0.0001
Gleason score								
≤6	0.62 (0.38–1.02)	0.0576	1.25 (0.66–2.37)	0.4953	0.81 (0.54–1.21)	0.3003	1.03 (0.55–1.95)	0.9193
7	1		1		1		1	

(Continued)

TABLE 5 Continued

Variable	Cancer- specific survival				Overall survival			
	2004–2013		2014–2018		2004–2013		2014–2018	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
8	1.07 (0.85–1.34)	0.5692	1.13 (0.85–1.50)	0.3998	1.03 (0.83–1.27)	0.8039	1.12 (0.86–1.44)	0.4043
9–10	1.52 (1.25–1.84)	<0.0001	2.12 (1.65–2.72)	<0.0001	1.43 (1.20–1.70)	<0.0001	2.01 (1.60–2.53)	<0.0001
Unknown	1.40 (1.11–1.77)	0.005	2.22 (1.66–2.97)	<0.0001	1.35 (1.09–1.67)	0.0065	2.21 (1.70–2.88)	<0.0001
Local treatment								
Nol treatment			1				1	
Radiotherapy only			1.09 (0.96–1.25)	0.1882			1.06 (0.94–1.20)	0.3266
Surgery only			0.89 (0.71–1.11)	0.3077			0.93 (0.75–1.14)	0.4543
Radiotherapy and surgery			1.65 (1.19–2.28)	0.0024			1.45 (1.06–1.99)	0.0200
Chemotherapy								
No	1		1		1		1	
Yes	1.57 (1.39–1.77)	<0.0001	0.85 (0.76–0.95)	0.0030	1.48 (1.33–1.65)	<0.0001	0.78 (0.70–0.86)	<0.0001
Marital status								
Married					1			
Unmarried#					1.21 (1.07–1.37)	0.0028		
Unknown					1.04 (0.82–1.33)	0.7425		
Race								
White	0.89 (0.76–1.04)	0.1372	1				1	
Black	1		1.02 (0.88–1.18)	0.8188			1.04 (0.91–1.20)	0.5424
Other	0.70 (0.49–1.00)	0.0473	0.62 (0.48–0.82)	0.0007			0.66 (0.52–0.85)	0.0013
Unknown			0.36 (0.09–1.44)	0.1483			0.31 (0.08–1.26)	0.1015
Region								
West	1				1		1	
South	1.20 (1.03–1.40)	0.0185			1.27 (1.11–1.45)	0.0007	1.14 (1.00–1.29)	0.0448
Midwest	1.13 (0.94–1.36)	0.1823			1.18 (1.00–1.40)	0.052	0.98 (0.82–1.16)	0.7858
Northeast	0.94 (0.79–1.13)	0.5243			1.05 (0.90–1.24)	0.5217	1.07 (0.92–1.25)	0.3815

Unmarried including divorced, separated, single (never married), unmarried or domestic partner, and widowed.

TABLE 6 Descriptive characteristics of patients with a *de novo* diagnosis of prostate neuroendocrine carcinoma who were initially treated with or without chemotherapy during 2004–2013 and 2014–2018.

Variable	2004–2013 n (%)			2014–2018 n (%)		
	No chemotherapy 177 (48)	Chemotherapy 192 (52)	*p*-value	No chemotherapy 133 (44)	Chemotherapy 169 (56)	*p*-value
Age (years)						
Mean ± SD	72.8 ± 11.1	67.9 ± 11.3	<0.0001	73.3 ± 11.2	67.2 ± 9.3	<0.0001
Median (range)	73 (44–96)	68 (30–92)		73 (44–96)	67 (39–92)	
Distribution						
≤50	3 (2)	11 (6)	0.0009	3 (2)	10 (6)	<0.0001

(Continued)

10.3389/fonc.2023.1165188

TABLE 6 Continued

Variable	2004–2013 n (%)			2014–2018 n (%)		
	No chemotherapy 177 (48)	Chemotherapy 192 (52)	*p*-value	No chemotherapy 133 (44)	Chemotherapy 169 (56)	*p*-value
51–60	21 (12)	41 (21)		16 (12)	22 (13)	
61–70	55 (31)	61 (32)		37 (28)	75 (44)	
71–80	46 (26)	51 (27)		38 (29)	51 (30)	
>80	52 (29)	28 (15)		39 (29)	11 (7)	
PSA (ng/ml)						
Mean ± SD	34.7 ± 39.7	22 ± 33.9	0.0010	35.7 ± 39.3	27.3 ± 36.8	0.0787
Median (range)	10.5 (0.1–99.8)	5.8 (0.1–99.8)		13.6 (0.1–99.8)	6.1 (0.1–99.8)	
Distribution			0.0422			0.0113
< 20.0	91 (51)	121 (63)		64 (48)	105 (62)	
20.0–90.0	23 (13)	16 (8)		18 (14)	24 (14)	
>90.0	36 (20)	23 (12)		25 (19)	27 (16)	
Unknown	27 (15)	32 (17)		26 (20)	13 (8)	
T stage			0.0002			0.0530
T1	36 (20)	30 (16)		27 (20)	20 (12)	
T2	69 (39)	41 (21)		36 (27)	38 (22)	
T3	18 (10)	36 (19)		23 (17)	27 (16)	
T4	54 (31)	85 (44)		47 (35)	84 (50)	
N stage			0.0003			0.0016
N0	118 (67)	92 (48)		77 (58)	67 (40)	
N1	59 (33)	100 (52)		56 (42)	102 (60)	
M stage			0.0006			0.0002
M0	102 (58)	74 (39)		68 (51)	44 (26)	
M1a	9 (5)	11 (6)		8 (6)	11 (7)	
M1b	27 (15)	36 (19)		21 (16)	41 (24)	
M1c	39 (22)	62 (32)		35 (26)	65 (38)	
M1X	0 (0)	9 (5)		1 (1)	8 (5)	
Gleason score			0.2540			0.0162
≤6	7 (4)	11 (6)		2 (2)	0 (0)	
7	8 (5)	3 (2)		10 (8)	5 (3)	
8	11 (6)	8 (4)		10 (8)	8 (5)	
9–10	53 (30)	50 (26)		45 (34)	43 (25)	
Unknown	98 (55)	120 (63)		66 (50)	113 (67)	
Local treatment						
No	76 (43)	65 (34)	<0.0001	66 (50)	70 (41)	0.0013
Radiotherapy only	24 (14)	65 (34)		18 (14)	53 (31)	
Surgery only	66 (37)	34 (18)		37 (28)	28 (17)	

(Continued)

TABLE 6 Continued

Variable	2004–2013 n (%)			2014–2018 n (%)		
	No chemotherapy 177 (48)	Chemotherapy 192 (52)	*p*-value	No chemotherapy 133 (44)	Chemotherapy 169 (56)	*p*-value
Radiotherapy and surgery	11 (6)	28 (15)		12 (9)	18 (11)	
Marital status						
Married	109 (62)	139 (72)	0.0546	83 (62)	126 (75)	0.0510
Unmarried[#]	56 (32)	47 (24)		44 (33)	35 (21)	
Unknown	12 (7)	6 (3)		6 (5)	8 (5)	
Race						
White	150 (85)	175 (91)	0.1040	106 (80)	138 (82)	0.9540
Black	16 (9)	12 (6)		16 (12)	17 (10)	
Other	11 (6)	4 (2)		10 (8)	13 (8)	
Unknown	0 (0)	1 (1)		1 (1)	1 (1)	
Region						
West	96 (54)	108 (56)	0.9203	70 (53)	98 (58)	0.0007
South	37 (21)	39 (20)		42 (32)	28 (17)	
Midwest	15 (8)	18 (9)		17 (13)	20 (12)	
Northeast	29 (16)	27 (14)		4 (3)	23 (14)	

[#]Unmarried including divorced, separated, single (never married), unmarried or domestic partner, and widowed.

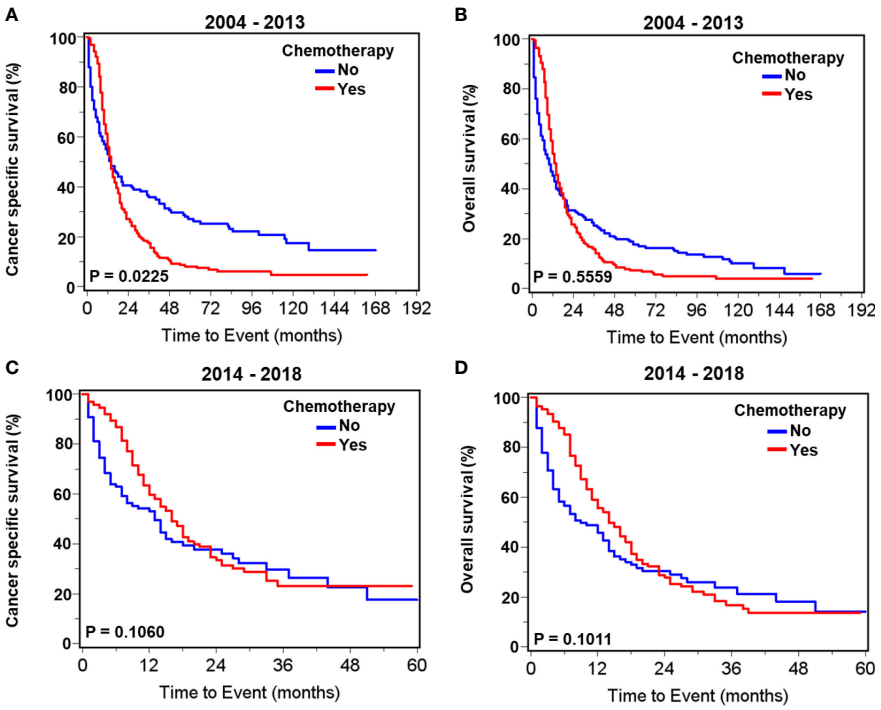


FIGURE 3
Kaplan–Meier survival curves for cancer-specific and overall survival in patients with *de novo* neuroendocrine prostate carcinoma with or without chemotherapy. For patients diagnosed during 2004–2013, curves of cancer- specific survival **(A)** and overall survival **(B)**. For patients diagnosed during 2014–2018, curves of cancer- specific survival **(C)** and overall survival **(D)**.

TABLE 7 Multivariate analyses of risk factors related to survival in patients with a *de novo* diagnosis of prostate neuroendocrine carcinoma during 2004–2013 and 2014–2018.

	Cancer- specific survival				Overall survival			
	2004–2013		2014–2018		2004–2013		2014–2018	
Variable	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Ag (years)								
≤50	1		1		1		1	
51–60	1.30 (0.70–2.41)	0.4163	1.15 (0.44–3)	0.7763	1.30 (0.69–2.45)	0.4226	0.92 (0.38–2.23)	0.8509
61–70	1.26 (0.68–2.31)	0.4622	1.61 (0.93–2.77)	0.0897	1.25 (0.68–2.33)	0.4724	1.37 (0.62–3.03)	0.4371
71–80	2.11 (1.14–3.90)	0.0181	1.47 (0.83–2.62)	0.1881	2.40 (1.29–4.45)	0.0057	1.42 (0.63–3.20)	0.3983
>80	2.52 (1.34–4.75)	0.0041	2.64 (1.45–4.83)	0.0016	2.89 (1.53–5.44)	0.001	2.52 (1.09–5.84)	0.0313
PSA (ng/ml)								
< 20.0	1				1			
20.0–90.0	0.61 (0.39–0.94)	0.0243			0.64 (0.43–0.95)	0.0274		
>90	1.01 (0.71–1.42)	0.9767			1.04 (0.75–1.45)	0.8021		
Unknown	1.14 (0.80–1.62)	0.4645			1.29 (0.95–1.77)	0.1039		
T stage								
T1	1				1			
T2	0.87 (0.61–1.24)	0.4383			1.41 (0.93–2.14)	0.1029		
T3	0.76 (0.56–1.04)	0.0892			1.32 (0.91–1.91)	0.1381		
T4	0.64 (0.44–0.93)	0.0194			1.73 (1.22–2.47)	0.0023		
N stage								
N0	1				1			
N1	1.36 (1.04–1.76)	0.0226			1.34 (1.04–1.71)	0.0219		
M stage								
M0	1		1		1		1	
M1a	1.54 (0.88–2.71)	0.1342	0.41 (0.15–1.13)	0.0842	1.37 (0.81–2.31)	0.2474	0.48 (0.21–1.11)	0.0851
M1b	1.84 (1.29–2.63)	0.0008	1.16 (0.73–1.85)	0.5334	1.61 (1.15–2.25)	0.0058	1.15 (0.76–1.73)	0.5191
M1c	2.31 (1.68–3.18)	<0.0001	3.88 (2.62–5.75)	<.0001	2.47 (1.84–3.31)	<.0001	3.24 (2.26–4.65)	<0.0001
M1x	1.95 (0.92–4.14)	0.0827	1.32 (0.52–3.36)	0.5613	1.37 (0.81–2.31)	0.2474	1.21 (0.52–2.83)	0.6649
Gleason score								
≤6	1							
7	1.05 (0.35–3.11)	0.9337						
8	0.96 (0.41–2.27)	0.9270						
9–10	1.42 (0.71–2.82)	0.3216						
Unknown	2.04 (1.03–4.01)	0.0399						
Chemotherapy								
No	1		1		1		1	
Yes	0.99 (0.76–1.29)	0.9138	0.62 (0.45–0.87)	0.0055	0.89 (0.70–1.14)	0.3540	0.69 (0.51–0.94)	0.0176
Marital status								
Married			1		1		1	

(Continued)

TABLE 7 Continued

Variable	Cancer- specific survival				Overall survival			
	2004–2013		2014–2018		2004–2013		2014–2018	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Unmarried#					1.33 (1.03–1.71)	0.0284	1.38 (1.01–1.89)	0.0419
Unknown					0.80 (0.46–1.38)	0.4164	0.8 (0.37–1.75)	0.5756
Race								
White	1				1			
Black	1.08 (0.70–1.66)	0.7311			0.89 (0.58–1.39)	0.6127		
Other	2.06 (1.09–3.90)	0.0267			1.87 (1.05–3.34)	0.0346		
Unknown	1.72 (0.23–12.94)	0.5976			1.78 (0.23–13.51)	0.5791		
Region								
West					1			
South					1.45 (1.08–1.95)	0.0133		
Midwest					0.94 (0.63–1.42)	0.7784		
Northeast					0.96 (0.69–1.34)	0.8289		

#Unmarried including divorced, separated, single (never married), unmarried or domestic partner, and widowed.

referral patterns with the urologist typically serving as the initial focal point for diagnosis and perhaps not having medical oncology engaged at the time of the initial treatment plan. Clearly, with only 30% of men receiving chemotherapy, the perceived benefits do not exceed risks in the minds of practitioners and or patients.

Interestingly, we found that from 2004 to 2013, the administration of chemotherapy was associated with worse prognosis compared with no chemotherapy. As chemotherapy became more widespread, we observed a significantly improved cancer- specific and overall survival during 2014–2018. Notably, these findings are verified in subsequent propensity score matching analyses. The poorer outcomes in 2004–2013 likely represents the use of initial chemotherapy for men with greater cancer volume and more aggressive disease, a subgroup destined to have a shorter life expectancy. The lack of a standard of care chemotherapy regimen (agents, dose, and duration) for *de novo* distant metastatic patients may also attribute to the worse outcome during the period. Multiple factors, such as cancer grade, distribution of lesions and volume of disease, PSA, age, and other variables impact offering the chemotherapy option. A previous publication, employing an older version of SEER database (2014–2015) with far less data, also found that chemotherapy-exposed prostate cancer patients exhibited significantly better overall survival (HR=0.82, 95% CI: 0.72–0.96, p=0.01) compared to their chemotherapy- naive counterparts (35). The finding was confirmed in propensity score matching analyses (multivariable HR: 0.77, 95% CI: 0.66-0.90, $p<0.001$) (35). Utilizing a large national cancer database in the United States (2014–2015), another retrospective cohort study revealed that upfront chemotherapy was associated with longer overall survival (HR= 0.78, 95% CI: 68–0.89, $p < 0.001$) in men with *de novo*, treatment-naive metastatic prostate cancer after adjusting for patient and clinical variables (36).

Due to lack of precise data in the SEER database regarding lesion numbers, size, and locations of metastasis, it is not possible to precisely define the high-volume disease. Our multivariate survival analysis suggests that chemotherapy displayed a more beneficial impact for men with potentially higher volume. Men with the nodal metastasis experience no benefit, whereas those with bone metastasis fare better with chemotherapy, and the greatest benefit is seen in men with the visceral disease with or without bone and nodal metastasis.

Not surprisingly, age is shown to be a risk factor for death and particularly strong over the age of 80 years. This and other studies suggest that chemotherapy is less often prescribed for older patients with metastatic prostate cancers (37, 38) and likely due perceived risks associated with frailty, accumulating comorbidities, and poor resilience. Interestingly, we find the benefit of chemotherapy to be best in the ages of 70 –80 years, both on cancer- specific and overall survival. Those younger than 70 and older than 80 tended to gain little or no benefit from chemotherapy. Perhaps, younger men with *de novo* metastatic prostate cancer have a more aggressive disease that is less sensitive to therapy, while older men have comorbidities impacting resilience and tolerability. Similar to our finding, another report suggests that chemotherapy plus ADT, compared to ADT alone, was associated with improved overall survival in *de novo* metastatic prostate cancer patients ≥70 years but not in patients <70 years (39).

Our multivariate survival analyses quantitate the impact of several relevant variables on survival in this cohort. We have

limited data on the impact of integrating radiotherapy and surgery but do see a worse outcome noted for those receiving both. It is possible that men with significant and symptomatic local disease have a more aggressive phenotype or medical complications that impact survival. We see a clear trend for married men doing better in survival, perhaps a marker for stronger support systems and compliance with care plans. We did not detect a difference in response based on black vs. white race, but the Southern region of the United States consistently shows poorer survival than the Midwest, with the West and Northeast being similar, perhaps reflecting the impact of social and economic issues on health care access and quality (40, 41). Higher grade cancers and greater disease burden, as indicated by PSA, TNM staging, and Gleason scoring, were strongly related to poor outcomes for those presenting with *de novo* metastatic adenocarcinoma.

Neuroendocrine carcinoma is the rare histological type of prostate cancer with the worst prognosis (25, 42). The disease is typically defined histopathologically and often characterized by lower PSA secretion, higher risk of metastasis, an inferior response to ADT, and poor prognosis (26, 43–45). Small early studies supported the use of chemotherapy with agents often used for cancers of other tissue origins with neuroendocrine features (46). Chemotherapy, radiotherapy, or combinations are associated with improved overall survival compared with palliative therapy (47). We observe that an average of 54% of patients with neuroendocrine prostate cancer are treated with first-line chemotherapy and is steady from 2004 to 2018. Chemotherapy is associated with improved cancer-specific and overall survival during 2014–2018, but not during 2004–2013, perhaps due to improved supportive care plans and better patient selection. Hence, our findings may support the use of chemotherapy for both *de novo* and treatment-emergent neuroendocrine prostate cancer due to the potential survival benefits.

This retrospective study has limitations. Our study is subject to the constraints of the SEER database, including the precision of data collection and the number of variables collected. There is a lack of information on the specific chemotherapy regime, dose of drug, compliance, and dose intensity including the number of cycles. The database has no information on concurrent ADT and the types or duration of agents provided. Indeed, we suspect that the increased use of effective agents impacting androgen receptor signaling may reduce the frequency of selecting taxane-based chemotherapy in the up-front approach to *de novo* metastatic disease since 2016 (Figure 1). There is no information on other important outcomes such as toxicities of chemotherapy, quality of life, recurrence of cancer and additional therapy, and the dynamic change in PSA. Our study is limited by the inherent challenges of a retrospective cohort design. For example, it is likely that patients with better performance status were selected for chemotherapy, which may contribute to better survival outcomes. Hence, selection bias is inevitable but is clearly a component of practice decisions. The strength of this study is the very large sample size providing accurate insight into practice patterns in a real world setting during a period when new data were emerging.

Conclusions

Chemotherapy has been increasingly employed in the community for men with *de novo* metastatic adenocarcinoma at diagnosis following a series of publications in 2013–2014, yet for less than one-third of men. Our data suggest that both medical practitioners and patients may be carefully considering the risks and benefits for each individual based upon age, histopathological features, PSA, staging criteria, comorbidities, and a number of factors such as overall performance status. Findings of this study support the initial treatment with chemotherapy in men in the 70–80 age group presenting *de novo* with more aggressive features or greater volume of metastatic disease. Clearly, our work suggests that shared decision making is the strategy in the community for men presenting with metastatic adenocarcinoma who are mostly seniors and often with comorbidities. In contrast, the treatment of neuroendocrine prostate cancer with initial chemotherapy has been stable at approximately 50%, but with improving outcomes in recent years.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

Concept and design: SW and SC. Data acquisition and analysis: SW and SC. Interpretation of data: all authors. Drafting of the manuscript: SW and SC. Critical revision of the manuscript for important intellectual content: all authors. Supervision: SC. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the Prostate Cancer Prevention Development Fund supported by the Karlsberger Family (SKC, 302024) and The Biostatistics Shared Resource supported by The Ohio State University Comprehensive Cancer Center (NIH P30CA016058).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1165188/full#supplementary-material

References

1. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA: Cancer J Clin* (2023) 73(1):17–48. doi: 10.3322/caac.21763

2. Moyer VA, Force USPST. Screening for prostate cancer: U.S. preventive services task force recommendation statement. *Ann Intern Med* (2012) 157(2):120–34. doi: 10.1059/0003-4819-157-2-201207170-00459

3. Desai MM, Cacciamani GE, Gill K, Zhang J, Liu L, Abreu A, et al. Trends in incidence of metastatic prostate cancer in the US. *JAMA Netw Open* (2022) 5(3): e222246. doi: 10.1001/jamanetworkopen.2022.2246

4. Jemal A, Fedewa SA, Ma J, Siegel R, Lin CC, Brawley O, et al. Prostate cancer incidence and PSA testing patterns in relation to USPSTF screening recommendations. *JAMA* (2015) 314(19):2054–61. doi: 10.1001/jama.2015.14905

5. Eapen RS, Herlemann A, Washington SL3rd, Cooperberg MR. Impact of the united states preventive services task force 'D' recommendation on prostate cancer screening and staging. *Curr Opin Urol* (2017) 27(3):205–9. doi: 10.1097/MOU.0000000000000383

6. Huggins C HC. Studies on prostatic cancer. i. the effect of castration, of estrogen and androgen injection on serum phosphatases in metastatic carcinoma of the prostate. 93–7. *Cancer Res* (1941) 1:293–97.

7. Chi KN, Agarwal N, Bjartell A, Chung BH, Pereira de Santana Gomes AJ, Given R, et al. Apalutamide for metastatic, castration-sensitive prostate cancer. *N Engl J Med* (2019) 381(1):13–24. doi: 10.1056/NEJMoa1903307

8. de Wit R, de Bono J, Sternberg CN, Fizazi K, Tombal B, Wulfing C, et al. Cabazitaxel versus abiraterone or enzalutamide in metastatic prostate cancer. *N Engl J Med* (2019) 381(26):2506–18. doi: 10.1056/NEJMoa1911206

9. James ND, de Bono JS, Spears MR, Clarke NW, Mason MD, Dearnaley DP, et al. Abiraterone for prostate cancer not previously treated with hormone therapy. *N Engl J Med* (2017) 377(4):338–51. doi: 10.1056/NEJMoa1702900

10. Sweeney CJ, Chen YH, Carducci M, Liu G, Jarrard DF, Eisenberger M, et al. Chemohormonal therapy in metastatic hormone-sensitive prostate cancer. *New Engl J Med* (2015) 373(8):737–46. doi: 10.1056/NEJMoa1503747

11. Kyriakopoulos CE, Chen YH, Carducci MA, Liu G, Jarrard DF, Hahn NM, et al. Chemohormonal therapy in metastatic hormone-sensitive prostate cancer: long-term survival analysis of the randomized phase III E3805 CHAARTED trial. *J Clin Oncol* (2018) 36(11):1080–7. doi: 10.1200/JCO.2017.75.3657

12. Siegel DA, O'Neil ME, Richards TB, Dowling NF, Weir HK. Prostate cancer incidence and survival, by stage and Race/Ethnicity - united states, 2001-2017. *MMWR Morbidity mortality weekly Rep* (2020) 69(41):1473–80. doi: 10.15585/mmwr.mm6941a1

13. Armstrong AJ, Lin P, Tombal B, Saad F, Higano CS, Joshua AM, et al. Five-year survival prediction and safety outcomes with enzalutamide in men with chemotherapy-naive metastatic castration-resistant prostate cancer from the PREVAIL trial. *Eur Urol* (2020) 78(3):347–57. doi: 10.1016/j.eururo.2020.04.061

14. Halabi S, Lin CY, Kelly WK, Fizazi KS, Moul JW, Kaplan EB, et al. Updated prognostic model for predicting overall survival in first-line chemotherapy for patients with metastatic castration-resistant prostate cancer. *J Clin Oncol* (2014) 32(7):671–7. doi: 10.1200/JCO.2013.52.3696

15. Tangen CM, Hussain MH, Higano CS, Eisenberger MA, Small EJ, Wilding G, et al. Improved overall survival trends of men with newly diagnosed M1 prostate cancer: a SWOG phase III trial experience (S8494, S8894 and S9346). *J Urol* (2012) 188 (4):1164–9. doi: 10.1016/j.juro.2012.06.046

16. Kantoff PW, Halabi S, Conaway M, Picus J, Kirshner J, Hars V, et al. Hydrocortisone with or without mitoxantrone in men with hormone-refractory prostate cancer: results of the cancer and leukemia group b 9182 study. *J Clin Oncol* (1999) 17(8):2506–13. doi: 10.1200/JCO.1999.17.8.2506

17. Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, Chi KN, et al. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. *N Engl J Med* (2004) 351(15):1502–12. doi: 10.1056/NEJMoa040720

18. Petrylak DP, Tangen CM, Hussain MH, Lara PNJr., Jones JA, Taplin ME, et al. Docetaxel and estramustine compared with mitoxantrone and prednisone for advanced refractory prostate cancer. *N Engl J Med* (2004) 351(15):1513–20. doi: 10.1056/NEJMoa041318

19. Armstrong AJ, Carducci MA. Chemotherapy for advanced prostate cancer: results of new clinical trials and future studies. *Curr Oncol Rep* (2005) 7(3):220–7. doi: 10.1007/s11912-005-0077-y

20. Berry W, Eisenberger M. Achieving treatment goals for hormone-refractory prostate cancer with chemotherapy. *Oncologist* (2005) 10 Suppl 3:30–9. doi: 10.1634/theoncologist.10-90003-30

21. de Bono JS, Oudard S, Ozguroglu M, Hansen S, Machiels JP, Kocak I, et al. Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: a randomised open-label trial. *Lancet* (2010) 376(9747):1147–54. doi: 10.1016/S0140-6736(10)61389-X

22. Clarke NW, Ali A, Ingleby FC, Hoyle A, Amos CL, Attard G, et al. Addition of docetaxel to hormonal therapy in low- and high-burden metastatic hormone sensitive prostate cancer: long-term survival results from the STAMPEDE trial. *Ann Oncol* (2019) 30(12):1992–2003. doi: 10.1093/annonc/mdz396

23. Gravis G, Fizazi K, Joly F, Oudard S, Priou F, Esterni B, et al. Androgen-deprivation therapy alone or with docetaxel in non-castrate metastatic prostate cancer (GETUG-AFU 15): a randomised, open-label, phase 3 trial. *Lancet Oncol* (2013) 14 (2):149–58. doi: 10.1016/S1470-2045(12)70560-0

24. Mohler JL, Kantoff PW, Armstrong AJ, Bahnson RR, Cohen MB, D'Amico AV, et al. NCCN clinical practice guidelines in oncology, in: *Prostate cancer. version 1* (2014). NCCNorg (Accessed Novemver 08, 2022).

25. Bronkema C, Arora S, Sood A, Dalela D, Keeley J, Borchert A, et al. Rare histological variants of prostate adenocarcinoma: a national cancer database analysis. *J Urol* (2020) 204(2):260–6. doi: 10.1097/JU.0000000000001011

26. Yao J, Liu Y, Liang X, Shao J, Zhang Y, Yang J, et al. Neuroendocrine carcinoma as an independent prognostic factor for patients with prostate cancer: a population-based study. *Front Endocrinol (Lausanne)* (2021) 12:778758. doi: 10.3389/fendo.2021.778758

27. Kashaf MS, McGill E. Does shared decision making in cancer treatment improve quality of life? a systematic literature review. *Med Decis Making* (2015) 35(8):1037–48. doi: 10.1177/0272989X15598529

28. Rosenthal SA, Hunt D, Sartor AO, Pienta KJ, Gomella L, Grignon D, et al. A phase 3 trial of 2 years of androgen suppression and radiation therapy with or without adjuvant chemotherapy for high-risk prostate cancer: final results of radiation therapy oncology group phase 3 randomized trial NRG oncology RTOG 9902. *Int J Radiat Oncol Biol Phys* (2015) 93(2):294–302. doi: 10.1016/j.ijrobp.2015.05.024

29. Bouman-Wammes EW, van den Berg HP, de Munck L, Beeker A, Smorenburg CH, Vervenne WL, et al. A randomised phase II trial of docetaxel versus docetaxel plus carboplatin in patients with castration-resistant prostate cancer who have progressed after response to prior docetaxel chemotherapy: the RECARDO trial. *Eur J Cancer* (2018) 90:1–9. doi: 10.1016/j.ejca.2017.11.021

30. Fléchon A, Pouessel D, Ferlay C, Perol D, Beuzeboc P, Gravis G, et al. Phase II study of carboplatin and etoposide in patients with anaplastic progressive metastatic castration-resistant prostate cancer (mCRPC) with or without neuroendocrine differentiation: results of the French genito-urinary tumor group (GETUG) P01 trial. *Ann Oncol* (2011) 22(11):2476–81. doi: 10.1093/annonc/mdr004

31. Berthold DR, Pond GR, Soban F, de Wit R, Eisenberger M, Tannock IF. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer: updated survival in the TAX 327 study. *J Clin Oncol* (2008) 26(2):242–5. doi: 10.1200/JCO.2007.12.4008

32. Kushnir I, Mallick R, Ong M, Canil C, Bosse D, Koczka K, et al. Docetaxel dose-intensity effect on overall survival in patients with metastatic castrate-sensitive prostate

cancer. *Cancer Chemother Pharmacol* (2020) 85(5):863–8. doi: 10.1007/s00280-020-04063-7

33. Nuhn P, De Bono JS, Fizazi K, Freedland SJ, Grilli M, Kantoff PW, et al. Update on systemic prostate cancer therapies: management of metastatic castration-resistant prostate cancer in the era of precision oncology. *Eur Urol* (2019) 75(1):88–99. doi: 10.1016/j.eururo.2018.03.028

34. Zhao J, Mao Z, Fedewa SA, Nogueira L, Yabroff KR, Jemal A, et al. The affordable care act and access to care across the cancer control continuum: a review at 10 years. *CA: Cancer J Clin* (2020) 70(3):165–81. doi: 10.3322/caac.21604

35. Hoeh B, Wurnschimmel C, Flammia RS, Horlemann B, Sorce G, Chierigo F, et al. Effect of chemotherapy on overall survival in contemporary metastatic prostate cancer patients. *Front Oncol* (2021) 11:778858. doi: 10.3389/fonc.2021.778858

36. Weiner AB, Ko OS, Li EV, Vo AX, Desai AS, Breen KJ, et al. Survival following upfront chemotherapy for treatment-naive metastatic prostate cancer: a real-world retrospective cohort study. *Prostate Cancer Prostatic Dis* (2021) 24(1):261–7. doi: 10.1038/s41391-020-00278-0

37. Jha GG, Anand V, Soubra A, Konety BR. Challenges of managing elderly men with prostate cancer. *Nat Rev Clin Oncol* (2014) 11(6):354–64. doi: 10.1038/nrclinonc.2014.71

38. Oh WK, Tully P, Kantoff PW, Regan MM. Physician attitudes toward cytotoxic chemotherapy use in patients with advanced prostate carcinoma. *Cancer*. (2003) 97 (9):2171–9. doi: 10.1002/cncr.11344

39. Lage DE, Michaelson MD, Lee RJ, Greer JA, Temel JS, Sweeney CJ. Outcomes of older men receiving docetaxel for metastatic hormone-sensitive prostate cancer. *Prostate Cancer Prostatic Dis* (2021) 24(4):1181–8. doi: 10.1038/s41391-021-00389-2

40. Oates GR, Jackson BE, Partridge EE, Singh KP, Fouad MN, Bae S. Sociodemographic patterns of chronic disease: how the mid-south region compares to the rest of the country. *Am J Prev Med* (2017) 52(1S1):S31–S9. doi: 10.1016/j.amepre.2016.09.004

41. Islami F, Guerra CE, Minihan A, Yabroff KR, Fedewa SA, Sloan K, et al. American Cancer society's report on the status of cancer disparities in the united states, 2021. *CA Cancer J Clin* (2022) 72(2):112–43. doi: 10.3322/caac.21703

42. Alanee S, Moore A, Nutt M, Holland B, Dynda D, El-Zawahry A, et al. Contemporary incidence and mortality rates of neuroendocrine prostate cancer. *Anticancer Res* (2015) 35(7):4145–50.

43. Zaffuto E, Pompe R, Zanaty M, Bondarenko HD, Leyh-Bannurah SR, Moschini M, et al. Contemporary incidence and cancer control outcomes of primary neuroendocrine prostate cancer: a SEER database analysis. *Clin Genitourin cancer* (2017) 15(5):e793–800. doi: 10.1016/j.clgc.2017.04.006

44. Zhu J, Liang X, Wu D, Chen S, Yang B, Mao W, et al. Clinicopathological characteristics and survival outcomes in neuroendocrine prostate cancer: a population-based study. *Med (Baltimore)* (2021) 100(15):e25237. doi: 10.1097/MD.0000000000025237

45. Conteduca V, Oromendia C, Eng KW, Bareja R, Sigouros M, Molina A, et al. Clinical features of neuroendocrine prostate cancer. *Eur J Cancer* (2019) 121:7–18. doi: 10.1016/j.ejca.2019.08.011

46. Moore SR, Reinberg Y, Zhang G. Small cell carcinoma of prostate: effectiveness of hormonal versus chemotherapy. *Urology* (1992) 39(5):411–6. doi: 10.1016/0090-4295(92)90235-O

47. Wang HT, Yao YH, Li BG, Tang Y, Chang JW, Zhang J. Neuroendocrine prostate cancer (NEPC) progressing from conventional prostatic adenocarcinoma: factors associated with time to development of NEPC and survival from NEPC diagnosis-a systematic review and pooled analysis. *J Clin Oncol* (2014) 32(30):3383–90. doi: 10.1200/JCO.2013.54.3553

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 14 June 2023
DOI 10.3389/fonc.2023.1170397

OPEN ACCESS

EDITED BY
Sifeng Qu,
Shandong University, China

REVIEWED BY
Yijian Zhang,
Shanghai Jiao Tong University, China
Yupeng Wu,
First Affiliated Hospital of Fujian Medical University, China

*CORRESPONDENCE
Yong Xu
xymnwk@163.com

RECEIVED 20 February 2023
ACCEPTED 01 June 2023
PUBLISHED 14 June 2023

CITATION
Zhang H, Liu D, Qin Z, Yi B, Zhu L, Xu S, Wang K, Yang S, Liu R, Yang K and Xu Y (2023) CHMP4C as a novel marker regulates prostate cancer progression through cycle pathways and contributes to immunotherapy.
Front. Oncol. 13:1170397.
doi: 10.3389/fonc.2023.1170397

CHMP4C as a novel marker regulates prostate cancer progression through cycle pathways and contributes to immunotherapy

Hongtuan Zhang, Dongze Liu, Zheng Qin, Bocun Yi, Liang Zhu, Shengxian Xu, Kaibin Wang, Shaobo Yang, Ranlu Liu, Kuo Yang and Yong Xu*

Institute of Urology, the Second Hospital of Tianjin Medical University, Tianjin, China

Background: CHMP4C is one of the charged multivesicular protein (CHMP), and is involved in the composition of the endosomal sorting complex required for transport III (ESCRT-III), facilitating the necessary separation of daughter cells. CHMP4C has been proposed to be involved in the progression of different carcinomas. However, the value of CHMP4C in prostate cancer has not yet been explored. Prostate cancer is the most frequently occurring malignancy among male and remains a leading cause of deaths in cancers. So far, clinical therapy of prostate cancer is more inclined to molecular classification and specific clinical treatment and research. Our study investigated the expression and clinical prognosis of CHMP4C and explored its potential regulatory mechanism in prostate cancer. The immune status of CHMP4C in prostate cancer and relative immunotherapy were then analyzed in our study. Based on CHMP4C expression, a new subtype of prostate cancer was established for precision treatment.

Methods: We studied the expression of CHMP4C and relative clinical outcome using the online databases TIMER, GEPIA2, UALCAN, and multiple R packages. Meanwhile, the biological function, immune microenvironment and immunotherapy value of CHMP4C in prostate cancer were further explored on the R software platform with different R packages. Then we performed qRT-PCR, Western Blotting, transwell, CCK8, wound healing assay, colony formation assay and immunohistochemistry to verify the expression of CHMP4C, carcinogenesis and potential regulatory mechanisms in prostate cancer.

Results: We found that the expression of CHMP4C is significant in prostate cancer and the high expression of CHMP4C represents a poor clinical prognosis and malignant progression of prostate cancer. In subsequent vitro validation, CHMP4C promoted the malignant biological behavior of prostate cancer cell lines by adjusting the cell cycle. Based on CHMP4C expression, we established two new subtypes of prostate cancer and found that low CHMP4C expression

has a better immune response while high CHMP4C expression was more sensitive to paclitaxel and 5-fluorouracil. Above findings revealed a new diagnostic marker for prostate cancer and facilitated the subsequent precise treatment of prostate cancer.

KEYWORDS

CHMP4C, prostate cancer, diagnostic and prognostic biomarkers, accurate treatment, anti-tumor

1 Introduction

In male population, prostate cancer is still the 2nd most commonly diagnosed tumor worldwide. There were approximately 1,400,000 new cases and 375,000 deaths in 2020 and the incidence has been increasing worldwide (1) (2). Prostate cancer is geographically most prevalent in the Nordic population and is associated with many risk factors such as family history, race and hereditary syndromes (3). The high degree of heterogeneity in prostate cancer treatment decisions and outcomes dictates appropriate risk stratification of patients. This requires that we distinguish between the relatively benign state of prostate cancer and the more aggressive state, so the inclusion of prognostic and predictive biomarkers of clinical value is urgent. The progression and development of prostate cancer are complex and heterogeneous, with approximately 20-30% of male patients with limited prostate cancer recur after treatment and a 5-year survival rate of only 30% when metastases occur. More importantly, the therapy of male patients with metastatic and castration-resistant prostate cancer remains unsatisfactory. Immunotherapy is currently a hot topic in prostate cancer treatment. In immunotherapy for prostate cancer, cytotoxic T lymphocyte-associated antigen 4 (CTLA4), programmed death ligand-1 (PD-L1), and programmed death-1 (PD-1) inhibitors have shown promising outcomes in terms of anti-tumor immune therapy. However, some clinical tests on immunotherapy in prostate cancer patients have not been as effective as expected. In the era of precision medicine, specific and targeted treatment for different tumor subtypes of patients is considered to be the best measure to achieve the maximum therapeutic effect (4) (5). Therefore, exploring and establishing new subtypes of prostate cancer will help to address above issues and improve the clinical prognosis of patients.

Chromatin modifying protein 4C (CHMP4C), one of the chromatin modifying protein (CHMP), is a constituent of the endosomal sorting complex needed for transport (6) (7). CHMP4C transports the required endosomal sorting complex involved in cell division of daughter cells (8) and plays a greatly significant role in many processes such as cancer pathogenesis and progression in the form of extracellular vesicles (9). CHMP4C has been shown to have a regulatory effect in numerous tumors, including human ovarian cancer (10), lung cancer (11) (12) and cervical cancer (13). However, the role of CHMP4C in prostate cancer is rarely mentioned. Previous studies have shown that CHMP4C is abundantly expressed as a component of extracellular vesicles in patients with high Gleason scores and as a novel signature of pyroptosis that affects the prognosis of prostate cancer patients (14) (15). Therefore, further validation of CHMP4C expression in prostate cancer, and whether CHMP4C affects the biological behavior of prostate cancer and the regulatory pathways involved, will help us to identify new biomarkers and new therapeutic options.

2 Materials and methods

2.1 Online database and R packages for analyzing CHMP4C expression

The timer database (TIMER2.0 (cistrome.org)) was applied to compare the differences in the expression of CHMP4C between prostate tumor samples and normal tissue samples (16). Prostate cancer transcriptome data were got from the TCGA database (https://cancergenome.nih.gov/) and used for validation of the difference analysis and paired difference analysis with “ggpubr” and “limma” packages (17).

2.2 Online database and R packages for analysis of CHMP4C clinicopathological correlations

The GEPIA2 database (http://gepia2.cancer-pku.cn/#analysis), “survival” package and “survminer” package were applied to analyze the prognosis of CHMP4C in prostate cancer (18). UALCAN database (UALCAN (uab.edu)) was applied to find the relationship between CHMP4C expression and prostate cancer Gleason score, lymph node metastasis status and TP53 mutation status (19) (20).

2.3 Biological functional analysis of CHMP4C

To further investigate the regulatory role of CHMP4C in cell proliferation, we performed co-expression gene analysis and GSEA analysis using the “limma” package and “enrichplot” package and identified important regulatory roles of CHMP4C in the cell cycle.

To deepen our understanding of the biological functions of CHMP4C, we conducted grouping differences analysis of CHMP4C using the "limma" package and performed KEGG and GO analysis based on the grouping results. KEGG and GO gene sets were got from the Gene Set Enrichment Analysis (GSEA) website (GSEA (gsea-msigdb.org) (21). Then the R packages 'enrichplot', 'ggplot2', 'clusterProfiler' and "org.Hs.eg.db" were utilized to perform the GO and KEGG analysis.

2.4 Immune infiltration analysis of CHMP4C in prostate cancer

limma package, estimate package and reshape2 package were used to analyze the immune and mesenchymal scores of CHMP4C in prostate cancer. CIBERSORT is a deconvolution algorithm that can be used to transform the normalized gene expression array into the composition of infiltrating immune cells. Based on the result of CIBERSORT, we obtained the relationship of CHMP4C with the infiltration level of 22 immune cells using "limma" packages. We then explored the relationship of CHMP4C expression with 49 immune checkpoint genes with "ggplot2" and "reshape2" R packages and set the p-value filter to 0.001.

2.5 Analysis of the potential therapeutic value of CHMP4C in prostate cancer

IPS-CTLA4 blocker and IPS-PD1/PD-L1 blocker data for prostate cancer from TCGA were got in TCIA (https://tcia.at) and utilized to predict the response to ICI in patients with low and high expression of CHMP4C. External independent immune validation queue data from GEO database (https://www.ncbi.nlm.nih.gov/geo/, GSE67501). The "pRRophetic" package was applied to predict the drug sensitivity of CHMP4C.

2.6 Cell culture

The prostate cancer cell lines PC-3 and DU-145 were got from the Affiliated Cell Resource Center of the Chinese Academy of Medical Sciences. Culture medium was RPMI1640 medium (Biological Industries) with 10% added fetal bovine serum (Biological Industries) and 1% penicillin and streptomycin (100units/ml, Solarbio). The environmental conditions for incubation were 37 degrees, 5% CO2 humidified incubator.

2.7 Quantitative real-time PCR and transfection of si-RNA molecules

Total RNA Kit (Omega Bio-Tek, USA) was used to isolate and purify total RNA from cell lines, and BIOG cDNA Synthesis Kit (BioDai, Changzhou, China) was used for reverse transcription.

In qRT-PCR, GAPDH primers were designed as follows (GAPDH-F: GGAAGGTGAAGGTCG GAGTCA, GAPDH-R: GTCATTGATGGCAACAATATATCCACT) and CHMP4C primers were designed as follows (CHMP4C-F: AGACTGAG GAGATGCTGGGCAA, CHMP4C-R: TAGTGC CTGTAATG CAGCTCGC). Relative expression differences were calculated using the 2-ΔΔCt method with the GAPDH gene as a control. si-NC and si-RNA of CHMP4C were designed as follows (si-NC: CCUCUGGCAUUAGAAUUAUTT, si- CHMP4C: CCUGCGU CUC UACAACUAU).

2.8 Western blot

RIPA buffer with PMSF was used to get the total protein, and the total protein concentration was determined with the BCA method (solarbio, Beijing, China). The protein was separated on 10% SDS/PAGE gels and transferred to PVDF membranes. After transferring, the membranes were blocked with 5% skim milk and incubated overnight in primary antibody at 4°C. Bound antibodies were detected by horseradish peroxidase-labeled secondary antibody. Western blot analysis was performed with ECL luminescent reagent (solarbio, Beijing, China). The antibodies were displayed as follows (CHMP4C: Abcam, Cambridge, UK, ab168205, CDK2: Abcam, Cambridge, UK, ab32147, CCNA2: Abcam, Cambridge, UK, ab181591, GAPDH: Abcam, Cambridge, UK, ab8245).

2.9 Immunohistochemistry

Pathology slides from patients with prostate cancer and benign prostate hyperplasia (BPH), and we cut paraffin sections of the corresponding tissues. Immunohistochemical antibodies were obtained from Abcam, Cambridge, UK, ab272638. DAB reagent was used for staining (Zhongshan Jinqiao, ZLI-9018).

2.10 Cellular functional assays

In CCK8 assay, si-NC and si-CHMP4C cells were inoculated into 96-well plates at a density of 2 × 103 cells/well, incubated at 37 degrees for 3 hours, and absorbance was measured at 450nm for 3 consecutive days. In the colony formation assay, 2 groups of cells were inoculated in 6-well plates at a density of 1000 cells/well and cultured until visible colonies were formed. Cell colonies were fixed in 4% paraformaldehyde (Solarbio, Beijing, China) for 20 min and then stained with 0.1% crystal violet solution (Solarbio, Beijing, China) for 20 min. In the wound healing assay, we inoculated cells on 6-well plates and cultured cells until fusion reached 80%-90%, using pipette tips for cell scoring and PBS for cell rinsing. Photographs were taken under the microscope at 15h, 30h, and 45h, respectively. In the transwell assay, the matrigel was melted and spread in a 24-well transwell chamber. The lower chamber was added with 10% fetal bovine serum. After 12 h of starvation, cells were transferred to transwell chambers and incubated at 37° C for 36 h. The remaining cells in the upper chamber were erased with cotton swabs and fixed with paraformaldehyde for 20 min. The cells were stained with 0.1% crystal violet solution for 20min.

2.11 statistical analysis

Statistical analysis of bioinformatics was conducted on the R software platform and experimental data were counted using GraphPad Prism version 9.0. We adopted a t-test to compare differences between the two groups and all statistical tests were set as two-sided (P<0.05 was considered statistically significant. ***, **, * stood for P value <0.001, P value <0.01, P value <0.05, respectively).

2.12 Ethics declaration

All studies involving human tissues in this study have been reviewed and approved by the Medical Ethics Committee of the Second Hospital of Tianjin Medical University (KY2023K038), and all experiments were conducted in accordance with relevant requirements and guidelines.

3 Results

3.1 High expression of CHMP4C in prostate cancer

We first performed immunohistochemical staining on prostate cancer tissues and benign prostatic hyperplasia tissues, revealing that CHMP4C was higher in prostate cancer tissues (Figure 1A). To select appropriate cell lines for a subsequent cell functional experiment, we tested the expression of CHMP4C in six prostate cancer cell lines (RWPE-1, LnCap, 22RVI, C4-2, PC-3, DU145). The results showed that the expression levels of PC-3 and DU-145 were the highest compared to RWPE-1. (Figures 1B, C). Then the level of CHMP4C expression was verified again by the timer database. CHMP4C was shown to be hyper-expressed among most cancer types, especially prostate cancer (Figure 1D). Next, we performed variance analysis and paired difference analysis using prostate cancer data from the TCGA database, with results consistent with those described above (Figure 1E). All of the results reveal that CHMP4C is a potential diagnostic biomarker for prostate cancer.

3.2 Correlation of CHMP4C expression with prostate cancer clinicopathology and prognosis

In this study, we utilized the GEPIA2 database and the packages "survival" and "survminer" to explore the correlation of CHMP4C expression levels with prognosis to determine whether CHMP4C can be regarded as a diagnostic biomarker for prostate cancer. We found that up-regulated CHMP4C was generally accompanied by a poor prognosis in terms of DFS and PFS (Figure 2A). Next, CHMP4C expression in prostate cancer was investigated in different pathological parameters using the UALCAN database. Regarding Gleason score, the expression of Gleason6/7/8/9 was significantly upregulated compared to normal controls. Although no significant difference was found in CHMP4C expression in Gleason10 samples, this may be due to the small sample size in this category (n=4). Future studies with larger sample sizes may clarify the role of CHMP4C in high-grade prostate cancer (Figure 2B). In lymph node metastasis, CHMP4C expression gradually increased as the number of lymph node metastases increased (Figure 2C). Similarly, CHMP4C expression was also upregulated in both TP53 mutant and non-mutant compared to normal control (Figure 2D). Taken together, the findings suggested that CHMP4C was indeed correlated with prostate cancer progression and invasion.

3.3 CHMP4C *in vitro* validation of DU-145 and PC-3 cell lines

PC-3 and DU-145 cells were transfected with 50 nM si-CHMP4C or si-con using Lipofectamine 2000. After 48 h, the cells were harvested for analysis. The expression level of cells was detected by Western blot. The results suggested that the CHMP4C protein expression levels of PC-3 and DU-145 were significantly decreased after si-CHMP4C transfection (n=3, t-test, p<0.05) (Figure 3A). We then performed a cellular function experiment to investigate whether CHMP4C induces prostate cancer cell malignancy. CCK-8 cell proliferation assay results showed that CHMP4C knockdown reduced PC-3 and DU-145 proliferation viability compared to controls (n=3, t-test, p<0.05) (Figure 3B). Similar conclusions have also been verified in colony formation experiments. We found a significant decrease in the number of si-CHMP4C clones compared to the si-con group (n=3, t-test, p<0.01) (Figure 3C). In the invasiveness assay transwell, the number of invading and metastatic cells of PC-3 and DU-145 transfected with si-CHMP4C was reduced compared to the control (n=3, t-test, p<0.001) (Figure 3D). In the wound healing assay, knockdown of CHMP4C would further diminish the migration distance of PC-3 and DU-145 (Figure 3E). The above results suggested that CHMP4C is a positive factor of prostate cancer cell proliferation and invasion.

3.4 Co-expression analysis of CHMP4C in prostate cancer

In order to further investigate the possible mechanism of action of CHMP4C in prostate cancer, we performed a co-expression analysis of CHMP4C using the TCGA database (Supplementary Table 1). The results of the co-analysis suggested that CHMP4C was positively correlated with cell cycle-related genes including CCNE2, DCTN2, NSMCE2, ORC4, PRKAG1, BCCIP, CDKN3, CCNT1, CDK2, CCNB1 (Figure 4A). GSEA results also showed that CHMP4C was mainly involved in DNA replication in GOBP and in cell cycle regulation in KEGG (Figure 4B). Cyclin-dependent kinases (CDKs) and associated

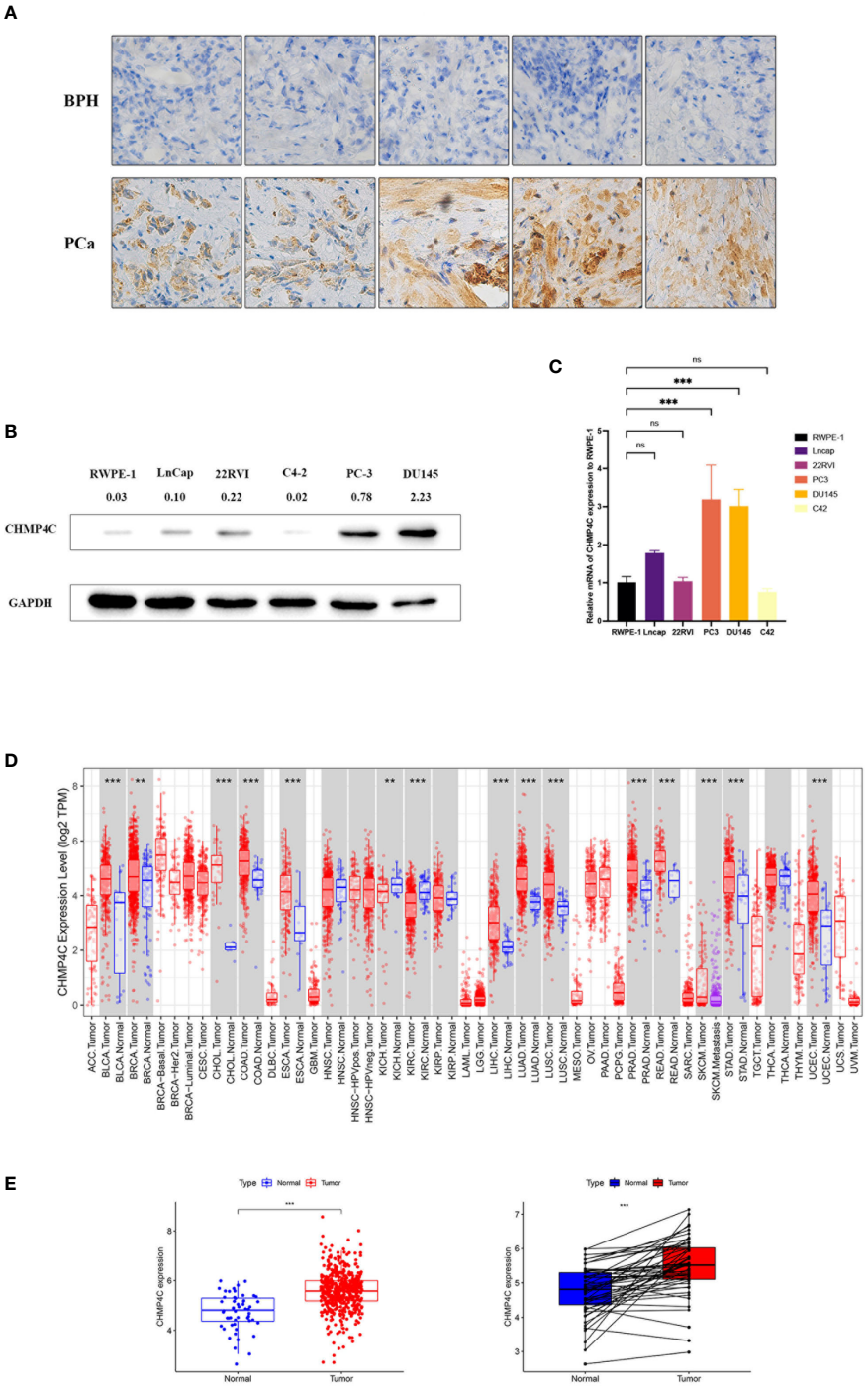


FIGURE 1
CHMP4C expression in prostate cancer. Immunohistochemical staining of CHMP4C in prostate cancer and benign prostatic hyperplasia **(A)**. Western blot of CHMP4C in cell lines **(B)**. qRT-PCR of CHMP4C in cell lines **(C)**. Validation of CHMP4C expression in timer online **(D)**. Validation of CHMP4C expression in the TCGA database **(E)**. ***, **, ns stood for P value <0.001, P value <0.01, P value >0.05, respectively.

cell cycle chaperone proteins are the main regulators of the cell cycle. The CDK2 and cyclinA2 proteins have been shown to play a major role in the regulation of the cell cycle by regulating the transition from the G1 to the S phase. Our experimental results showed that CHMP4C knockdown resulted in a significant decrease in the expression of CDK2 and CyclinA2 (Figure 3A). The above findings showed that CHMP4C is likely to be involved in regulating the prostate cancer cell cycle.

3.5 Analysis of related biological functions of CHMP4C

We performed a grouped variance analysis of CHMP4C and presented it with a heat map (Figure 5A). Then GO and KEGG function enrichment analysis were conducted for these differential genes. The results suggested that the GO analysis was mostly related to

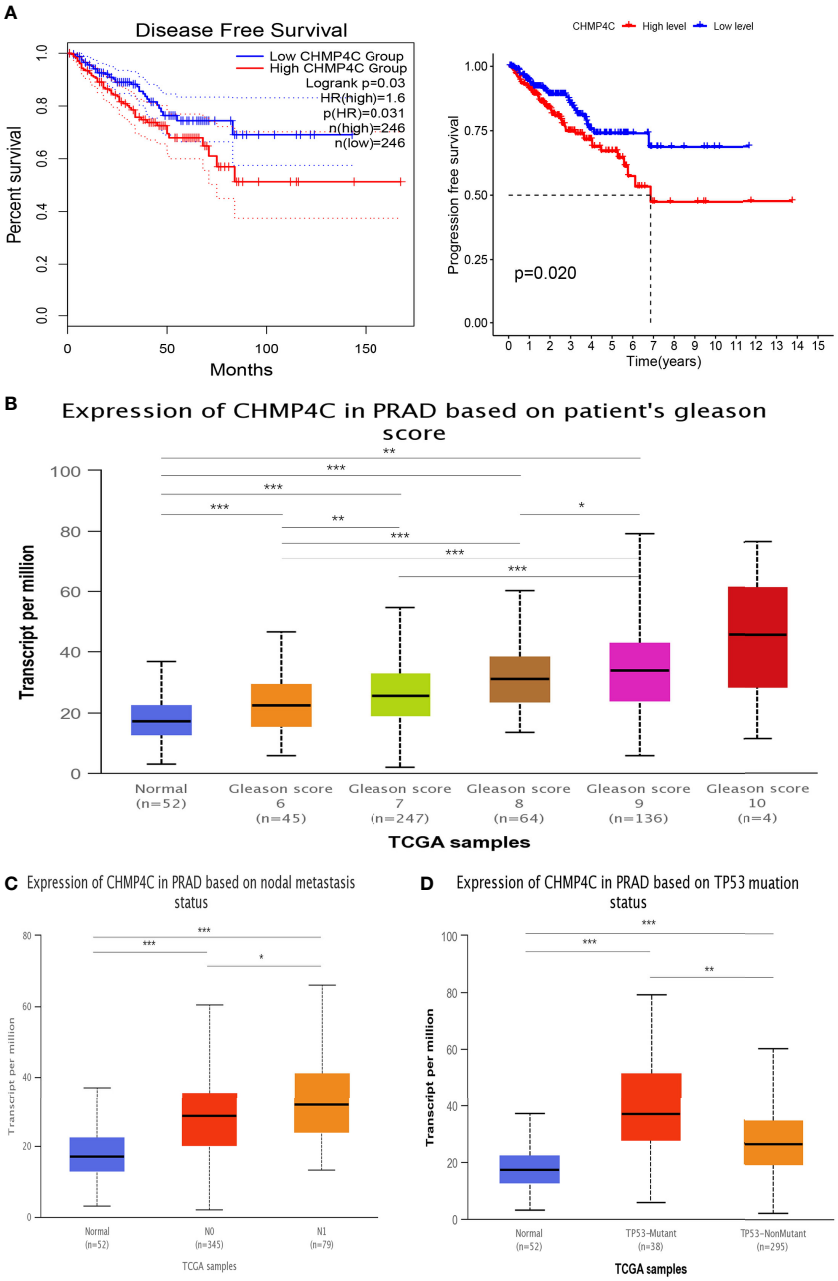


FIGURE 2
Association between CHMP4C and clinicopathological features. Survival analysis of CHMP4C **(A)**. Correlation of CHMP4C with the gleason score **(B)**, lymph node metastasis **(C)**, and TP53 mutation status **(D)**. ***, **, * stood for P value <0.001, P value <0.01, P value <0.05, respectively.

the regulation of immune function (Figure 5B). KEGG analysis also suggested that these genes are also involved in regulating immune function. (Figure 5C). The above findings suggested the potential value of our CHMP4C in the mediation of immune function.

3.6 Differential analysis of CHMP4C immune cell infiltration

To further investigate how CHMP4C relates to immune function, we explored the correlation between the ratio of immune and mesenchymal components and the expression of CHMP4C. Next, the estimate score, stromal score, immune score of the low and high expression groups of CHMP4C were evaluated. The results suggested that low expression of CHMP4C had a higher score (Figure 6A, p<0.01). The correlation between CHMP4C and 22 infiltrating immune cells was then analyzed (Supplementary Table 2). The results revealed that immunosuppressive M2 macrophages were enriched in the high CHMP4C expression group (Figure 6B). We then explored the relationship between CHMP4C and 49 immune checkpoints and found that CHMP4C had a negative correlation with most immune checkpoints (Figure 6C, p<0.001). Above findings suggested to us that the high expression level of CHMP4C in prostate cancer may represent a worse prognosis after immunotherapy.

10.3389/fonc.2023.1170397

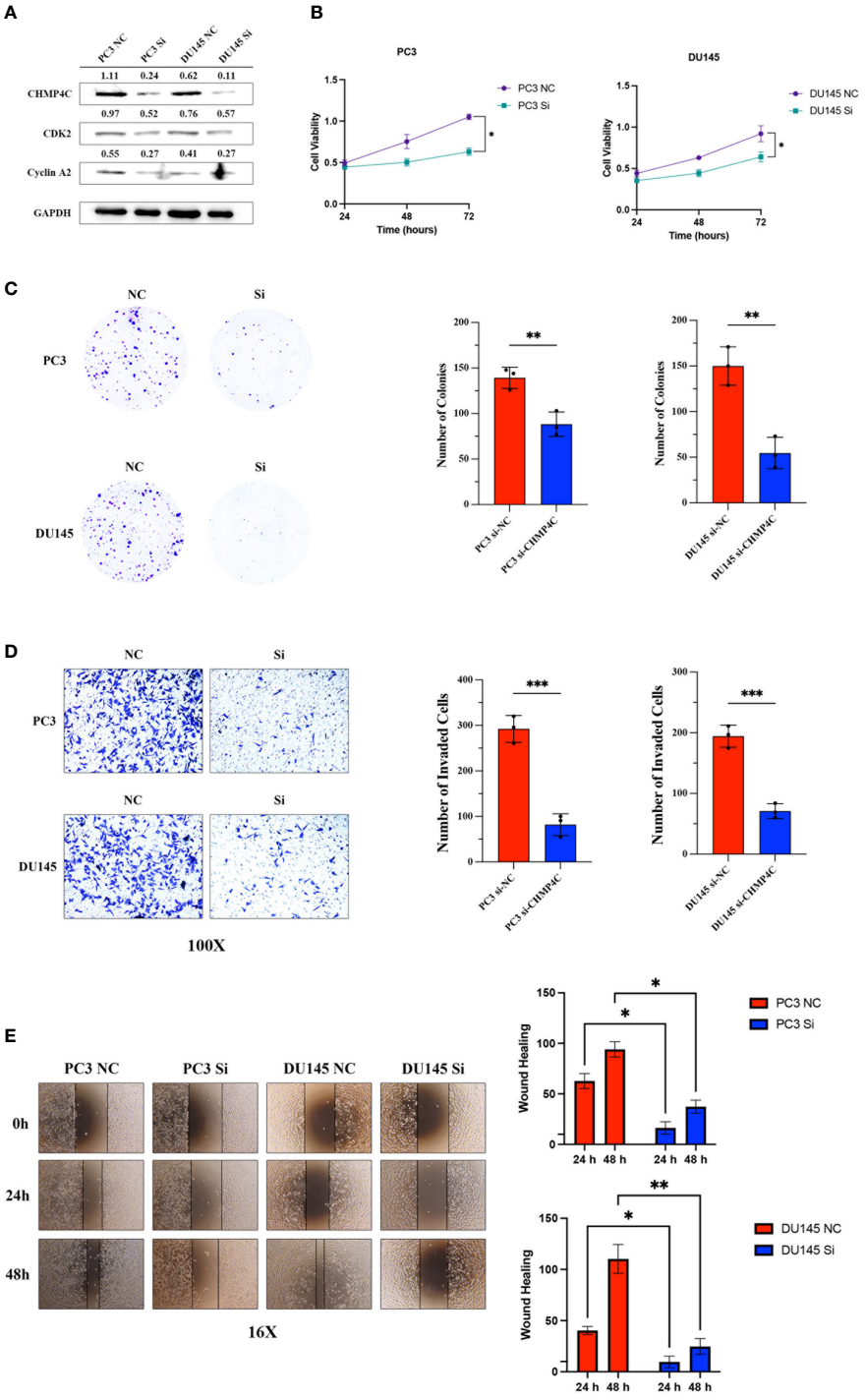


FIGURE 3
CHMP4C promoted the proliferation, invasion, and metastasis of PC-3 and DU-145 cell lines *in vitro*. Knockdown efficiency of CHMP4C and changes of downstream proteins CDK2 and cyclinA2 **(A)**. CHMP4C knockdown inhibited the migration of CHMP4C inhibited PC-3 and DU-145 cell proliferation tested by CCK-8 assay **(B)**. Knockdown of CHMP4C inhibited the colony formation ability of PC-3 and DU-145 cells **(C)**. Transwell invasion assays were conducted in PC-3 and DU-145 cells **(D)**. Knockdown of PC-3 and DU-145 cells migration **(E)**. ***, **, * stood for P value <0.001, P value <0.01, P value <0.05, respectively.

3.7 Targeted drug and immunotherapy response prediction for CHMP4C

The application of immune checkpoint inhibitors (ICI) has achieved success in tumor immunotherapy. To forecast the response to ICI, we computed scores for four subtypes based on machine learning. The results suggested that the group with lower expression of CHMP4C was more likely to respond to anti-PD1, anti-CTLA-4, and comprehensive therapy (Figure 7A). Combined with the above study, we concluded that the CHMP4C low

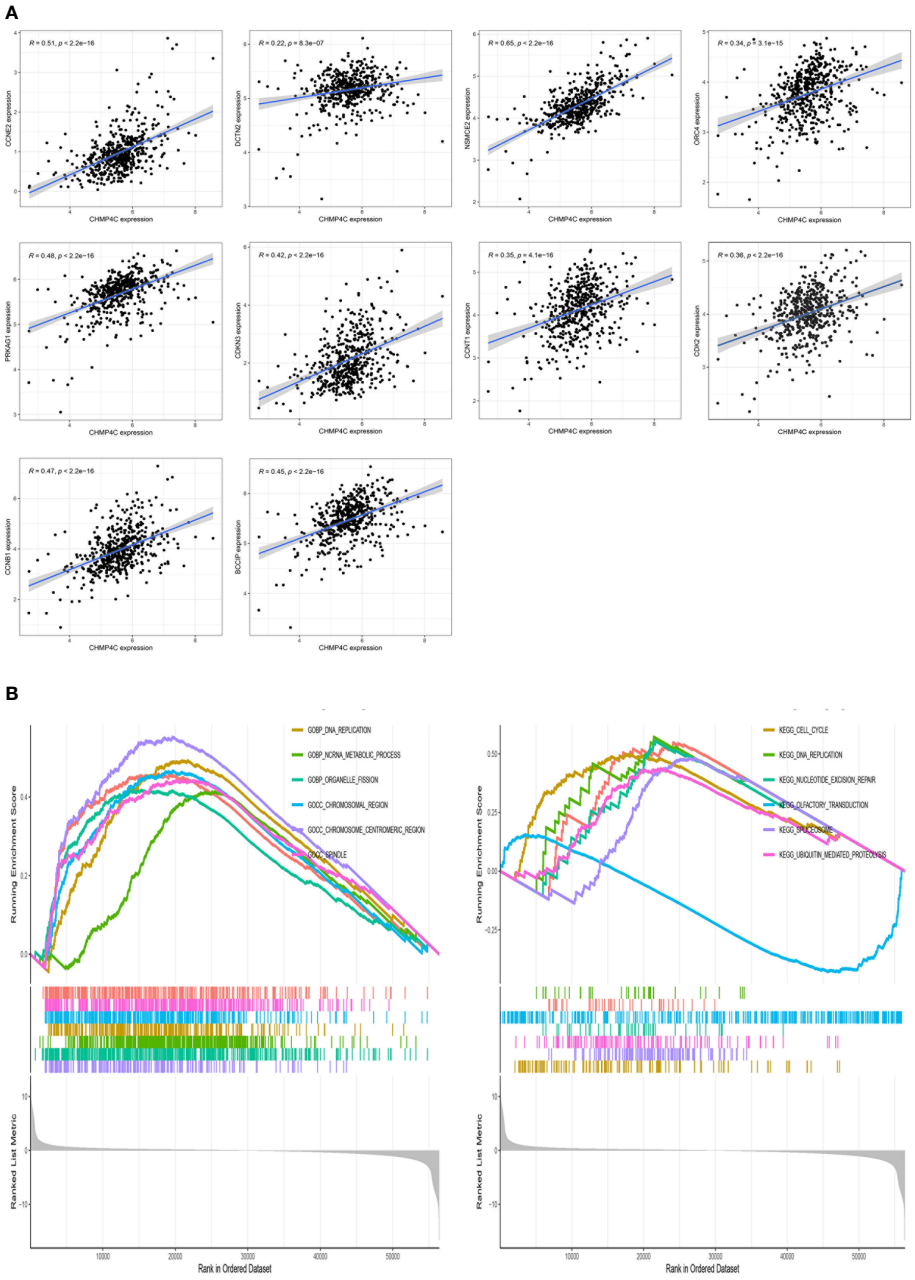


FIGURE 4
CHMP4C is involved in the cell cycle regulation of PC-3 and DU-145. CHMP4C co-expressed genes associated with the cell cycle **(A)**. GSEA analysis of CHMP4C in prostate cancer **(B)**.

expression group had better effect on immunotherapy and more clinical benefits for patients. The GSE67501 cohort is often used as an external independent cohort to assess immunotherapy effects (22). The predictive value of CHMP4C expression was further validated in an external cohort of 11 renal cell carcinoma patients who received PD-1/PD-L1 immunotherapy (GSE67501). The 'stat_compare_means' function showed that the patients with low CHMP4C expression exhibited a significantly higher response rate to the therapy (Figure 7B). We then utilized the R package 'pRRophetic' to predict vitro drug sensitivity according to the expression level of CHMP4C. The results suggested that the group with high expression of CHMP4C could benefit better when treated with paclitaxel and 5-fluorouracil (Figures 7C, D). Further analysis showed that bortezomib had lower IC50 values in the low expression group of CHMP4C, revealing that the bortezomib was more effective in patients with low CHMP4C (Figure 7E).

4 Discussion

Prostate cancer is the second most prevalent tumor in men, with a 5-year survival rate of 60% in Asia. In the United States, the 10-year survival rate for localized prostate cancer is nearly 100%.

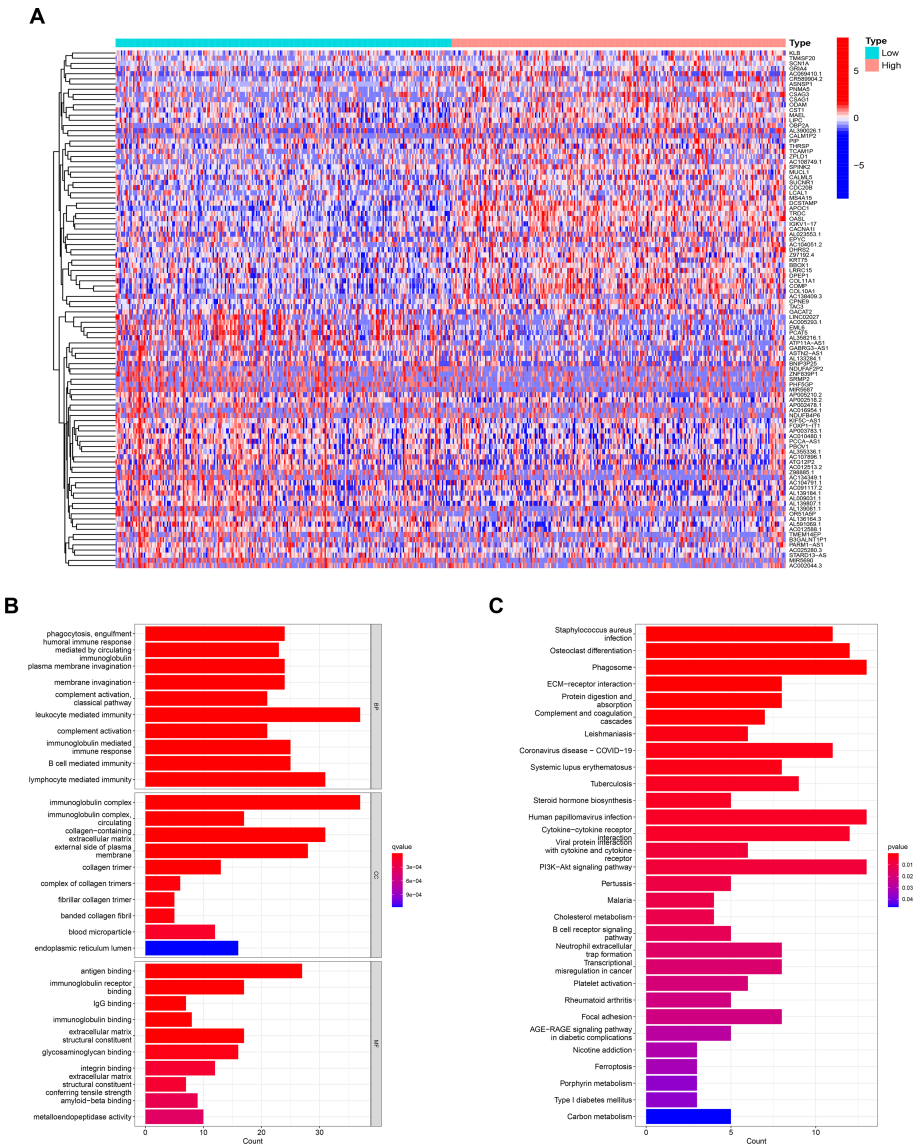


FIGURE 5
Analysis of related biological functions of CHMP4C. Differential genes grouped by CHMP4C **(A)**. GO analysis of CHMP4C **(B)**. KEGG analysis of CHMP4C **(C)**.

However, when distant metastasis occurs, the 5-year survival rate is only 32.3% (23) (24). Globally, the incidence of prostate cancer increased from 30.5 per 100,000 people in 1900 to 37.9 per 100,000 people in 2017 (25). Most prostate cancer will subsequently progress to the castration-resistant stage and result in the eventual death of prostate patients. Therefore, as a global health problem, prostate cancer seriously endangers the physical and mental health of men. Although PSA is the preferred serum marker for prostate cancer, it is not clear whether PSA is effective in reducing the risk of death in patients with prostate cancer (26). Hence, the search for biomarkers for the diagnosis, prognosis and treatment of prostate cancer is urgent. Pyroptosis as a form of programmed cell death plays an important role in the regulation of immune and inflammatory responses. Previous studies have demonstrated that pyroptosis-related genes can be used as new diagnostic and prognostic markers for tumors and contribute to the sensitivity analysis of immunotherapy, especially CHMP4C has an important role in cervical cancer, hepatocellular carcinoma and bladder cancer prognostic models (27) (28) (29). Therefore, the diagnostic, prognostic, and therapeutic value of CHMP4C in prostate cancer also deserves to be fully explored. The ESCRT mechanism (the endosomal cell sorting complex for translation) was involved in the normal separation of the genetic material of daughter cells during normal cell division. CHMP4C, as a component of endosomal sorting complex required for transport III (ESCRT-III), checked cell kinetics shedding by abscission checkpoint (11). The above mechanisms prevented excessive accumulation of DNA damage. In the absence of CHMP4C, cell

10.3389/fonc.2023.1170397

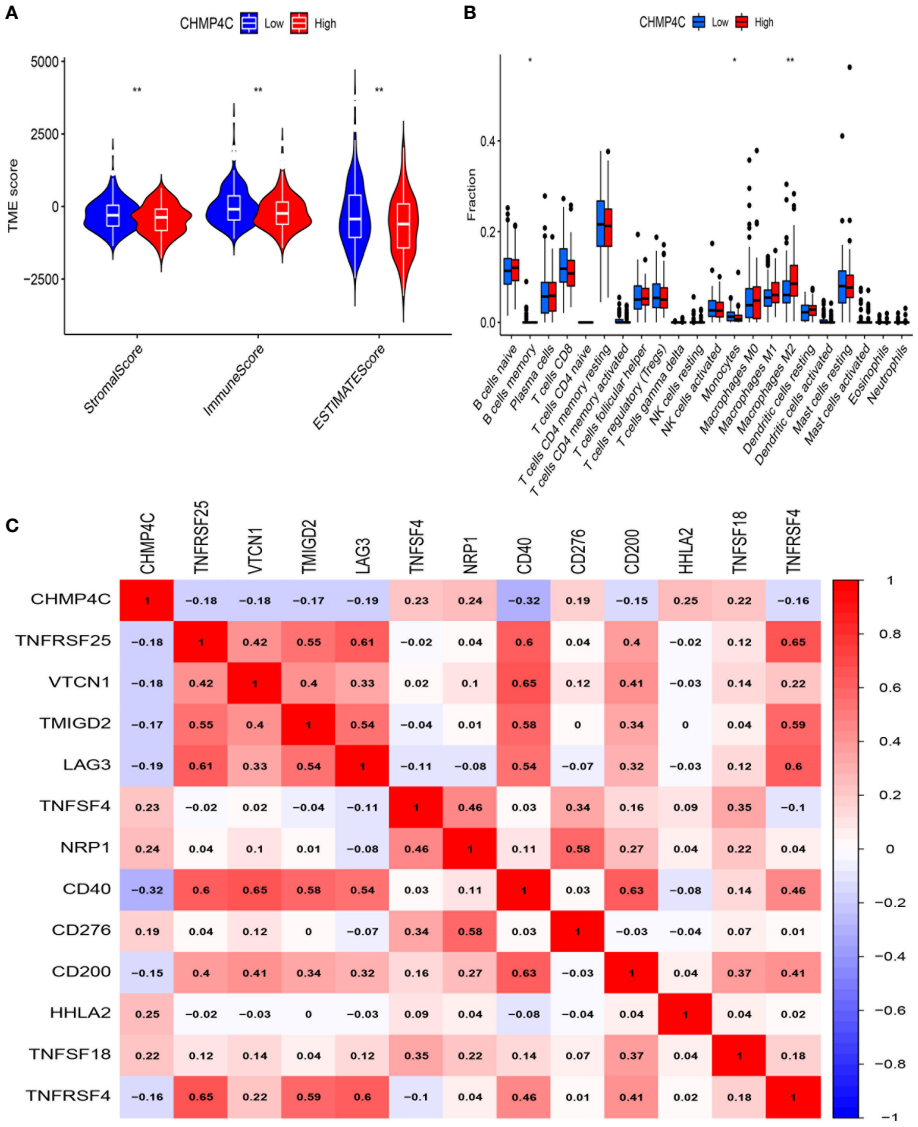


FIGURE 6
Immune microenvironment analysis of CHMP4C. Tumor microenvironment (TME) scores of CHMP4C **(A)**. 22 immune cell infiltration analysis of CHMP4C **(B)**. Correlation analysis between CHMP4C and immune checkpoints **(C)**. **, * stood for P value <0.01, P value <0.05, respectively.

shedding checks failed and damaged cells rapidly progressed from M to S phase, resulting in accumulation of DNA damage and genomic instability (12).

Our work explored the diagnostic, prognostic and therapeutic value of CHMP4C in the prostate cancer. We initially used the TCGA and TIMER databases to pinpoint the high expression of CHMP4C in prostate cancer, and we then confirmed the high expression of target genes at the molecular and protein levels. We then found that the CHMP4C expression level was significantly related to gleason score and lymph node status and at the same time high expression of CHMP4C was associated with poor prognosis. Subsequently, it was demonstrated that CHMP4C was highly expressed in the prostate cancer and was related to the advances in malignant biology. Moreover, *in vitro* prostate cancer cell growth, invasion, and metastasis were all considerably reduced when CHMP4C was knocked down. The above results fully illustrated that CHMP4C could be regarded as a novel diagnostic and prognostic marker in prostate cancer. CHMP4C has been shown to be up-regulated in a variety of tumors and is related to malignant behavior. CHMP4C as a model gene for pyroptosis was more closely associated with prostate cancer prognosis implying prognostic value of CHMP4C in prostate cancer (14). High enrichment of CHMP4C in the urine of patients with high Gleason score prostate cancer suggested the potential of CHMP4C as a novel diagnostic marker for prostate cancer (15). Among other cancers, CHMP4C was up-regulated in lung cancer and regulated tumor proliferation by modulating cell cycle progression (12). Meanwhile, high expression of CHMP4C also increased cell viability and anti-apoptosis in lung cancer under radiation conditions (11). Increased expression of CHMP4C in cervical cancer facilitated cervical cancer cell proliferation and invasion (13). Pancreatic cancer cell growth and invasion were

10.3389/fonc.2023.1170397

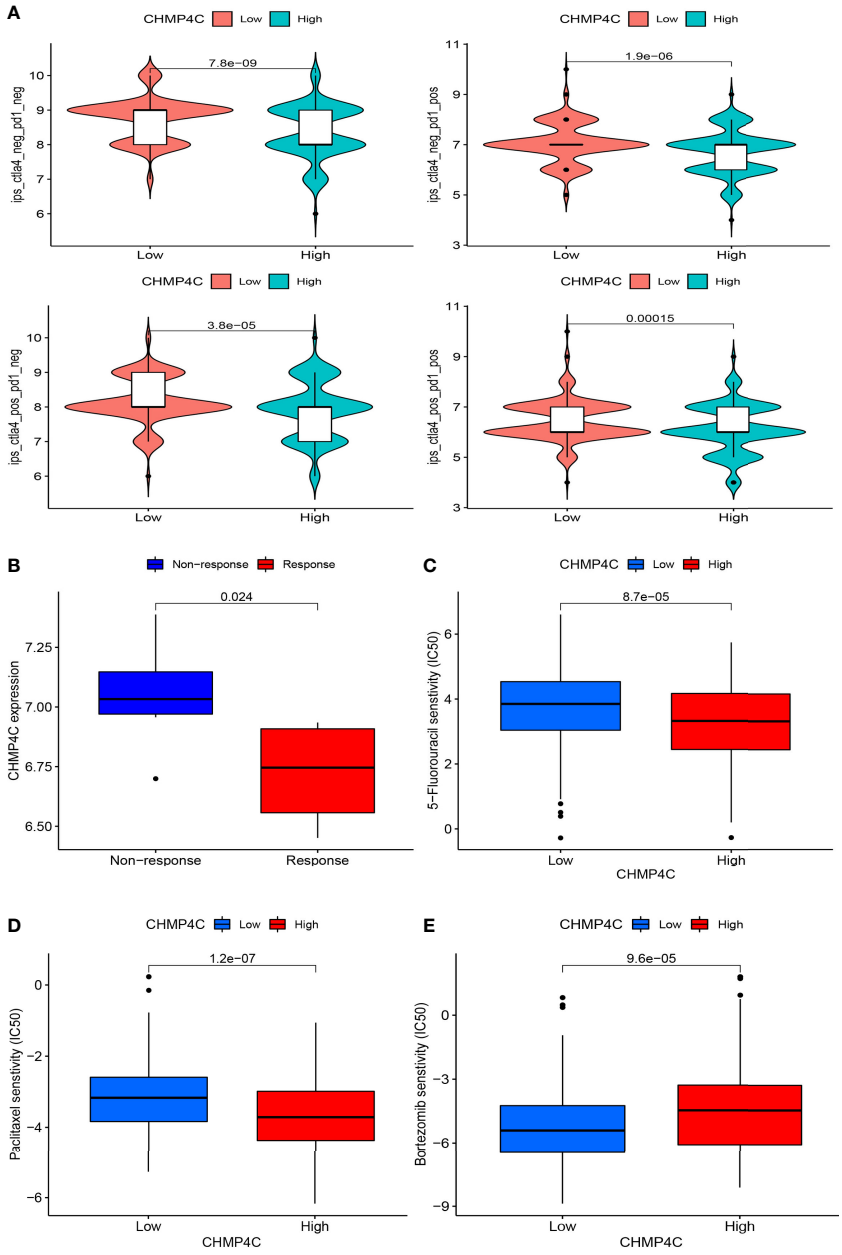


FIGURE 7
The immunotherapy and chemotherapy agents value of CHMP4C. Immune checkpoint inhibitor anti-PD1 and anti-CTLA-4 sensitivity analysis of four subtypes **(A)**. Prediction of sensitivity in an external PD-1/PD-L1 immunotherapy cohort **(B)**. Sensitivity analysis of 5-fluorouracil **(C)**, paclitaxel **(D)**, and bortezomib **(E)**.

markedly reduced when CHMP4C was knocked down (30). These results demonstrated the role of CHMP4C as an oncogene in tumors and were consistent with our findings.

To further explore the oncogenic role of CHMP4C, we performed a co-expression analysis using the ggplot2 and ggExtra R packages. We found that some of these genes are involved in cell cycle regulation especially CDK2 protein, suggesting that CHMP4C may affect prostate cancer progression in part by regulating the cell cycle. The results of GSEA analysis in GOBP and KEGG were also similar with the above findings. According to reports, CDK2 and cyclinA2 have significant regulatory functions in cell cycle and proliferation (31). This further validated the role of CHMP4C as a member of ESCRT in the regulation of cell cycle and proliferation and was consistent with our conjecture. Knockdown of CHMP4C led to reduced expression of CDK2 and cyclinA2. The above results demonstrated that CHMP4C partially mediated the regulation of cell proliferation by regulating the cell cycle.

Importantly, our subsequent GO and KEGG analysis of CHMP4C grouped differential genes revealed that CHMP4C may have been involved in the regulation of immune function in prostate cancer. Immunotherapy had bright promise in the treatment of prostate cancer (32). Regulation of immune function by CHMP4C

may contribute to our understanding of immunotherapy in prostate cancer. We already know that the tumor microenvironment affected the efficacy and acted a crucial regulatory function in immunotherapy (33). We found that high expression of CHMP4C in tumor environment tended to have a lower immune score suggesting less lymphoid T-cell infiltration. This result suggested that high expression of CHMP4C had an immunosuppressive microenvironment compared to low expression groups. In the following immune infiltration cell analysis, we also found that macrophages M2 were abundantly enriched in the CHMP4C high expression group. Macrophage M2 promoted tumor cell development and metastasis and promoted tumor angiogenesis leading to tumor progression. Meanwhile, it also inhibited the T cell-mediated anti-tumor immune response (34). Above results suggested that CHMP4C may act as an immunosuppressive role in prostate cancer. In terms of immunotherapy, great progress has been made through immune checkpoint inhibitors (ICI) in urinary tumors, including kidney, bladder, and prostate cancers (35). However, the efficacy of ICI is not always satisfactory, and the degree of T-cell infiltration affects the final outcomes. Hot tumors with a large infiltration of T cells have a stronger response to ICI, while cold tumors are the opposite (36). In prostate cancer, immunosuppressive microenvironment and cold tumors were common immune features of prostate cancer and the efficacy of ICI was limited to specific subtypes of prostate cancer (35) (37). Therefore, immunotherapy for prostate cancer should be precisely classified to achieve maximum clinical benefit for prostate cancer patients. Our study proposed that CHMP4C based on low expression may have a stronger response to ICI to achieve precision therapy and improved the efficacy of ICI in prostate cancer. In our research, we discovered that CHMP4C was negatively related to most of the immune checkpoints. This result suggested that ICI might not be sensitive to the high expression group of CHMP4C. To confirm our above conjecture, we used an external database to predict the immunotherapeutic value of CHMP4C. CTLA4 and PD1 are currently the common immune targets in our immunotherapy, and the combination of both blockers has shown good efficacy in cancer immunotherapy (38) (39). In the clinical prediction of different treatment regimens for prostate cancer anti-PD1 and anti-CTLA4, the low CHMP4C expression group always showed better response compared to the high CHMP4C expression group. GSE67501 has been used as an independent cohort for immunotherapy (PD-1/PD-L1) to predict the value of target genes in immunotherapy (40) (41). In the GSE67501 cohort, the low expression CHMP4C group was also more sensitive to immunotherapy, which is consistent with our findings. In summary, the low-expression group of CHMP4C has a higher immune score and a more active immune microenvironment in prostate cancer, which is more favorable for immunotherapy of prostate cancer. In terms of other chemotherapeutic drug treatments *in vitro*, the treatment of paclitaxel and 5-fluorouracil has been very successful in prostate cancer (42). We predicted the sensitivity of paclitaxel and 5-fluorouracil according to the expression level of CHMP4C. Interestingly, the high CHMP4C expression group has a higher sensitivity to paclitaxel and 5-fluorouracil. Therefore, we can classify prostate cancer into different groups according to the expression of CHMP4C and adopted different treatment plans for the low and high expression groups of CHMP4C to achieve precise treatment of prostate cancer and maximize the clinical benefit of prostate cancer patients. However, our research still has certain shortcomings. Our analysis is mainly based on bioinformatics analysis and only a small amount of experimental verification has been carried out. Therefore, further research is required in the future to understand the precise mechanisms by which CHMP4C regulates the cell cycle and influences immunotherapy response in prostate cancer.

5 Conclusion

CHMP4C is highly expressed in prostate cancer tissues and plays a role in CHMP4C cell proliferation and metastasis by regulating the cell cycle. Importantly, CHMP4C is closely correlated with prostate cancer clinicopathological parameters and prognosis, indicating that CHMP4C can be used as a novel diagnostic and prognostic molecular marker for prostate cancer. Meanwhile, the expression can help to predict immunotherapy response in prostate cancer and implement different therapeutic regimens to achieve clinical benefit for prostate cancer patients.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

The studies involving human participants were reviewed and approved by The Medical Ethics Committee of the Second Hospital of Tianjin Medical University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

ZH researched and designed all bioinformatics analyses. DL, BY, LZ did the basic experimental part, and SX, DL, ZQ, SY and KW critically reviewed the manuscript. YX, HZ, KY and RL provided administrative, technical, and material support. All authors contributed to the article and approved the submitted version.

Funding

This research was supported by grants from the National Natural Science Foundation of China (81972412 and 81772758). We are grateful for the financial support from YX.

Acknowledgments

We also thank the TCGA databases for the availability of the data.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1170397/full#supplementary-material

SUPPLEMENTARY TABLE 1
co-expression analysis of CHMP4C.

SUPPLEMENTARY TABLE 2
the correlation between CHMP4C and 22 infiltrating immune cells

References

1. Kimura T, Egawa S. Epidemiology of prostate cancer in Asian countries. *Int J Urol* (2018) 25(6):524–31. doi: 10.1111/iju.13593

2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* (2021) 71(3):209–49. doi: 10.3322/caac.21660

3. Gandaglia G, Leni R, Bray F, Fleshner N, Freedland SJ, Kibel A, et al. Epidemiology and prevention of prostate cancer. *Eur Urol Oncol* (2021) 4(6):877–92. doi: 10.1016/j.euo.2021.09.006

4. Yu G, Bao J, Zhan M, Wang J, Li X, Gu X, et al. Comprehensive analysis of m5C methylation regulatory genes and tumor microenvironment in prostate cancer. *Front Immunol* (2022) 13:914577. doi: 10.3389/fimmu.2022.914577

5. Adamaki M, Zoumpourlis V. Prostate cancer biomarkers: from diagnosis to prognosis and precision-guided therapeutics. *Pharmacol Ther* (2021) 228:107932. doi: 10.1016/j.pharmthera.2021.107932

6. Petsalaki E, Dandoulaki M, Zachos G. The ESCRT protein Chmp4c regulates mitotic spindle checkpoint signaling. *J Cell Biol* (2018) 217(3):861–76. doi: 10.1083/jcb.201709005

7. Petsalaki E, Zachos G. CHMP4C: a novel regulator of the mitotic spindle checkpoint. *Mol Cell Oncol* (2018) 5(3):e1445944. doi: 10.1080/23723556.2018.1445944

8. Lata S, Schoehn G, Solomons J, Pires R, Göttlinger HG, Weissenhorn W. Structure and function of ESCRT-III. *Biochem Soc Trans* (2009) 37(Pt 1):156–60. doi: 10.1042/BST0370156

9. Juan T, Fürthauer M. Biogenesis and function of ESCRT-dependent extracellular vesicles. *Semin Cell Dev Biol* (2018) 74:66–77. doi: 10.1016/j.semcdb.2017.08.022

10. Gusev A, Lawrenson K, Lin X, Lyra PCJr, Kar S, Vavra KC, et al. A transcriptome-wide association study of high-grade serous epithelial ovarian cancer identifies new susceptibility genes and splice variants. *Nat Genet* (2019) 51(5):815–23. doi: 10.1038/s41588-019-0395-x

11. Li K, Liu J, Tian M, Gao G, Qi X, Pan Y, et al. CHMP4C disruption sensitizes the human lung cancer cells to irradiation. *Int J Mol Sci* (2015) 17(1):18. doi: 10.3390/ijms17010018

12. Liu B, Guo S, Li GH, Liu Y, Liu XZ, Yue JB, et al. CHMP4C regulates lung squamous carcinogenesis and progression through cell cycle pathway. *J Thorac Dis* (2021) 13(8):4762–74. doi: 10.21037/jtd-21-583

13. Lin SL, Wang M, Cao QQ, Li Q. Chromatin modified protein 4C (CHMP4C) facilitates the malignant development of cervical cancer cells. *FEBS Open Bio* (2020) 10 (7):1295–303. doi: 10.1002/2211-5463.12880

14. Xiao X, Li J, Wan S, Wu M, Li Z, Tian J, et al. A novel signature based on pyroptosis-related genes for predicting prognosis and treatment response in prostate cancer patients. *Front Genet* (2022) 13:1006151. doi: 10.3389/fgene.2022.1006151

15. Fujita K, Kume H, Matsuzaki K, Kawashima A, Ujike T, Nagahara A, et al. Proteomic analysis of urinary extracellular vesicles from high Gleason score prostate cancer. *Sci Rep* (2017) 7:42961. doi: 10.1038/srep42961

16. Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, et al. TIMER: a web server for comprehensive analysis of tumor-infiltrating immune cells. *Cancer Res* (2017) 77(21): e108–10. doi: 10.1158/0008-5472.CAN-17-0307

17. Tomczak K, Czerwińska P, Wiznerowicz M. The cancer genome atlas (TCGA): an immeasurable source of knowledge. *Contemp Oncol (Pozn)* (2015) 19(1A):A68–77. doi: 10.5114/wo.2014.47136

18. Tang Z, Kang B, Li C, Chen T, Zhang Z. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. *Nucleic Acids Res* (2019) 47 (W1):W556–60. doi: 10.1093/nar/gkz430

19. Chandrashekar DS, Karthikeyan SK, Korla PK, Patel H, Shovon AR, Athar M, et al. UALCAN: an update to the integrated cancer data analysis platform. *Neoplasia* (2022) 25:18–27. doi: 10.1016/j.neo.2022.01.001

20. Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi BVSK, et al. UALCAN: a portal for facilitating tumor subgroup gene expression and survival analyses. *Neoplasia* (2017) 19(8):649–58. doi: 10.1016/j.neo.2017.05.002

21. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci USA* (2005) 102(43):15545–50. doi: 10.1073/pnas.0506580102

22. Zhao Z, Yang Y, Liu Z, Chen H, Guan X, Jiang Z, et al. Prognostic and immunotherapeutic significance of mannose receptor c type II in 33 cancers: an integrated analysis. *Front Mol Biosci* (2022) 9:951636. doi: 10.3389/fmolb.2022.951636

23. Hassanipour S, Delam H, Arab-Zozani M, Abdzadeh E, Hosseini SA, Nikbakht HA, et al. Survival rate of prostate cancer in Asian countries: a systematic review and meta-analysis. *Ann Glob Health* (2020) 86(1):2. doi: 10.5334/aogh.2607

24. Siegel DA, O'Neil ME, Richards TB, Dowling NF, Weir HK. Prostate cancer incidence and survival, by stage and Race/Ethnicity - united states, 2001-2017. *MMWR Morb Mortal Wkly Rep* (2020) 69(41):1473–80. doi: 10.15585/mmwr.mm6941a1

25. Zhai Z, Zheng Y, Li N, Deng Y, Zhou L, Tian T, et al. Incidence and disease burden of prostate cancer from 1990 to 2017: results from the global burden of disease study 2017. *Cancer* (2020) 126(9):1969–78. doi: 10.1002/cncr.32733

26. Mei W, Jia X, Xin S, Liu X, Jin L, Sun X, et al. A N7-Methylguanine-Related gene signature applicable for the prognosis and microenvironment of prostate cancer. *J Oncol* (2022) 2022:8604216. doi: 10.1155/2022/8604216

27. Hu H, Yang M, Dong W, Yin B, Ding J, Huang B, et al. A pyroptosis-related gene panel for predicting the prognosis and immune microenvironment of cervical cancer. *Front Oncol* (2022) 12:873725. doi: 10.3389/fonc.2022.873725

28. Xing M, Li J. Diagnostic and prognostic values of pyroptosis-related genes for the hepatocellular carcinoma. *BMC Bioinf* (2022) 23(1):177. doi: 10.1186/s12859-022-04726-7

29. Wu T, Li S, Yu C, Wu Y, Long H. A risk model based on pyroptosis subtypes predicts tumor immune microenvironment and guides chemotherapy and

immunotherapy in bladder cancer. *Sci Rep* (2022) 12(1):21467. doi: 10.1038/s41598-022-26110-4

30. Chen Y, Liu Y, Wang M. Identification of a pyroptosis-related gene signature and effect of silencing the CHMP4C and CASP4 in pancreatic adenocarcinoma. *Int J Gen Med* (2022) 15:3199–213. doi: 10.2147/IJGM.S353849

31. Kong Y, Wu R, Zhang S, Zhao M, Wu H, Lu Q, et al. Wilms' tumor 1-associating protein contributes to psoriasis by promoting keratinocytes proliferation via regulating cyclinA2 and CDK2. *Int Immunopharmacol* (2020) 88:106918. doi: 10.1016/j.intimp.2020.106918

32. Cha HR, Lee JH, Ponnazhagan S. Revisiting immunotherapy: a focus on prostate cancer. *Cancer Res* (2020) 80(8):1615–23. doi: 10.1158/0008-5472.CAN-19-2948

33. Petitprez F, Meylan M, de Reyniès A, Sautès-Fridman C, Fridman WH. The tumor microenvironment in the response to immune checkpoint blockade therapies. *Front Immunol* (2020) 11:784. doi: 10.3389/fimmu.2020.00784

34. Pan Y, Yu Y, Wang X, Zhang T. Tumor-associated macrophages in tumor immunity. *Front Immunol* (2020) 11:583084. doi: 10.3389/fimmu.2020.583084

35. Gandhy S U, Madan RA, Aragon-Ching JB. The immunotherapy revolution in genitourinary malignancies. *Immunotherapy* (2020) 12(11):819–31. doi: 10.2217/imt-2020-0054

36. Liu D, Xu S, Chang T, Ma S, Wang K, Sun G, et al. Predicting prognosis and distinguishing cold and hot tumors in bladder urothelial carcinoma based on necroptosis-associated lncRNAs. *Front Immunol* (2022) 13:916800. doi: 10.3389/fimmu.2022.916800

37. Mukherjee AG, Wanjari UR, Prabakaran DS, Ganesan R, Renu K, Dey A, et al. The cellular and molecular immunotherapy in prostate cancer. *Vaccines (Basel)* (2022) 10(8):1370. doi: 10.3390/vaccines10081370

38. Rowshanravan B, Halliday N, Sansom DM. CTLA-4: a moving target in immunotherapy. *Blood* (2018) 131(1):58–67. doi: 10.1182/blood-2017-06-741033

39. Rotte A. Combination of CTLA-4 and PD-1 blockers for treatment of cancer. *J Exp Clin Cancer Res* (2019) 38(1):255. doi: 10.1186/s13046-019-1259-z

40. Ye Y, Jiang H, Wu Y, Wang G, Huang Y, Sun W, et al. Role of ARRB1 in prognosis and immunotherapy: a pan-cancer analysis. *Front Mol Biosci* (2022) 9:1001225. doi: 10.3389/fmolb.2022.1001225

41. He S, Yu J, Sun W, Sun Y, Tang M, Meng B, et al. A comprehensive pancancer analysis reveals the potential value of RAR-related orphan receptor c (RORC) for cancer immunotherapy. *Front Genet* (2022) 13:969476. doi: 10.3389/fgene.2022.969476

42. Lin CC, Hsu CH, Hour TC, Cheng AL, Huang CY, Huang KH, et al. Weekly paclitaxel and high-dose 5-fluorouracil plus leucovorin in hormone-refractory prostate cancer: *in vitro* combined effects and a phase II trial. *Urol Oncol* (2007) 25(3):207–13. doi: 10.1016/j.urolonc.2006.06.002

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 11 August 2023
DOI 10.3389/fonc.2023.1135052

OPEN ACCESS

EDITED BY
Sifeng Qu,
Shandong University, China

REVIEWED BY
Donald Blake Fuller,
Genesis Healthcare Partners, United States
Yan-Feng Li,
Daping Hospital, China

*CORRESPONDENCE
Peihe Liang
302478@cqmu.edu.cn

†These authors have contributed equally to this work and share first authorship

RECEIVED 31 December 2022
ACCEPTED 26 July 2023
PUBLISHED 11 August 2023

CITATION
Xie X, Zhang P, Ran C, Liu L, Hu J, Lei P and Liang P (2023) Global research status and hotspots of radiotherapy for prostate cancer: a bibliometric analysis based on Web of Science from 2010-2022. *Front. Oncol.* 13:1135052. doi: 10.3389/fonc.2023.1135052

Global research status and hotspots of radiotherapy for prostate cancer: a bibliometric analysis based on Web of Science from 2010-2022

Xiaodu Xie[1†], Peng Zhang[1†], Chongjun Ran[1†], Lumiao Liu[2], Jian Hu[1], Pan Lei[1] and Peihe Liang[1*]

[1]Department of Urology, The Second Affiliated Hospital of Chongqing Medical University, Chongqing, China, [2]Department of Anesthesiology, The Second Affiliated Hospital of Chongqing Medical University, Chongqing, China

Background: Radiotherapy (RT) is one of the important treatments for various cancer types and its application to prostate cancer (PCa) has also gradually gained increasing attention. However, there is a lack of comprehensive and objective studies on the overall status of research on RT for PCa. This article aims to summarize and quantify the dynamic trends of RT in PCa by using bibliometrics.

Methods: Studies on RT for PCa were screened from the Web of Science Core Collection (WoSCC) database between 1 January 2010 and 21 November 2022 to collate and quantify information characteristics by analyzing parameters including annual publications, countries/regions, institutions and authors with the aid of the bibliometric software CiteSpace and VOSviewer. In addition, research trends and hotspots were explored by analyzing keywords and co-cited references.

Results: A total of 21338 documents were retrieved. The United States of America (USA) ranked first and maintained the leading position among all countries in the number of publications (8489) and total citations (266342). The University of Toronto was the most active institution in total publications (n=587). Paul L Nguyen enjoyed the most publications (n=179), and Michael J Zelefsky enjoyed the most co-citations (n=3376). *INTERNATIONAL JOURNAL OF RADIATION ONCOLOGY BIOLOGY PHYSICS* published the most papers (n=1026), and was the most frequently co-cited journal (n=78550). The largest and closest cluster in the reference cluster analysis was "oligorecurrent prostate cancer". The timeline view of keywords reveals that cluster "biochemical recurrence(BCR)" is ongoing. Moreover, keywords burstness analysis showed that "radiation dosimetry", "dose rate brachytherapy(BT)", "salvage radiotherapy", "stereotactic body radiotherapy(SBRT)", "guideline", and "multicenter" were the terms with great bursts in the past a few years.

Conclusion: The application of RT targeting oligometastatic prostate cancer (OMPC) has garnered considerable attention among researchers. SBRT and BT

have become hot topics in the field. Additionally, the BCR of PCa has long been a critical issue requiring extensive research and resolution, and salvage radiotherapy has currently emerged as a closely related research focus. Related large-scale multicenter studies have been conducted over the past few years, providing valuable insights. More high-quality research is expected to be employed to guide clinical decision-making.

KEYWORDS

radiotherapy, prostate cancer, bibliometric, CiteSpace, VOSviewer, hotspots

1 Introduction

Prostate cancer (PCa) is an epithelial malignant tumor occurring in the male prostate gland and is the most common malignant tumor of the male genitourinary system. According to data released by the National Comprehensive Cancer Network (NCCN) in 2018, PCa has surpassed lung cancer as the most common malignancy in men and ranks the second leading cause of cancer-related death in men worldwide (1). Radical prostatectomy (RP), radiotherapy (RT) and endocrine therapy remain the principal treatments for PCa at present. RT plays an irreplaceable role in radical RT, postoperative adjuvant or salvage RT and palliative care due to minimal trauma, high safety and the reliable curative effect. RT for PCa, eighter used alone or in combination with other treatments, is a widely accepted. The use of RT as adjuvant treatment after radical prostatectomy has proved to improve progression-free survival (PFS) and reduce the incidence of associated adverse events (2, 3). With the deeper understanding of the radiobiological behavior of PCa and the advent of new techniques, more advances have been made in RT of PCa patients.

In recent years, bibliometrics has emerged as a crucial academic field, focusing on quantifying and evaluating the quantitative attributes, developmental trends, and scholarly impact of scientific literature. While a quantitative overview can be drawn on many methods such as traditional reviews, meta-analysis, and evidence maps, only bibliometrics allows for a qualitative and quantitative analysis of data characteristics such as countries, institutions, authors, and journals, as well as an assessment of trends and profiles of research topics (4, 5). RT, as a pivotal modality in the management of PCa, encompasses a diverse array of treatment modalities, including external beam radiation therapy (EBRT), brachytherapy (BT), and proton therapy, among others. Several research teams have embarked on bibliometric investigations about the application of EBRT in PCa, uncovering salient trends and focal points of interest in this domain (6). However, it is noteworthy that there is currently a lack of sufficient bibliometric research specifically addressing the entire domain of RT applications in PCa. Conducting a bibliometric analysis encompassing the entire domain of RT to explore its focal points and advancements in PCa will facilitate a macroscopic comprehension of the potential strengths and challenges of RT in PCa treatment, consequently furnishing more robust scientific grounds for future clinical practices and therapeutic strategies.

Based on the above background and theoretical support, this paper aims to provide an overall picture of research on RT in PCa and address the research progress, hotspots and trends in the last decade by using two bibliometric software VOSviewer and CiteSpace, in an attempt to provide useful references for future research in this field.

2 Materials and methods

2.1 Data collection

Web of Science (WoS) is an important platform for obtaining global academic information, containing databases such as Science Citation Index Expanded (SCIE), Social Science Citation Index (SSCI), and Conference Proceedings Citation Index (CPCI-S), which include more than 10,000 authoritative and high-impact international academic journals. In this study, we collected and analyzed data by searching the Science Citation Index Expanded Web of Science Core Collection (WoSCC) database. To avoid omissions caused by frequent updates of the database, document retrieval and data download were completed within one day (November 21, 2022). The search formula and process of data screening are shown in Figure 1, with the publication year ranging from January 1st, 2010 to November 21st, 2022. Only reviews and original articles published in English were included in this study. The search process was conducted independently by two individuals, and in case of disagreement, the final decision would be made by the third more experienced corresponding author.

2.2 Bibliometric analysis

Bibliometric analysis and visualization were performed by using CiteSpace 6.1.R2, VOSviewer 1.6.18.0, and Microsoft Excel 2019, knowing that CiteSpace is a Java application for identifying and displaying new trends and developments in the scientific literature

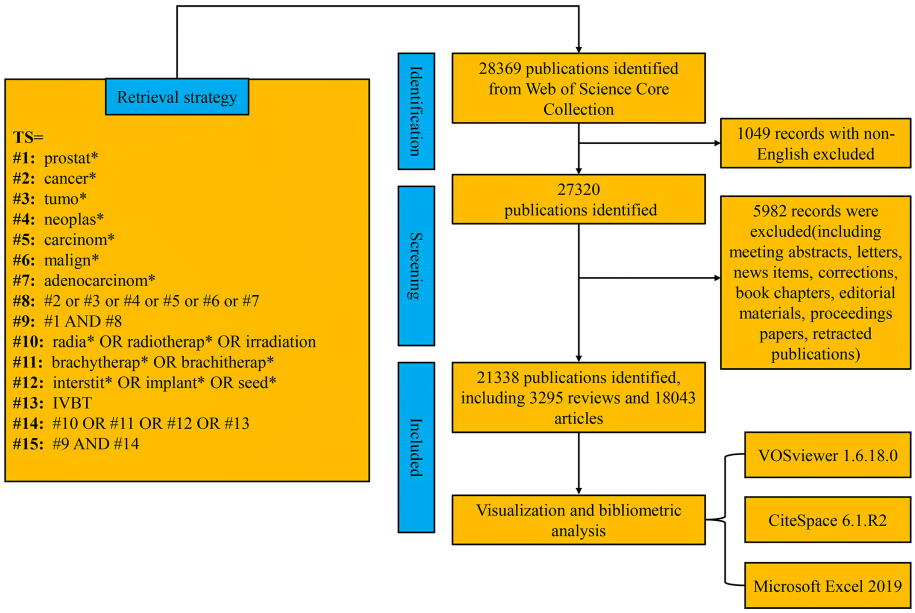


FIGURE 1
Flow chart of data screening. TS, topic search; *, truncation operator; #, connection character.

developed by Professor Chen Chaomei (7), and CiteSpace software makes it possible to find out research advances and current research frontiers in a certain subject area and its corresponding knowledge base (8, 9). We deployed CiteSpace to perform the dual-map overlay of journals, cluster and burstness analysis of references, and timeline and burstness analysis of keywords. The parameters were set as follows: the minimum burst duration (1 year), time span (January 2010 to December 2022), pruning (painfinder and pruning sliced networks), and selection criteria (Top N=50). The cluster analysis was performed by the log-likelihood ratio (LLR) algorithm, and other parameters were set to default values. In addition, we further calculated the nodes with high betweenness centrality (≥0.1) in the keywords to identify the important pivots within a domain (7, 9).

VOSviewer is another professional bibliometric analysis and knowledge graph visualization software suitable for large-scale data analysis, which supports labeled views, overlay views, density views, and cluster views (10). In this study, VOSviewer software was used to map the country/region collaboration network, author collaboration network along with co-citation network, journals co-citation network, references co-citation network, and co-occurrence network of keywords. All the contents were analyzed by the fractional counting method, with the cartographic thresholds shown in the corresponding sections.

Excel software was used to collate data characteristics. The graph of the annual publication quantity in the top 10 countries/regions was created with the help of an online website (https://bibliometric.com/). In addition, Journal Citation Reports (JCR), as an authoritative multidisciplinary journal evaluation tool, is an important indicator to measure the value of scientific research. The H-index can also accurately measure an author's academic achievement (11). We obtained the JCR division and impact factor (IF) of journals in 2021, as well as the H-index of researchers through the WoS database.

3 Results

3.1 Contributions of countries/regions and institutions to global publications

A total of 21338 papers (18043 original articles and 3295 reviews) were screened from the WoSCC database, involving 129 countries/regions and 14184 institutions (Figure 1). Over the past 10 years, studies related to RT in PCa have increased steadily. The United States of America (USA) took the lead in the annual publication volume (Figure 2A). The top 10 countries/regions and institutions by the number of publications are shown in Table 1. The USA enjoyed the largest number of papers (n=8489), followed by China (n=2198), Canada (n=2028), and Germany (n=1972) (Figure 2B). However, China only ranked eight in citations (n= 38827), with the USA taking the first place (n= 266342), and the United Kingdom (UK) the second place (n= 69001), and Canada in third place (n= 67039). As shown in the country cooperation map, the intensity of cooperation between China and the USA was the strongest, and the cooperation between the other countries was comparatively weak (Figure 3A). The national cooperation as a whole needed to be strengthened in future. Figure 3B further demonstrates the dominance of *the Occident*, showing that the USA took the lead in this field. The institutional cooperation network in Figure 3C shows that most of the top 10 publishers

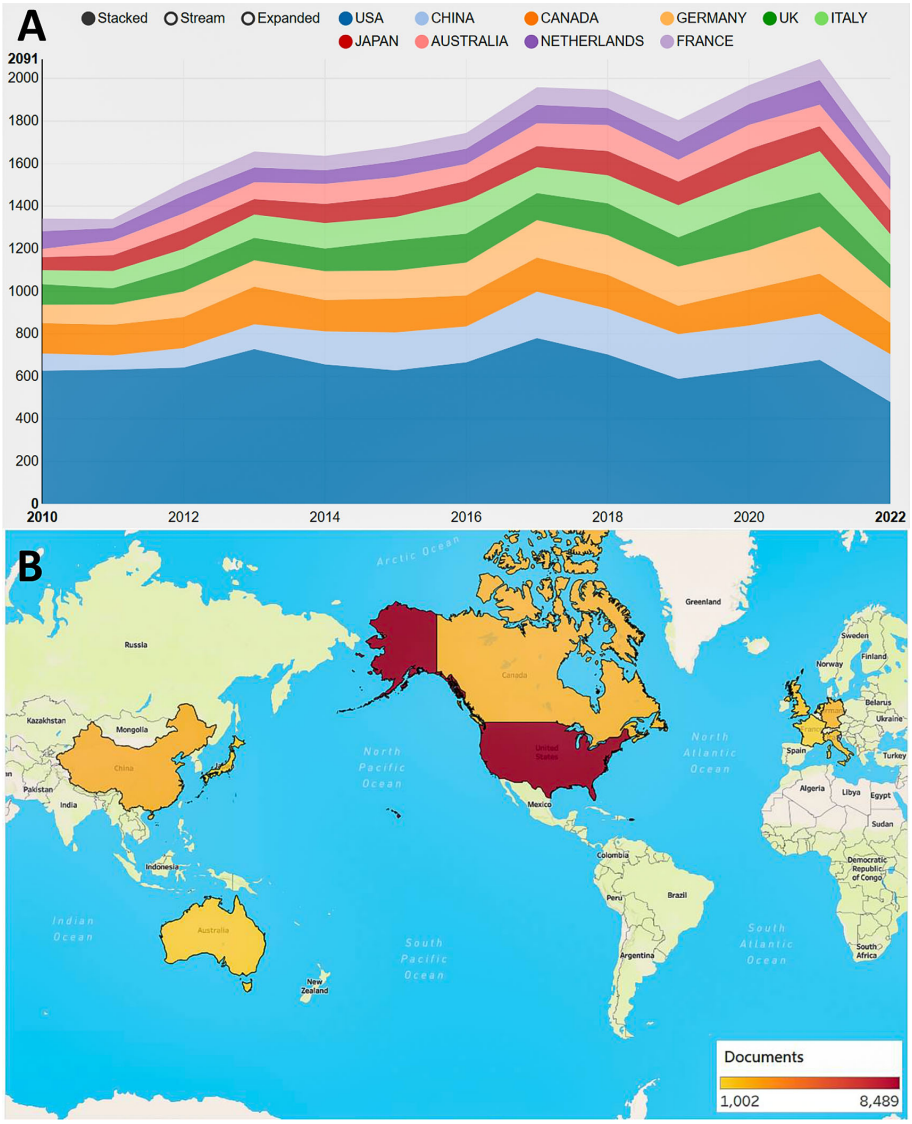


FIGURE 2
Analysis of publications in countries/regions. **(A)** Annual publication trends for the top 10 countries by the article number. **(B)** Geographical map of the top 10 countries/regions in the number of publications.

TABLE 1 Top 10 countries/regions and institutions related to radiotherapy for prostate cancer.

Rank	Country/Regions	Count	Citations	Rank	Institution	Count	Citations
1	United States	8489	266342	1	University of Toronto, Canada	587	21029
2	China	2198	38827	2	Memorial Sloan-Kettering Cancer Center, USA	553	25803
3	Canada	2028	67039	3	The University of Texas MD Anderson Cancer Center, USA	551	20415
4	Germany	1972	64985	4	University of California - San Francisco, USA	484	17668
5	Italy	1634	49370	5	University of Michigan, USA	448	16889
6	United Kingdom	1563	69001	6	University of California, Los Angeles, USA	409	16097
7	Japan	1280	18821	7	Mayo Clinic, USA	407	15101
8	Australia	1186	36198	8	The Johns Hopkins University, USA	327	12115
9	Netherlands	1037	44013	9	Duke University, USA	327	13249
10	France	1002	40915	10	Harvard University, USA	307	19204

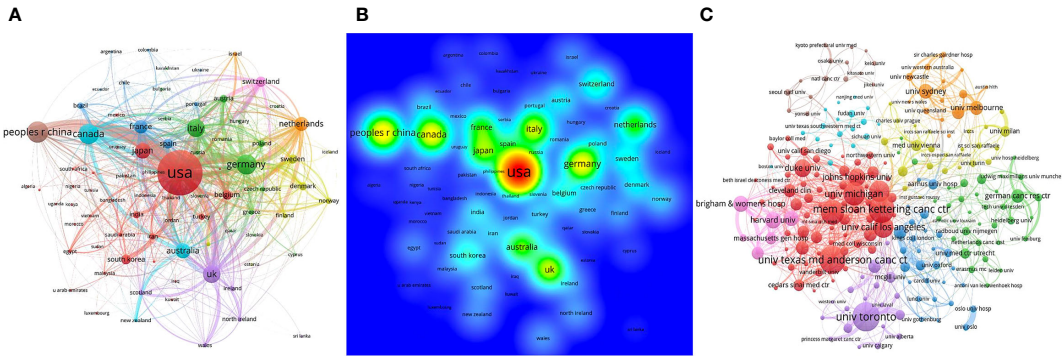


FIGURE 3
Analysis of cooperation networks in countries/regions and institutions. **(A)** National cooperation network. The size of the node represents the number of documents, and the thickness of links represents the strength of collaboration. **(B)** Density map of countries and regions. **(C)** Cooperation network visualization between institutions.

were in the USA (n=9, 90%). It is noteworthy that the University of Toronto was the institution with the largest number of papers (n=587). The contribution of Canada to the field of RT in PCa also deserves close attention.

3.2 Analysis of authors

Of the 82648 authors selected by VOSviewer, 71 authors had 50 or more publications, based on which an author collaboration network was drawn (Figure 4A). The six colors in the cooperation network represent different clusters. The high cooperation intensity mainly occurred in the same cluster, such as "D'amico, Anthony V." and "Chen, Ming-Hui", "Graefen, Markus" and "Tilki, Derya". It is clear that "Briganti, Alberto" was at the center of the collaborative network, with a high level of collaboration with other authors. The author with the largest number of articles in the field was "Nguyen, Paul L."(n=179), followed by "Briganti, Alberto" (n=168) and "Montorsi, Francesco"(n=121) (Table 2).

In addition, co-cited authors refer to two or more authors who are simultaneously cited in one or more papers. Among the 209840 co-cited authors, 332 authors enjoyed more than 200 co-citations (Figure 4B). Larger nodes represent more citations. The top three authors with the most co-citations were "Zelefsky, Michael J. "(n=3376), "D'amico, Anthony V."(n=3347), and "Bolla, Michel"(n=2650) (Table 2).

3.3 Analysis of journals

A total of 2005 journals published articles on RT in PCa, of which the top 10 journals published 4447 publications, accounting for 22.4% of all papers (Table 3). The journal with the most publications was *INTERNATIONAL JOURNAL OF RADIATION ONCOLOGY BIOLOGY PHYSICS* (n=1026, IF=8.013), followed by *RADIOTHERAPY AND ONCOLOGY* (n=641, IF=6.901) and *MEDICAL PHYSICS* (n=515, IF=4.506) (Figure 5A). In addition, analysis of co-cited journals can determine the core or marginal position of a journal in a discipline. Highly co-cited journals represent their significant influence in a specific field. Of the 34902 co-cited journals, 544 journals were cited more than 200 times, with *INTERNATIONAL JOURNAL OF RADIATION ONCOLOGY BIOLOGY PHYSICS* (n=78550, IF=8.013) taking the

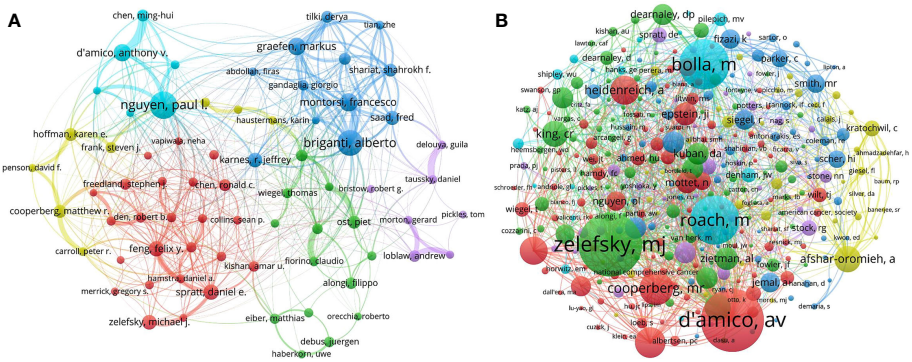


FIGURE 4
Visualization map of authors. **(A)** Author collaboration network. The size of the node indicates the number of papers, and the thickness of the links represents the intensity of the cooperation. **(B)** Author co-citation analysis.

TABLE 2 Top 10 authors and co-cited authors related to radiotherapy for prostate cancer.

Rank	Author	Documents	H-Index	Author	Co-citations	H-Index
1	Nguyen, Paul L.	179	56	Zelefsky, Michael J.	3376	86
2	Briganti, Alberto	168	83	D'amico, Anthony V.	3347	71
3	Montorsi, Francesco	121	115	Bolla, Michel	2650	47
4	Graefen, Markus	117	85	Roach, Mack	2574	61
5	D'amico, Anthony V.	114	71	Cooperberg, Matthew R.	1731	65
6	Feng, Felix Y.	100	79	Thompson, Ian M.	1568	82
7	Spratt, Daniel E.	100	27	Heidenreich, Axel	1481	67
8	Karnes, R. Jeffrey	96	64	Stephenson, Andrew J.	1443	52
9	Zelefsky, Michael J.	96	90	Afshar-Oromieh, Ali	1349	37
10	Saad, Fred	93	86	Pollack, Alan	1344	51

lead, followed by *JOURNAL OF CLINICAL ONCOLOGY* (n=30749, IF=50.739) and *JOURNAL OF UROLOGY* (n=29698, IF=7.641) (Table 3). The corresponding co-citation network diagram is shown in Figure 5B, which contains five clusters.

Additionally, the topic distribution of academic journals is represented by conducting the dual-map overlay of journals (Figure 6). Citing journals are on the left and cited journals are on the right, with colored lines standing for citation relationships. It can be seen that there are mainly four paths, from Medicine/Medical/Clinical journals to Molecular/Biology/Genetics journals, Medicine/Medical/Clinical journals to Health/Nursing/Medicine journals, Molecular/Biology/Immunology journals to Molecular/Biology/Genetics journals, and Molecular/Biology/Immunology journals to Health/Nursing/Medicine journals.

3.4 Co-citation network of references

Of the 417830 cited references, 112 were cited at least 200 times, and the corresponding co-citation network is shown in Figure 7A. Table 4 presents the top 10 cited references, all of which are articles. The most cited reference was written by Mack Roach 3rd et al. in *INTERNATIONAL JOURNAL OF RADIATION ONCOLOGY BIOLOGY PHYSICS* in 2006, which is entitled *Defining biochemical failure following radiotherapy with or without hormonal therapy in men with clinically localized prostate cancer: recommendations of the RTOG-ASTRO Phoenix Consensus Conference* (n=1285). We then performed a cluster analysis of the references. The largest 11 clusters are summarized in Figure 7B. The clustering color tends to be yellow to indicate a more recent

TABLE 3 Top 10 journals and co-cited journals related to radiotherapy for prostate cancer.

Rank	Journal	Documents	JCR (2021)	IF (2021)	Co-cited journal	Citations	JCR (2021)	IF (2021)
1	INTERNATIONAL JOURNAL OF RADIATION ONCOLOGY BIOLOGY PHYSICS	1026	Q1	8.013	INTERNATIONAL JOURNAL OF RADIATION ONCOLOGY BIOLOGY PHYSICS	78550	Q1	8.013
2	RADIOTHERAPY AND ONCOLOGY	641	Q1	6.901	JOURNAL OF CLINICAL ONCOLOGY	30749	Q1	50.739
3	MEDICAL PHYSICS	515	Q2	4.506	JOURNAL OF UROLOGY	29698	Q1	7.641
4	BRACHYTHERAPY	501	Q3	2.441	EUROPEAN UROLOGY	25582	Q1	24.344
5	RADIATION ONCOLOGY	425	Q2	4.309	RADIOTHERAPY AND ONCOLOGY	25472	Q1	6.901
6	BJU INTERNATIONAL	390	Q1	5.969	CANCER RESEARCH	17051	Q1	13.312
7	PHYSICS IN MEDICINE AND BIOLOGY	348	Q2	4.174	MEDICAL PHYSICS	16903	Q2	4.506
8	FRONTIERS IN ONCOLOGY	326	Q2	5.738	NEW ENGLAND JOURNAL OF MEDICINE	15671	Q1	176.082
9	JOURNAL OF UROLOGY	305	Q1	7.641	UROLOGY	14217	Q3	2.633
10	EUROPEAN UROLOGY	300	Q1	24.344	BJU INTERNATIONAL	13346	Q1	5.969

IF, impact factor; JCR, journal citation reports; Q, quartile in category.

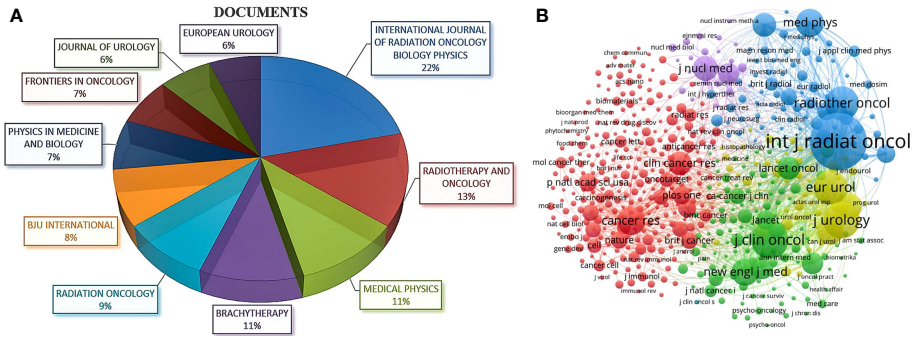


FIGURE 5
Visualization map of journals. **(A)** Pie chart of top 10 journals that published the largest number of documents. **(B)** Co-citation network of journals based on the reference sources. The size of the nodes indicates the co-citations of each journal, and the lines between the nodes represent the link strength.

occurrence. The largest and closest cluster was #0(oligorecurrent prostate cancer), to which the most relevant citer was *French ccafu guidelines - update 2020-2022: prostate cancer*. These updated French guidelines highlight the need for early salvage RT in the presence of biochemical recurrence (BCR) after RP and point out that the application of RT as a localized treatment modality for PCa can improve survival in synchronous OMPC patients (12). OMPC has emerged as a prominent research focus in recent years, prompting numerous clinical trials. For instance, Ren et al. conducted the world's first phase I/II prospective clinical trial on the "sandwich" therapy of OMPC, demonstrating the favorable tolerability of neoadjuvant radiohormonal therapy in OMPC patients (13). This breakthrough study provides a novel perspective for the treatment of advanced prostate cancer patients, presenting a new avenue for therapeutic exploration.

Furthermore, the burstness analysis provides insights into the development of research hotspots and trends over a period. We performed a burstness analysis of the references, and the top 25 are listed in Figure 8. Bray F, 2018, CA-CANCER J CLIN, V68, P394 had the highest burst strength (n=129.82), entitled *Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries*, with citation burstness from 2020 to 2022. Notably, two references are still frequently cited in the last two years. Respectively, Ryan Phillips et al. determined that stereotactic ablative radiotherapy (SABR) could improve oncological outcomes in patients with oligometastatic prostate cancer (MPC); Michael S Hofman et al. highlighted that prostate-specific membrane antigen (PSMA) PET-CT could provide a more accurate and effective basis for the management for PCa patients prior to RT or for the detection of BCR after radical RT.

3.5 Analysis of keywords

3.5.1 Co-occurrence analysis of keywords

Among the 24267 keywords, 165 appeared at least 50 times (Figure 9A). Table 5 shows the top 10 keywords about frequency and centrality. The most frequent term was "prostate cancer" (n=6755), followed by "radiotherapy" (n=2165) and "brachytherapy" (n=1156). According to the centrality, the term with the highest centrality was "prostate cancer" (n=0.81), followed by "radical prostatectomy" (n=0.4) and "radiation therapy" (n=0.15). RT and RP exhibit distinct advantages in the comprehensive management of prostate cancer, and the

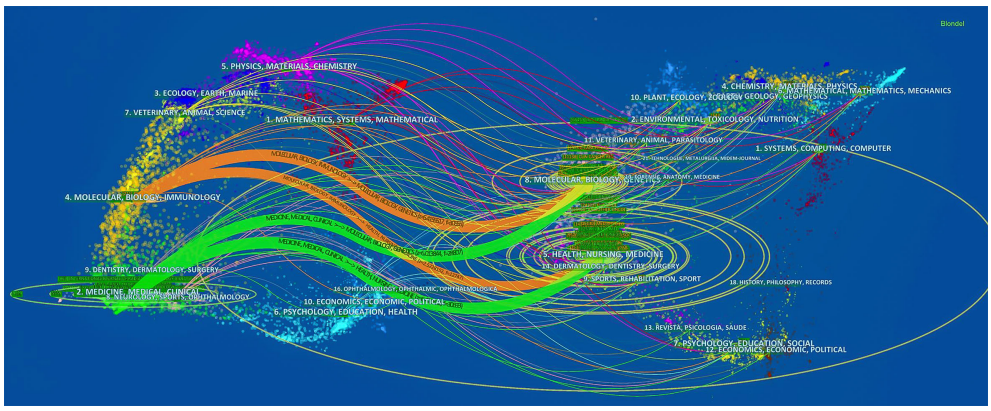


FIGURE 6
The dual-map overlay of journals on radiotherapy for prostate cancer.

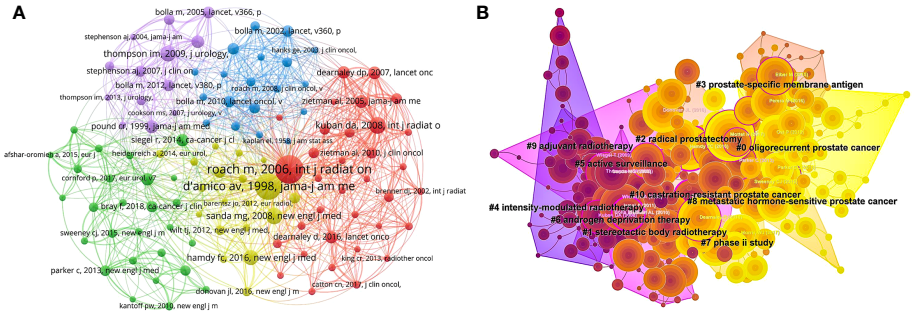


FIGURE 7
Visualization diagram of co-cited references. **(A)** Co-citation network of references. **(B)** Cluster analysis of cited references related to radiotherapy for prostate cancer. The color approaching yellow represents time getting closer.

comparative analysis of the two modalities has consistently remained a focal point of interest for researchers. A research report on a fifteen-year follow-up study of localized prostate cancer was recently published in *THE NEW ENGLAND JOURNAL OF MEDICINE* on April 27, 2023. The study findings reveal that both RP and RT demonstrate notably low prostate cancer-specific mortality (14). In addition, the timeline view of keywords shows the high-frequency keywords in each cluster over time (Figure 9B). The cluster #1(biochemical recurrence) is still ongoing, which provides researchers with a reference for research hotspots.

3.5.2 Burst keyword analysis

The bursts analysis is based on the word frequency growth to screen out words with high-frequency change rates and fast growth rates. As shown in Figure 10, the term with the strongest burst strength was "conformal radiotherapy"(n=83.1), followed by "localization" (n=70.18) and "dose escalation" (n=68.66).

TABLE 4 Top 10 co-cited references related to radiotherapy for prostate cancer.

Rank	Title	Type	Year	First Author	Journals	Citations
1	Defining biochemical failure following radiotherapy with or without hormonal therapy in men with clinically localized prostate cancer: recommendations of the RTOG-ASTRO Phoenix Consensus Conference	Article	2006	Mack Roach 3rd	International journal of radiation oncology, biology, physics	1285
2	Biochemical outcome after radical prostatectomy, external beam radiation therapy, or interstitial radiation therapy for clinically localized prostate cancer	Article	1998	A V D'Amico	JAMA	1182
3	Long-term results of the M. D. Anderson randomized dose-escalation trial for prostate cancer	Article	2008	Deborah A Kuban	International journal of radiation oncology, biology, physics	686
4	Quality of life and satisfaction with outcome among prostate-cancer survivors	Article	2008	Martin G Sanda	The New England journal of medicine	670
5	Adjuvant radiotherapy for pathological T3N0M0 prostate cancer significantly reduces risk of metastases and improves survival: long-term followup of a randomized clinical trial	Article	2009	Ian M Thompson	The Journal of urology	622
6	10-Year Outcomes after Monitoring, Surgery, or Radiotherapy for Localized Prostate Cancer	Article	2016	Freddie C Hamdy	The New England journal of medicine	593
7	Natural history of progression after PSA elevation following radical prostatectomy	Article	1999	C R Pound	JAMA	503
8	Comparison of conventional-dose vs high-dose conformal radiation therapy in clinically localized adenocarcinoma of the prostate: a randomized controlled trial	Article	2005	Anthony L Zietman	JAMA	498
9	Escalated-dose versus standard-dose conformal radiotherapy in prostate cancer: first results from the MRC RT01 randomised controlled trial	Article	2007	David P Dearnaley	The Lancet. Oncology	482
10	Phase III postoperative adjuvant radiotherapy after radical prostatectomy compared with radical prostatectomy alone in pT3 prostate cancer with postoperative undetectable prostate-specific antigen: ARO 96-02/AUO AP 09/95	Article	2009	Thomas Wiegel	Journal of clinical oncology : official journal of the American Society of Clinical Oncology	480

Top 25 References with the Strongest Citation Bursts

References	Year	Strength	Begin	End	2010 - 2022
Kuban DA, 2008, INT J RADIAT ONCOL, V70, P67, DOI 10.1016/j.ijrobp.2007.06.054, DOI	2008	98.66	2010	2013	
Thompson IM, 2009, J UROLOGY, V181, P956, DOI 10.1016/j.juro.2008.11.032, DOI	2009	95.04	2010	2014	
Sanda MG, 2008, NEW ENGL J MED, V358, P1250, DOI 10.1056/NEJMoa074311, DOI	2008	90.03	2010	2013	
Wiegel T, 2009, J CLIN ONCOL, V27, P2924, DOI 10.1200/JCO.2008.18.9563, DOI	2009	83.53	2010	2014	
Roach M, 2006, INT J RADIAT ONCOL, V65, P965, DOI 10.1016/j.ijrobp.2006.04.029, DOI	2006	82.6	2010	2011	
Jemal A, 2009, CA-CANCER J CLIN, V59, P225, DOI 10.3322/caac.20006, DOI	2009	72.39	2010	2013	
Dearnaley DP, 2007, LANCET ONCOL, V8, P475, DOI 10.1016/S1470-2045(07)70143-2, DOI	2007	68.71	2010	2012	
Bolla M, 2009, NEW ENGL J MED, V360, P2516, DOI 10.1056/NEJMoa0810095, DOI	2009	66.34	2010	2014	
Jemal A, 2011, CA-CANCER J CLIN, V61, P69, DOI 10.3322/caac.20107, DOI	2011	112.21	2011	2015	
Siegel R, 2012, CA-CANCER J CLIN, V62, P10, DOI 10.3322/caac.20138, DOI	2012	72.59	2012	2015	
Heidenreich A, 2011, EUR UROL, V59, P61, DOI 10.1016/j.eururo.2010.10.039, DOI	2011	70.05	2012	2016	
Wilt TJ, 2012, NEW ENGL J MED, V367, P203, DOI 10.1056/NEJMoa1113162, DOI	2012	81.47	2013	2017	
Bolla M, 2012, LANCET, V380, P2018, DOI 10.1016/S0140-6736(12)61253-7, DOI	2012	70.1	2014	2017	
Heidenreich A, 2014, EUR UROL, V65, P124, DOI 10.1016/j.eururo.2013.09.046, DOI	2014	74.93	2015	2018	
Heidenreich A, 2014, EUR UROL, V65, P467, DOI 10.1016/j.eururo.2013.11.002, DOI	2014	68.16	2015	2018	
Eiber M, 2015, J NUCL MED, V56, P668, DOI 10.2967/jnumed.115.154153, DOI	2015	64.18	2016	2020	
Hamdy FC, 2016, NEW ENGL J MED, V375, P1415, DOI 10.1056/NEJMoa1606220, DOI	2016	107.43	2017	2022	
Mottet N, 2017, EUR UROL, V71, P618, DOI 10.1016/j.eururo.2016.08.003, DOI	2017	87.03	2018	2022	
Dearnaley D, 2016, LANCET ONCOL, V17, P1047, DOI 10.1016/S1470-2045(16)30102-4, DOI	2016	84.19	2018	2022	
Ost P, 2018, J CLIN ONCOL, V36, P446, DOI 10.1200/JCO.2017.75.4853, DOI	2018	91.88	2019	2022	
Parker CC, 2018, LANCET, V392, P2353, DOI 10.1016/S0140-6736(18)32486-3, DOI	2018	68.21	2019	2022	
Bray F, 2018, CA-CANCER J CLIN, V68, P394, DOI 10.3322/caac.21492, DOI	2018	129.82	2020	2022	
Widmark A, 2019, LANCET, V394, P385, DOI 10.1016/S0140-6736(19)31131-6, DOI	2019	84.07	2020	2022	
Phillips R, 2020, JAMA ONCOL, V6, P650, DOI 10.1001/jamaoncol.2020.0147, DOI	2020	77.91	2021	2022	
Hofman MS, 2020, LANCET, V395, P1208, DOI 10.1016/S0140-6736(20)30314-7, DOI	2020	76.41	2021	2022	

FIGURE 8
Top 25 references with the strongest citation bursts.

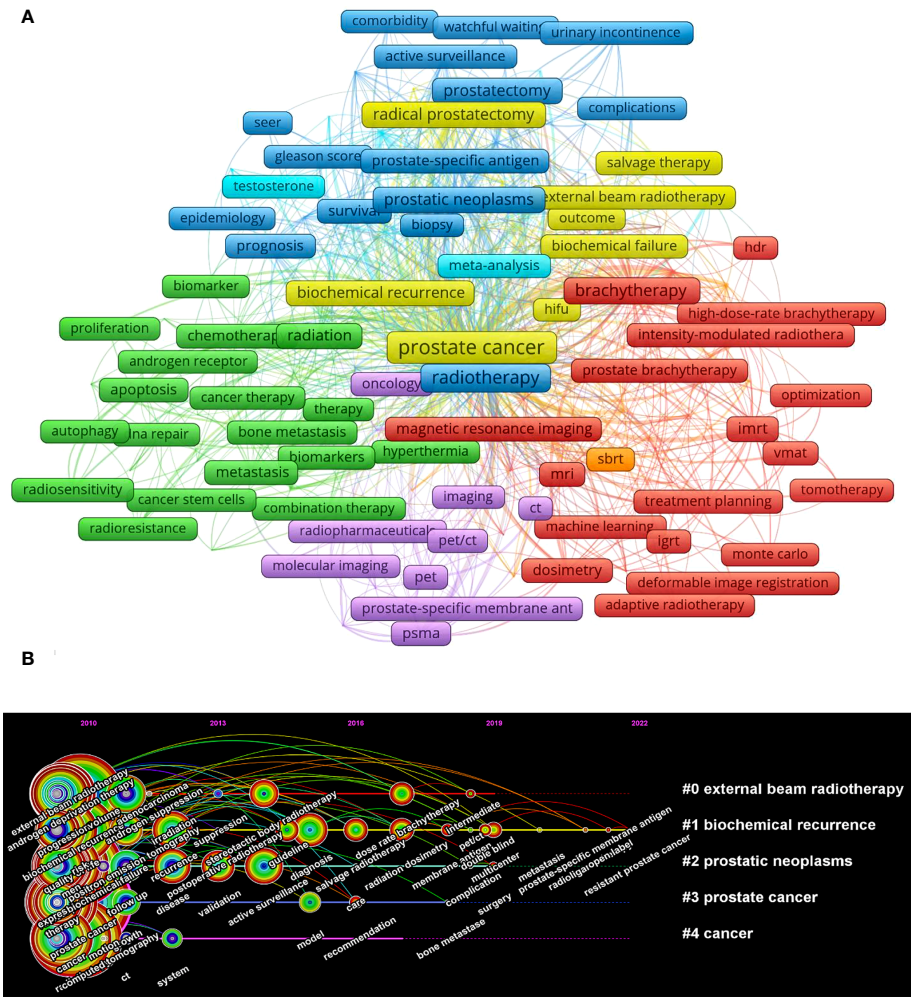


FIGURE 9
Visualization map of keywords in publications. **(A)** Occurrence analysis of keywords. **(B)** Timeline view of keywords. Each horizontal line indicates a cluster. The size of the circle indicates the frequency of occurrence, with the color approaching red representing the closer time.

10.3389/fonc.2023.1135052

TABLE 5 Top 10 keywords according to the frequency and centrality.

Rank	Keywords	Counts	Rank	Keywords	Centrality
1	Prostate cancer	6755	1	Prostate cancer	0.81
2	Radiotherapy	2165	2	Radical prostatectomy	0.4
3	Brachytherapy	1156	3	Radiation therapy	0.15
4	Prostatic neoplasms	735	4	Diagnosis	0.15
5	Radiation therapy	707	5	Progression	0.13
6	Radical prostatectomy	653	6	Dose escalation	0.13
7	Prostatectomy	559	7	Cancer	0.12
8	Quality of life	473	8	Androgen deprivation therapy	0.08
9	Radiation	389	9	Multicenter	0.08
10	Biochemical recurrence	355	10	Biochemical recurrence	0.07

Apparently, the word with the good burst strength in the past 2 years was “radiation dosimetry”, “dose rate brachytherapy”, “salvage radiotherapy”, “stereotactic body radiotherapy”, “guideline”, and “multicenter”.

4 Discussion

4.1 General information

In terms of the global publication volume over the last decade, there has been a general upward trend in research related to RT for PCa. Analysis of countries/regions shows that USA ranks first in the world in terms of the number of publications and citations. The majority (90%) of the top ten institutions are affiliated with the USA, further demonstrating the dominance of the USA in this field. This is due to the long-term advanced level in the medical field of European and American countries led by the USA. Among the university institutions, the University of Toronto, which is affiliated to Canada, ranks first in the number of articles published, and its contribution in the field also deserves our close attention. It is worth noting that while China holds the second position in terms of total publications, total citations for its research do not attain a leading position. This disparity implies that there exists potential for enhancing the innovativeness, breadth, or depth of China’s relevant research endeavors. Chinese researchers should

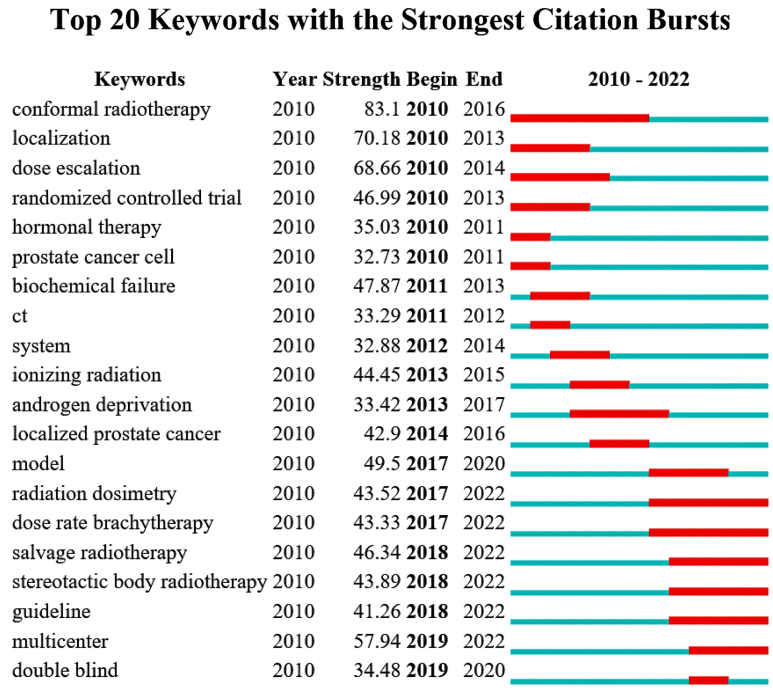


FIGURE 10
Top 25 keywords with the strongest citation bursts.

strengthen their collaboration and conduct more high-quality and innovative basic or clinical trials to increase China's academic influence. Furthermore, cooperation between countries/regions is mainly concentrated in countries such as the USA, UK, Netherlands, and China. Global cooperation requires to be further strengthened.

Among the top 10 authors according to publications and co-citations, A V D'Amico ranks among the top 5. A V D'Amico has made great contributions to the field of RT on PCa. In 1998, his team published an article, reporting that patients with intermediate and high-risk PCa who underwent RP or external beam radiation (EBRT) showed better BCR outcomes than those who received interstitial radiation therapy, which has been co-cited up to 1182 times in the field. Additionally, Paul L Nguyen, who has published the largest number of articles (n=179), has also made outstanding contributions to the research of RT on PCa. In 2018, Paul L Nguyen et al. published an article in the journal *CANCER* entitled *Travel distance and stereotactic body radiotherapy for localized prostate cancer*. The article emphasized the growing interest in the therapeutic effect and significance of definitive stereotactic body radiotherapy (SBRT) in localized PCa (15). Notably, the most co-cited author is Michael J Zelefsky (n=3376). Just in September 2022, Michael J Zelefsky et al. published an article entitled *Combined brachytherapy and ultra-hypofractionated radiotherapy for intermediate-risk prostate cancer: Comparison of toxicity outcomes using a high-dose-rate (HDR) versus low-dose-rate (LDR) brachytherapy boost*. They reported that both LDR and HDR brachytherapy boost combined with ultra-hypofractionated external beam radiation therapy (UH-EBRT) had good toxicity profiles, with a significant reduction in grade 2 + genitourinary toxicity found in patients receiving HDR (16).

Our journal analysis shows that the related journals are mainly concerned with clinical medicine, molecular biology, and immunology, which is consistent with the dual-map analysis. The journal *INTERNATIONAL JOURNAL OF RADIATION ONCOLOGY BIOLOGY PHYSICS* published the largest number of papers(n=1026) in the field, also ranking first in terms of co-citations (n=78550). This journal has received widespread attention from researchers. The journals *RADIOTHERAPY AND ONCOLOGY* and *MEDICAL PHYSICS* have an important influence on research, ranking second and third respectively in the number of publications. It is worth noting that although the journal *JAMA* is not in the top 10 in terms of publications and co-citations, three of the top 10 most co-cited articles were published in this journal, accounting for the largest proportion (30%), which deserves attention of the researchers. Important outputs in the field may later be published in the above journals. Researchers can regard these journals as an important source of theoretical references and ideal choices for publication in future.

The collation of high-frequency co-cited references provides an understanding about the knowledge base in the field. Among the top 10 co-cited references, seven mainly focus on the impact of RT on the outcome indicators such as BCR in PCa patients, and four explored the effectiveness of RT at different doses for PCa patients. Burstness analysis of references showed two references in burst which deserve our attention because they highlight the important role of PSMA PET-CT in guiding RT strategies.

4.2 Hotspots and trends of radiotherapy on prostate cancer

Amid the ongoing information explosion, it is vital for researchers to effectively grasp the developmental trends in their research field. In this paper, we utilized bibliometrics to explore emerging topics in the field through the cluster analysis and citation burstness analysis of references (5, 9, 17). Then, we evaluated the hotspots and frontiers through keyword co-occurrence analysis (18), keyword timeline (19), and burstness analysis of keywords.

Cluster analysis of references showed that RT for OMPC has been a hot spot in recent years. OMPC is a type of PCa between the state of tumor localization and extensive metastasis (20). The 2021 Updated European Association of Urology guidelines recommend a regimen of androgen deprivation therapy (ADT) combined with RT for patients with OMPC (21). SBRT, as a non-invasive treatment, can provide good control of local tumors with shorter treatment cycles and larger single doses (22). SBRT is a breakthrough treatment in the field of RT and has received widespread attention, which is in line with the results of our keyword burstness analysis. Some scholars evaluated 117 lesions in 74 patients with pelvic node oligorecurrent PCa who were treated with SBRT, and the result showed a 100% local control rate in all patients (23). In a prospective study, Deodato et al. selected 37 OMPC patients with bone metastases who received single fraction SBRT in the dose range of 12-24 Gy. During the median follow-up period of 25 months, few toxic events were observed in these patients, showing a high local control rate and prolonged *next-line systemic treatment-free* survival (NEST-FS) (24). In addition, the reference burstness analysis showed that (PSMA) PET-CT has gradually been widely used in recent years. Mazzola et al. conducted a prospective observational study involving 20 patients with castration sensitive oligorecurrent PCa who underwent PSMA-PET/CT guided SBRT by means of 1.5 T MRI-Linac, which initially confirmed the effectiveness and tolerability of this treatment (25). Other studies have shown that compared with choline-PET, PSMA-11-PET-guided SBRT resulted in a significantly longer response duration and ADT-free survival (26). A multi-institutional study in 2022 also demonstrated the superior performance of PSMA-PET guided SBRT in delaying the initiation of ADT in OMPC patients (27). PSMA-PET imaging holds great promise in the treatment of PCa. Nevertheless, the biological characteristics of OMPC are not fully understood, and there is no international consensus on the management of OMPC. The inclusion of SBRT in the routine management of PCa currently requires long-term clinical studies.

Our keyword burstness analysis showed that brachytherapy (BT) has also been a focus of research in the field of RT for PCa in the past 5 years. BT mainly consists of low-dose rate brachytherapy (LDR-BT) and high-dose rate brachytherapy (HDR-BT). Although HDR-BT

requires higher equipment costs than LDR-BT, several studies in the last two years have shown that HDR-BT is significantly better than LDR-BT in terms of postoperative adverse effects. Parry et al. conducted an observational cohort study of 54642 PCa patients and showed that both HDR-BT and LDR-BT exhibited similar degrees of genitourinary (GU) toxicity, whereas LDR-BT had significantly worse gastrointestinal (GI) toxicity (28). By enrolling 99 patients with intermediate-risk PCa, Kollmeier et al. demonstrated that patients receiving HDR-BT exhibited significantly less grade 2+ GU toxicity than those receiving LDR-BT (16). Other studies indicated that HDR-BT has better health related quality of life (HRQOL) in the irritative urinary domain compared with LDR-BT, although LDR-BT resulted in lower nadir prostate-specific antigen (nPSA) (29, 30). In addition, as an important modality for salvage RT, HDR-BT has similar efficacy to LDR-BT (31), while HDR-BT has potential advantages due to its biological characteristics and uneven dose distribution. Ménard et al. studied 88 patients from two institutions who underwent salvage HDR-BT at 22-26 Gy, and the 3-year and 5-year failure-free survival (FFS) rates were 67% and 49%, respectively (32). Kissel et al. reported 64 patients treated with salvage HDR-BT and showed a 2-year disease-free survival (DFS) rate of 58% in the whole population and 66% in hormone-sensitive patients (33). Given the lack of data from large-scale phase III clinical trials and no consensus on the optimal fractionation schedule, the potential of HDR-BT in the treatment of PCa needs to be further explored at a later stage.

Through the analysis of the timeline of keywords, it is apparent to see that the current research focus in the field of RT for PCa continues to revolve around the BCR after RT. BCR after RT is defined as a PSA value above the nadir of 2ng/ml after RT (34). The BCR has guided researchers to explore protocol options and treatment outcomes for multiple RT modalities, and has also prompted researchers to introduce more sensitive and accurate detection devices (such as PSMA-PET), which significantly enhanced the ability to localize PCa recurrence. These are inseparable from the implementation of many multicenter studies in the past two years (35–39), which is similar to the results of our keyword burstness analysis. In the field of RT for PCa, more multicenter clinical trials may emerge in the next few years, giving researchers new insights.

4.3 Strength and limitations

Compared with the previous meta-analyses and reviews, this bibliometric analysis provides more important data about the characteristics of RT for PCa, more objective references for the developmental trends and hotspots in the field, and a clearer picture of RT for PCa from multiple dimensions. Furthermore, different from previous investigations (6), this manuscript presents an immensely comprehensive and state-of-the-art data compilation and places particular emphasis on comprehensively exploring the panorama and advancements of RT in PCa from the entire spectrum of the field, aiming to contribute a wealth of content to the current knowledge system from a macro perspective. In data analysis, this paper has employed not only CiteSpace but also VOSviewer, another widely utilized tool in the field of bibliometrics. The latter furnishes an extensive array of visualization options, encompassing network visualization, density visualization, and overlay visualization, thereby empowering researchers to explore and present bibliometric data from diverse formats and perspectives. The synergistic amalgamation of these two tools enhances the visualization efficacy, credibility, and robustness of our research outcomes. In research hotspots, this study includes an essential analysis of keywords, including co-occurrence analysis, timeline, and burstness analysis. These analytical approaches, which have not been previously explored, provide novel insights into the underlying patterns and dynamics within the research domain. Novel hotspots and frontiers have been discerned, revealing the current research emphasis in RT for PCa revolving around the issue of BCR. Moreover, over the past two years, BT and SBRT have emerged as the central themes within this domain. Of the two, at least BT was overlooked in the previous study.

Certainly, there are inevitably some limitations in this study. This study only included original articles and reviews in English from the WoSCC database, which may differ slightly from the actual results. In addition, the constant updating of the database also had a subtle impact on the results of analysis, and more research needs to be included for future refinement.

5 Conclusion

Research on RT for PCa has been growing gradually worldwide over the last decade, with an emphasis on OMPC currently. The continuous advancement of imaging technologies has unveiled significant prospects for SBRT and BT in the realm of PCa treatment. Moreover, addressing the issue of BCR in PCa has long been a matter of importance. In this regard, salvage radiotherapy has garnered significant attention as a closely associated area of investigation at present. Several related large-scale multicenter studies have been conducted in recent years. More high-quality research is expected to be employed to guide clinical decision-making.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

Study concept and design: PHL, XX, PZ, and CR. Acquisition of data: XX and PZ. Analysis and interpretation of data: XX, PZ, CR, LL, JH, and PL. Drafting of the manuscript: XX, PZ, and CR. Critical revision of the manuscript for important intellectual content: XX and PHL. Statisticalanalysis: XX, PZ, CR, LL, JH, and PL. Supervision: PHL. All authors contributed to the article and approved the submitted version.

Funding

The work was funded by Natural Science Foundation of Chongqing, China (CSTB2023NSCQ-MSX0195), High-level Medical Reserved Personnel Training Project of Chongqing (the 4th batch) and Innovation Program for Chongqing's Overseas Returnees (cx2019146).

Acknowledgments

The authors would like to extend sincere gratitude to Professor Peihe Liang for instructive advice and useful suggestions in preparing the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: Cancer J For Clin* (2018) 68(6):394–424. doi: 10.3322/caac.21492

2. Lehrer EJ, Singh R, Wang M, Chinchilli VM, Trifiletti DM, Ost P, et al. Safety and survival rates associated with ablative stereotactic radiotherapy for patients with oligometastatic cancer: A systematic review and meta-analysis. *JAMA Oncol* (2021) 7 (1):92–106. doi: 10.1001/jamaoncol.2020.6146

3. Spratt DE, Malone S, Roy S, Grimes S, Eapen L, Morgan SC, et al. Prostate radiotherapy with adjuvant androgen deprivation therapy (ADT) improves metastasis-free survival compared to neoadjuvant ADT: an individual patient meta-analysis. *J Clin Oncol Off J Am Soc Clin Oncol* (2021) 39(2):136–44. doi: 10.1200/JCO.20.02438

4. Yeung AWK, Tosevska A, Klager E, Eibensteiner F, Laxar D, Stoyanov J, et al. Virtual and augmented reality applications in medicine: analysis of the scientific literature. *J Med Internet Res* (2021) 23(2):e25499. doi: 10.2196/25499

5. Chen C, Song M. Visualizing a field of research: A methodology of systematic scientometric reviews. *PloS One* (2019) 14(10):e0223994. doi: 10.1371/journal.pone.0223994

6. Li R, Liu X, Yang B, Qiu J. External beam radiotherapy for prostate cancer: What are the current research trends and hotspots? *Cancer Med* (2021) 10(2):772–82. doi: 10.1002/cam4.3700

7. Chen C. Searching for intellectual turning points: progressive knowledge domain visualization. *Proc Natl Acad Sci United States America* (2004) 101 Suppl 1:5303–10. doi: 10.1073/pnas.0307513100

8. Chen C. CiteSpace II: Detecting and visualizing emerging trends and transient patterns in scientific literature. *J Am Soc Inform Sci Technol* (2006) 57(3):359–77. doi: 10.1002/asi.20317

9. Chen C. Science mapping: A systematic review of the literature. *J Data Inf Sci* (2017) 2(2):1–40. doi: 10.1515/jdis-2017-0006

10. van Eck NJ, Waltman L. Software survey: VOSviewer, a computer program for bibliometric mapping. *Scientometrics* (2010) 84(2):523–38. doi: 10.1007/s11192-009-0146-3

11. Rad AE, Brinjikji W, Cloft HJ, Kallmes DF. The H-index in academic radiology. *Acad Radiol* (2010) 17(7):817–21. doi: 10.1016/j.acra.2010.03.011

12. Rozet F, Mongiat-Artus P, Hennequin C, Beauval JB, Beuzeboc P, Cormier L, et al. [French ccAFU guidelines - update 2020-2022: prostate cancer]. *Progres en Urologie J L'Association Francaise D'urologie Et la Societe Francaise D'urologie* (2020) 30(12S):S136–251. doi: 10.1016/S1166-7087(20)30752-1

13. Chang Y, Zhao X, Xiao Y, Yan S, Xu W, Wang Y, et al. Neoadjuvant radiohormonal therapy for oligo-metastatic prostate cancer: safety and efficacy outcomes from an open-label, dose-escalation, single-center, phase I/II clinical trial. *Front Med* (2023) 17(2):231–9. doi: 10.1007/s11684-022-0939-9

14. Hamdy FC, Donovan JL, Lane JA, Metcalfe C, Davis M, Turner EL, et al. Fifteen-year outcomes after monitoring, surgery, or radiotherapy for prostate cancer. *New Engl J Med* (2023) 388(17):1547–58. doi: 10.1056/NEJMoa2214122

15. Mahal BA, Chen Y-W, Sethi RV, Padilla OA, Yang DD, Chavez J, et al. Travel distance and stereotactic body radiotherapy for localized prostate cancer. *Cancer* (2018) 124(6):1141–9. doi: 10.1002/cncr.31190

16. Kollmeier MA, Gorovets D, Flynn J, McBride S, Brennan V, Beaudry J, et al. Combined brachytherapy and ultra-hypofractionated radiotherapy for intermediate-risk prostate cancer: Comparison of toxicity outcomes using a high-dose-rate (HDR) versus low-dose-rate (LDR) brachytherapy boost. *Brachytherapy* (2022) 21(5):599–604. doi: 10.1016/j.brachy.2022.04.006

17. Ma L, Ma J, Teng M, Li Y. Visual analysis of colorectal cancer immunotherapy: A bibliometric analysis from 2012 to 2021. *Front In Immunol* (2022) 13:843106. doi: 10.3389/fimmu.2022.843106

18. Xiao F, Li C, Sun J, Zhang L. Knowledge domain and emerging trends in organic photovoltaic technology: A scientometric review based on citeSpace analysis. *Front In Chem* (2017) 5:67. doi: 10.3389/fchem.2017.00067

19. Wang Y, Jia Y, Li M, Jiao S, Zhao H. Hotspot and frontier analysis of exercise training therapy for heart failure complicated with depression based on web of science database and big data analysis. *Front In Cardiovasc Med* (2021) 8:665993. doi: 10.3389/fcvm.2021.665993

20. Rossetti S, Di Napoli M, Pisano C, Cecere SC, Tambaro R, Ventriglia J, et al. Oligometastatic prostate cancer treatment. *Future Oncol (London England)* (2021) 17 (29):3893–9. doi: 10.2217/fon-2021-0126

21. Cornford P, van den Bergh RCN, Briers E, Broeck den Van T, Cumberbatch MG, Santis De M, 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(2):263–82. doi: 10.1016/j.eururo.2020.09.046

22. Ghadjar P, Wiegel T, De Bleser E, Jereczek-Fossa BA, Pasquier D, et al. Metastasis-directed therapy in treating nodal oligorecurrent prostate cancer: A multi-institutional analysis comparing the outcome and toxicity of stereotactic body radiotherapy and elective nodal radiotherapy. *Eur Urol* (2019) 76:732–9. doi: 10.1016/j.eururo.2019.07.009

23. Cozzi S, Botti A, Timon G, Blandino G, Najafi M, Manicone M, et al. Prognostic factors, efficacy, and toxicity of involved-node stereotactic body radiation therapy for lymph node oligorecurrent prostate cancer : An investigation of 117 pelvic lymph nodes. *Strahlentherapie Und Onkologie Organ Der Deutschen Rontgengesellschaft* (2022) 198(8):700–9. doi: 10.1007/s00066-021-01871-5

24. Deodato F, Pezzulla D, Cilla S, Ferro M, Romano C, Bonome P, et al. Stereotactic radiosurgery for bone metastases in oligometastatic prostate cancer patients: DESTROY-2 clinical trial subanalysis. *Clin Trans Oncol Off Publ Fed Spanish Oncol Societies Natl Cancer Institute Mexico* (2022) 24(6):1177–83. doi: 10.1007/s12094-021-02764-w

25. Mazzola R, Cuccia F, Figlia V, Rigo M, Nicosia L, Giaj-Levra N, et al. Stereotactic body radiotherapy for oligometastatic castration sensitive prostate cancer using 1.5 T MRI-Linac: preliminary data on feasibility and acute patient-reported outcomes. *La Radiologia Med* (2021) 126(7):989–97. doi: 10.1007/s11547-021-01352-w

26. Deijen CL, Vrijenhoek GL, Schaake EE, Vogel WV, Moonen LMF, Pos FJ, et al. PSMA-11-PET/CT versus choline-PET/CT to guide stereotactic ablative radiotherapy for androgen deprivation therapy deferral in patients with oligometastatic prostate cancer. *Clin Trans Radiat Oncol* (2021) 30:1–6. doi: 10.1016/j.ctro.2021.06.004

27. Mazzola R, Cuccia F, Pastorello E, Salgarello M, Francolini G, Livi L, et al. PSMA-guided metastases directed therapy for bone castration sensitive oligometastatic prostate cancer: a multi-institutional study. *Clin Exp Metastasis* (2022) 39(3):443–8. doi: 10.1007/s10585-022-10157-8

28. Parry MG, Nossiter J, SujenthIran A, Cowling TE, Patel RN, Morris M, et al. Impact of high-dose-rate and low-dose-rate brachytherapy boost on toxicity, functional and cancer outcomes in patients receiving external beam radiation therapy for prostate cancer: A national population-based study. *Int J Radiat Oncol Biol Phys* (2021) 109 (5):1219–29. doi: 10.1016/j.ijrobp.2020.11.023

29. Levin-Epstein R, Cook RR, Wong JK, Stock RG, Demanes Jeffrey D, Collins SP, et al. Prostate-specific antigen kinetics and biochemical control following stereotactic body radiation therapy, high dose rate brachytherapy, and low dose rate brachytherapy:

A multi-institutional analysis of 3502 patients. *Radiother Oncol J Eur Soc For Ther Radiol Oncol* (2020) 151:26–32. doi: 10.1016/j.radonc.2020.07.014

30. Reynaud T, Hathout L, Carignan D, Barkati M, Martin A-G, Foster W, et al. PSA outcomes and late toxicity of single-fraction HDR brachytherapy and LDR brachytherapy as monotherapy in localized prostate cancer: A phase 2 randomized pilot study. *Brachytherapy* (2021) 20(6):1090–8. doi: 10.1016/j.brachy.2021.05.010

31. Kollmeier MA, McBride S, Taggar A, Anderson E, Lin M, Pei X, et al. Salvage brachytherapy for recurrent prostate cancer after definitive radiation therapy: A comparison of low-dose-rate and high-dose-rate brachytherapy and the importance of prostate-specific antigen doubling time. *Brachytherapy* (2017) 16(6):1091–8. doi: 10.1016/j.brachy.2017.07.013

32. Ménard C, Navarro-Domenech I, Liu ZA, Joseph L, Barkati M, Berlin A, et al. MRI-guided focal or integrated boost high dose rate brachytherapy for recurrent prostate cancer. *Front In Oncol* (2022) 12:971344. doi: 10.3389/fonc.2022.971344

33. Kissel M, Pounou A, Ka K, Alexis A, Irani J, Jereczek-Fossa BA, et al. Efficacy and toxicity following salvage high-dose-rate brachytherapy for locally recurrent prostate cancer after radiotherapy. *Brachytherapy* (2022) 21(4):424–34. doi: 10.1016/j.brachy.2022.01.005

34. Cornford P, Bellmunt J, Bolla M, Briers E, De Santis M, Gross T, et al. EAU-ESTRO-SIOG guidelines on prostate cancer. Part II: treatment of relapsing, metastatic, and castration-resistant prostate cancer. *Eur Urol* (2017) 71(4):630–42. doi: 10.1016/j.eururo.2016.08.002

35. Cerci JJ, Fanti S, Lobato EE, Kunikowska J, Alonso O, Medina S, et al. Diagnostic performance and clinical impact of ga-PSMA-11 PET/CT imaging in early relapsed prostate cancer after radical therapy: A prospective multicenter study (IAEA-PSMA study). *J Nucl Med Off Publication Soc Nucl Med* (2022) 63(2):240–7. doi: 10.2967/jnumed.120.261886

36. Kirste S, Kroeze SGC, Henkenberens C, Schmidt-Hegemann N-S, Vogel MME, Becker J, et al. Combining ga-PSMA-PET/CT-directed and elective radiation therapy improves outcome in oligorecurrent prostate cancer: A retrospective multicenter study. *Front In Oncol* (2021) 11:640467. doi: 10.3389/fonc.2021.640467

37. Kishan AU, Wang X, Sun Y, Romero T, Michalski JM, Ma TM, et al. High-dose radiotherapy or androgen deprivation therapy (HEAT) as treatment intensification for localized prostate cancer: an individual patient-data network meta-analysis from the MARCAP consortium. *Eur Urol* (2022) 82(1):106–14. doi: 10.1016/j.eururo.2022.04.003

38. Kroeze SGC, Henkenberens C, Schmidt-Hegemann NS, Vogel MME, Kirste S, Becker J, et al. Prostate-specific membrane antigen positron emission tomography-detected oligorecurrent prostate cancer treated with metastases-directed radiotherapy: role of addition and duration of androgen deprivation. *Eur Urol Focus* (2021) 7(2):309–16. doi: 10.1016/j.euf.2019.08.012

39. Spohn SKB, Farolfi A, Schandeler S, Vogel MME, Ruf J, Mix M, et al. The maximum standardized uptake value in patients with recurrent or persistent prostate cancer after radical prostatectomy and PSMA-PET-guided salvage radiotherapy-a multicenter retrospective analysis. *Eur J Nucl Med Mol Imaging* (2022) 50(1):218–27. doi: 10.1007/s00259-022-05931-5

frontiers | Frontiers in Oncology

TYPE Case Report
PUBLISHED 27 September 2023
DOI 10.3389/fonc.2023.1185530

OPEN ACCESS

EDITED BY
Ran Xu,
Central South University, China

REVIEWED BY
Masayoshi Nagata,
Juntendo University, Japan
Martina Maggi,
Sapienza University of Rome, Italy

*CORRESPONDENCE
Ji Zheng
Jizheng@tmmu.edu.cn
Fan He
785517193@qq.com

RECEIVED 13 March 2023
ACCEPTED 05 September 2023
PUBLISHED 27 September 2023

CITATION
Ning W, Chang P, Zheng J and He F (2023) The second docetaxel rechallenge for metastatic castration-resistant prostate cancer: a case report. *Front. Oncol.* 13:1185530. doi: 10.3389/fonc.2023.1185530

The second docetaxel rechallenge for metastatic castration-resistant prostate cancer: a case report

Wei Ning[1], Pengkang Chang[1], Ji Zheng[1]* and Fan He[1,2]*

[1]Department of Urology, Second Affiliated Hospital, Army Medical University, Chongqing, China, [2]Urology Department, Institute of Urology (Laboratory of Reconstructive Urology), West China Hospital, Sichuan University, Chengdu, Sichuan, China

Background: Docetaxel combined with prednisone plus androgen deprivation therapy (ADT) is the preferred treatment option for metastatic hormone-sensitive prostate cancer (mHSPC) or metastatic castration-resistant prostate cancer (mCRPC). With the development of next-generation hormonal agents (NHAs) and poly (ADP-ribose) polymerase (PARP) inhibitors, more aggressive first-line or later-line treatment strategies have been added to the treatment of mHSPC and mCRPC. However, docetaxel rechallenge (DR) has special clinical significance in patients with "docetaxel-sensitive" prostate cancer. There are no reports on the efficacy and safety of the second DR in mCRPC patients.

Case presentation: We report one patient diagnosed with mCRPC who showed progression-free survival (PFS) and overall survival (OS) benefits and safety and good lower urinary tract function after the second DR.

Conclusion: The second DR as a potential alternative later-line treatment strategy should be considered for patients with mCRPC who worry about the high economic burden of multigene molecular testing and PARP inhibitors as well as repeated prostate needle biopsy.

KEYWORDS

prostate cancer (PCa), metastatic castration-resistant prostate cancer (mCRPC), second docetaxel rechallenge (DR), later-line treatment, case report

Introduction

The first case of prostate cancer (PCa) was described as a very rare disease by J. Adams at the London Hospital in 1853 (1). Currently, PCa ranks second in the incidence of male cancer and sixth in male cancer mortality worldwide (2). In China, the incidence and mortality of PCa have been rising rapidly for decades. In particular, Chinese patients with PCa have unique epidemiological characteristics, such as higher grading and staging of tumors and a worse disease prognosis (3). Malignant transformation of the normal

prostatic epithelium follows a complicated process (4). Metastatic spread of tumors is the main cause of death for patients with PCa. Bone metastases of patients with PCa always manifest as osteoblastic and osteolytic lesions, mainly osteoblastic features, which can lead to severe pain, pathological fractures, hypercalcemia, and nerve compression syndromes (5).

For decades, hormonal therapy, also known as androgen deprivation therapy (ADT), has played an important role in the treatment of patients with advanced PCa and is aimed at lowering testosterone levels. With the development of next-generation hormonal agents (NHAs) and chemotherapy, a more aggressive first-line treatment strategy has been added to the treatment of metastatic hormone-sensitive prostate cancer (mHSPC) (6). Although most patients suffering from mHSPC primarily respond to ADT, the duration of response is uncertain, and all patients ultimately develop metastatic castration-resistant prostate cancer (mCRPC) (7). The standard treatment of mCRPC refers to the combination of ADT and NHA (abiraterone acetate, enzalutamid) or chemotherapy (docetaxel or cabazitaxel). In addition, radium-223, an alpha emitter, can be considered as a treatment for symptomatic bone metastases of patients with PCa (8). Several therapies have been proven to improve the progression-free survival (PFS) and overall survival (OS) of patients with mCRPC. However, most patients eventually die from mCRPC within a few years (7).

Currently, docetaxel is approved for first-line treatment of mHSPC or mCRPC. Reintroduction of docetaxel, which is also known as docetaxel rechallenge (DR), lacks enough supporting evidence in patients with mCRPC (9). The concept of DR represents a special clinical significance in patients with "docetaxel-sensitive" PCa (10). Nevertheless, more high-level evidence is needed for DR as a potential alternative treatment in later lines. Here, we report one mCRPC patient with a second DR as an alternative to poly (ADP-ribose) polymerase (PARP) inhibitors or platinum-based chemotherapy.

Case presentation

A 70-year-old man was admitted to the Second Affiliated Hospital, Army Medical University on 27 December 2019, with the chief complaint of pollakiuria and urgent urination for half a year and dysuria for 13 days. The patient had urinary incontinence, nocturia, and intermittent hematuria but did not have other clinical symptoms or signs. The patient received transurethral resection of the prostate (TURP) at the local hospital 6 months prior, and the postoperative pathological diagnosis was uncertain. Urinary tract computed tomography (CT) on 14 December 2019, revealed a mass with mixed density in the pelvic region, unclearly displayed prostate and bladder, enlarged pelvic and retroperitoneal lymph nodes, and mildly dilated bilateral renal pelvis and ureter (Figure 1A). The prostate-specific antigen (PSA) level of the patient was 198 ng/mL. An enlarged prostate and a hard enlarged mass were palpated by digital rectal examination (DRE). The personal history, family history, and physical examination of the patient were not exceptional.

On 30 December 2019, the patient underwent TURP plus transperineal biopsy of the prostate, and invasion of the left wall of the bladder was observed during the operation. The postoperative pathological diagnosis showed prostate adenocarcinoma, Gleason score (GS) 5 + 4 = 9 (Figure 1B).

Positron emission tomography/computed tomography (PET/CT) on 7 January 2020, revealed that the mass in the pelvic region was considered a malignant tumor, and enlarged pelvic and retroperitoneal lymph nodes were considered metastatic carcinoma. The patient was eventually diagnosed with PCa (pT4N1M1a). Because of worrying about the adverse events of docetaxel chemotherapy and the high economic burden of NHA, at the beginning, the patient received ADT (goserelin, 3.6 mg, subcutaneous injection, per 28 days plus bicalutamide 50 mg, oral administration, once daily). To his disappointment, the reduction in the PSA level was unsatisfactory, dropping to only 7.04 ng/mL. Prostate-enhanced magnetic resonance imaging (MRI) on 18 June 2020 revealed an enlarged prostate with a maximum cross-sectional size of 50 × 37 mm, PCa with invasion of the left pelvic sidewall and left side of the bladder, unclearly displayed bilateral seminal vesicles, and enlarged left external and internal iliac lymph nodes (Figure 1C). According to the official definition of mCRPC by the European Association of Urology (EAU) guideline as well as Response Evaluation Criteria in Solid Tumors (RECIST) (11, 12), the patient showed radiological progression (an enlarged soft tissue lesion using RECIST), while the PSA level was more than three times as high as 2 ng/mL. The patient has become resistant to ADT with progression to mCRPC, despite serum testosterone remaining at castrate levels (< 50 ng/dL).

From 18 June to 23 October 2020, the patient accepted and started six cycles of docetaxel (75 mg/m^2, Day 1, intravenous injection, every 21 days) combined with prednisone (5 mg, oral administration, twice daily) plus goserelin. After six cycles of chemotherapy, the PSA level of the patient dropped to 0.97 ng/mL. Prostate-enhanced MRI on 15 March 2021, suggested an enlarged prostate with a maximum cross-sectional size of 50 ×3 7 mm and a significantly reduced volume of the prostate and left pelvic sidewall lesions compared to before treatment (Figure 1D). The patient had good lower urinary tract function and clinical efficacy and safety. However, he was still afraid of the adverse events of docetaxel chemotherapy and refused to accept chemotherapy sequentially. After 6 months of follow-up and maintenance ADT, the PSA level of the patient rose to 19.48 ng/mL. Prostate-enhanced MRI on 24 May 2021 revealed a significantly increased lesion volume in the left pelvic sidewall and new metastasis in the left femoral neck, sacrum, and bilateral iliac crest (Figure 1E).

We further analyzed the homologous recombination repair (HRR) gene panel of the patient by genetic testing of circulating tumor DNA (ctDNA), and the presence of HRR gene mutations (HRRm) was not found. Therefore, from 25 May to 7 December 2021, the patient accepted and started 10 cycles of docetaxel combined with prednisone (the first DR) plus goserelin and the addition of abiraterone acetate (1,000 mg, oral administration, once daily). Prostate-enhanced MRI on 17 November 2021 revealed a prostate with a maximum cross-sectional size of 37 × 29 mm and a

10.3389/fonc.2023.1185530

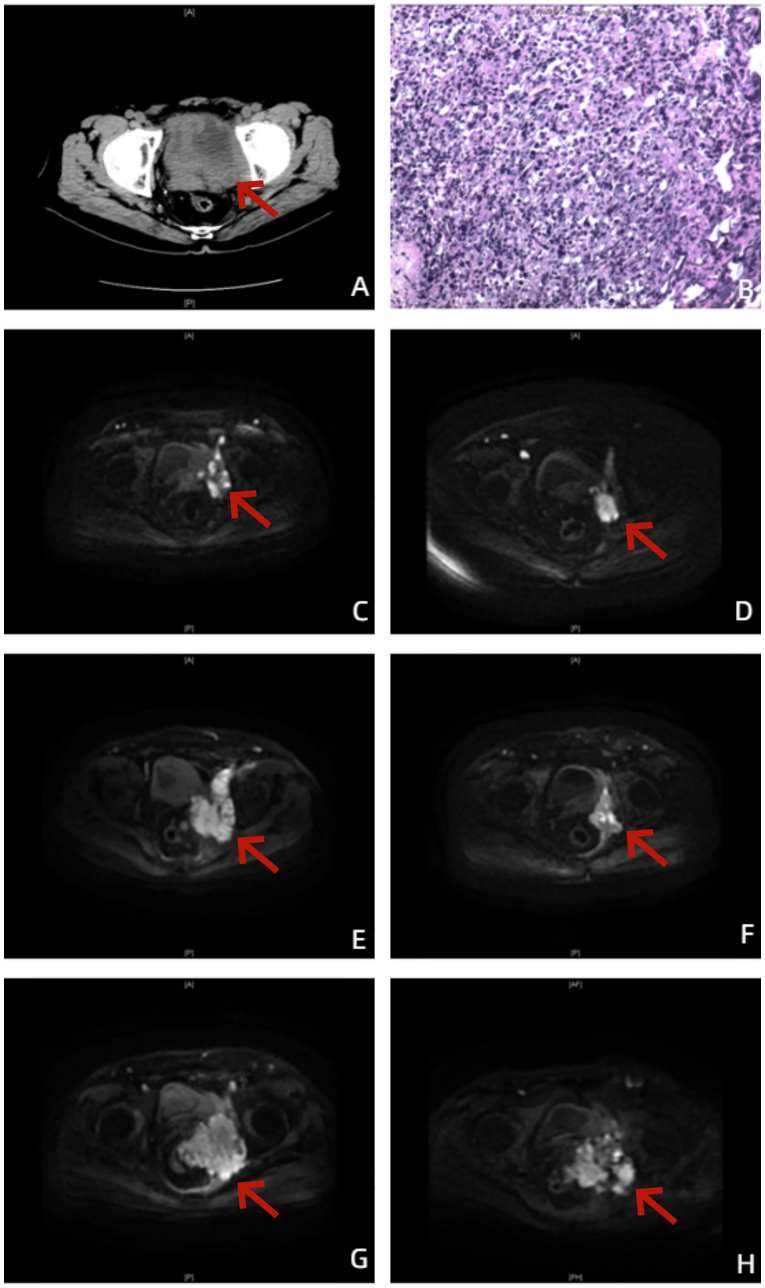


FIGURE 1
Images of the patient throughout the treatment. **(A)** Before treatment, urinary tract CT showed a localized mass with mixed density in the pelvic region, unclearly displayed prostate and bladder, enlarged pelvic and retroperitoneal lymph nodes, and mildly dilated bilateral renal pelvis and ureter. **(B)** The postoperative pathological diagnosis showed prostate adenocarcinoma, Gleason score (GS) 5 + 4 = 9. **(C)** On 18 June 2020, prostate-enhanced MRI cross-sectional DWI showed an enlarged prostate with a maximum cross-sectional size of 50 × 37 mm, prostate cancer with invasion of the left pelvic sidewall and left side of the bladder, unclearly displayed bilateral seminal vesicles, and enlarged left external and internal iliac lymph nodes. **(D)** On 15 March 2021, prostate-enhanced MRI cross-sectional DWI showed an enlarged prostate with a maximum cross-sectional size of 50 × 37 mm and a significantly reduced volume of the prostate and left pelvic sidewall lesions compared to before treatment. **(E)** On 24 May 2021, prostate-enhanced MRI cross-sectional DWI showed a significantly increased lesion volume in the left pelvic sidewall and new metastasis in the left femoral neck, sacrum, and bilateral iliac crest. **(F)** On 17 November 2021, prostate-enhanced MRI cross-sectional DWI showed prostate with a maximum cross-sectional size of 37 × 29 mm and significantly reduced volume of lesion of prostate and left pelvic sidewall. Except for the left femoral neck, no bone metastases were found in the other parts of the body. **(G)** On 23 March 2022, prostate-enhanced MRI cross-sectional DWI showed that the volume of the lesion of the left pelvic sidewall was significantly increased, and rectal invasion was not ruled out. **(H)** On 8 September 2022, prostate-enhanced MRI cross-sectional DWI did not show new confirmed progression of imaging.

significantly reduced lesion volume in the prostate and left pelvic sidewall. Except for the left femoral neck, no bone metastases were found in the other parts of the body (Figure 1F). After 10 cycles of chemotherapy, the PSA level of the patient dropped to 0.61 ng/mL again. Unfortunately, after less than 3 months of follow-up and maintenance goserelin plus abiraterone combined with prednisone, the PSA level of the patient rose to 21.78 ng/mL. In addition, prostate-enhanced MRI on 23 March 2022 revealed that the volume of the lesion on the left pelvic sidewall was significantly increased, and rectal invasion was not ruled out (Figure 1G).

After multidisciplinary team (MDT) discussion regarding the worries about the high economic burden of multigene molecular testing by tissue biopsy and PARP inhibitors as well as repeated prostate needle biopsy, on 30 March 2022, the patient accepted and started eight cycles of docetaxel combined with prednisone (the second DR) as well as maintenance goserelin plus abiraterone. Although the PSA response cannot reach a 97% PSA reduction as the first DR, the PSA level of the patient can still be maintained at 19–27 ng/mL. Full- body bone scan and prostate-enhanced MRI (Figure 1H) of follow-up did not show new confirmed progression of imaging. The changes in the PSA and serum testosterone levels throughout the treatment are shown in Figure 2.

Discussion

Currently, metastatic PCa (mPCa) remains incurable worldwide. Docetaxel was the first systemic therapy showing a survival benefit to patients with mHSPC or mCRPC (13). Before NHA became available in clinical practice, several studies showed the clinical efficacy of DR in selected patients with mCRPC (14). DR provided moderate clinical efficacy and a maximum PSA response rate of 48%, especially in patients with good PSA responses to first-line treatment with docetaxel.

Because of radiographic progress after ADT, the patient had become resistant to ADT with progression to mCRPC. According to the guidelines, the first-line treatment of docetaxel was administered to the patient with mCRPC, including six cycles of docetaxel combined with prednisone plus goserelin. The PSA level of the patient dropped to 0.97 ng/mL. Prostate-enhanced MRI suggested that the volume of the prostate and left pelvic sidewall lesions was significantly reduced compared to that before treatment. After 6 months of follow-up and maintenance ADT, the PSA level of the patient rose to 19.48 ng/mL. Metastasis of the left femoral neck, sacrum, and bilateral iliac crest was revealed by prostate-enhanced MRI at follow-up.

The E3805 CHAARTED trial revealed significant differences in the transcriptional profile of patients with mPCa, including luminal B subtype, basal subtype, lower androgen receptor activity (AR-A), and high Decipher risk disease. Patients with the luminal B subtype showed a significant OS benefit from ADT + docetaxel (HR 0.45, $p = 0.007$), whereas patients with the basal subtype showed no OS benefit (HR 0.85, $p = 0.58$). Lower AR-A and high Decipher risk were significantly related to poorer prognosis. In addition, patients with high Decipher risk had greater OS improvement from ADT + Docetaxel (HR 0.41, $p = 0.015$) (15). There was a retrospective study of 270 mCRPC patients with good response to first-line docetaxel. The median progression-free interval (PFI) was 6 months from the last chemotherapy of docetaxel. When it recurred, 223 patients received DR, and 47 received other therapy. The median OS for DR and other therapies was 18.2 vs. 16.8, respectively ($p = 0.35$). However, over 6 months of PFI indicated longer OS. Moreover, a good PSA response was more distinct on DR (40.4% vs. 10.6%, $p < 0.001$) (16). Another study showed that DR had OS improvement

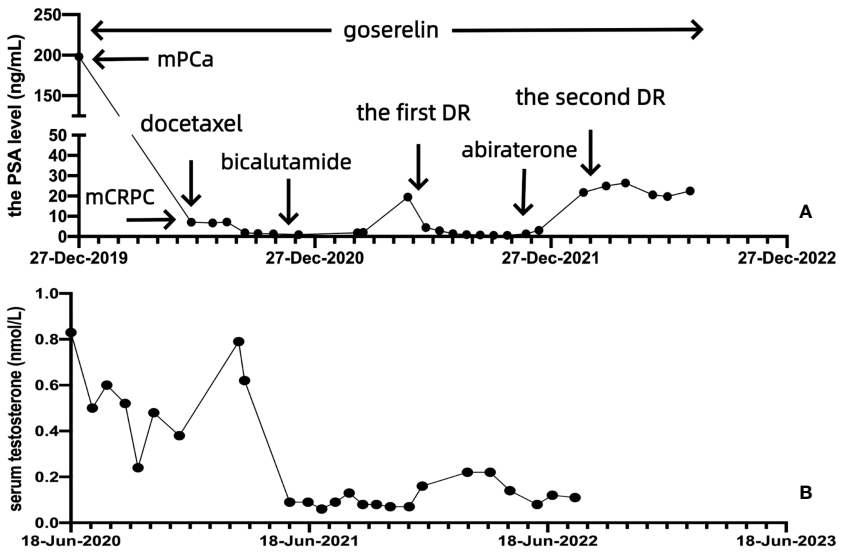


FIGURE 2
The PSA and serum testosterone levels of the patient throughout the treatment. **(A)** Change in the PSA level after docetaxel chemotherapy, the first DR, and the second DR. **(B)** Change in the serum testosterone level.

and safety in patients with a good response to docetaxel initially and more than 3 months of PFI (16). In addition, DR did not seem to increase the risk of adverse events, especially grade 3–4 events (14, 17). However, the GETUG-AFU 15 Phase 3 Trial suggested that only a limited number of patients who received first-line treatment with ADT + docetaxel for mHSPC benefited from DR at mCRPC. At this stage, NHAs such as abiraterone or enzalutamide can be used as a later-line treatment strategy (18). Because of the failure of HRRm testing, the patient received the first DR plus goserelin along with the addition of abiraterone acetate. The PSA level of the patient dropped to 0.61 ng/mL again. The volume of lesions of the prostate and left pelvic sidewall was significantly reduced by prostate-enhanced MRI. Except for the left femoral neck, no bone metastases were found in the other parts of the body. Unfortunately, after less than 3 months of follow-up and maintenance goserelin plus abiraterone, the PSA level of the patient rose to 21.78 ng/mL. In addition, the volume of the lesion of the left pelvic sidewall was significantly increased, and rectal invasion was not ruled out.

In the management of patients with mCRPC, regular MDT discussions can provide a more valuable and individualized treatment strategy, while patients can gain a more prolonged OS and better prognosis (19). After MDT discussion, because of the high economic burden of multigene molecular testing by tissue biopsy and PARP inhibitors, the patient could not receive the combination treatment of olaparib and abiraterone. Moreover, the patient refused prostate needle biopsy again; in turn, he could not confirm the pathological type of neuroendocrine prostate cancer (NEPC) and used platinum-based chemotherapy (20). PCa is a significantly increasing cause of mortality around the world and can also bring about a significantly increasing social and economic burden in modern society. FIRSTANA suggested that the median OS and PFS of patients with mCRPC were 24.3 months and 5.3 months, respectively, with docetaxel combined with prednisone (21). In the end, the patient received eight cycles of docetaxel combined with prednisone (the second DR) as well as maintenance goserelin plus abiraterone. To our excitement, although the PSA response cannot reach a 97% PSA reduction as the first DR, the PSA level of the patient can still be maintained at 19–27 ng/mL. Full-body bone scan and prostate-enhanced MRI during follow-up did not show new confirmed progression on imaging. More importantly, after a total of 24 cycles of docetaxel, the patient was still well-tolerated.

Conclusion

Overall, we demonstrated that the second DR was associated with further prolonged OS and PFS in patients with mCRPC. The PSA level, MRI progression of the lesion, and adverse events of the patient did not increase significantly. Therefore, this result can be added to the later-line treatment strategy of patients with mCRPC. In the future, more patients who worry about the high economic burden of testing and treatment as well as repeated prostate needle biopsy are advised to consider this later-line treatment strategy. We also believe that this strategy should be popularized by urological clinicians in hospitals.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethics Committee of Second Affiliated Hospital, Army Medical University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article. Written informed consent was obtained from the participant/patient(s) for the publication of this case report.

Author contributions

FH and JZ revised the manuscript. WN was responsible for data collection, data analysis, data interpretation, and writing the manuscript. PC was responsible for image design. All authors contributed to the article and approved the submitted version.

Funding

The current work was partly supported by the Natural Science Foundation of Chongqing [CSTC2020JCYJ-msxmX0513] and the Key Project for Clinical Innovation of Army Medical University [CX2019LC107].

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Nader R, El Amm J, Aragon-Ching JB. Role of chemotherapy in prostate cancer. *Asian J Androl* (2018) 20(3):221–9. doi: 10.4103/aja.aja_40_17

2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* (2018) 68(6):394–424. doi: 10.3322/caac.21492

3. Liu J, Dong L, Zhu Y, Dong B, Sha J, Zhu HH, et al. Prostate cancer treatment-China's perspective. *Cancer Lett* (2022) 550:215927. doi: 10.1016/j.canlet.2022.215927

4. Swami U, McFarland TR, Nussenzveig R, Agarwal N. Advanced prostate cancer: treatment advances and future directions. *Trends Cancer* (2020) 6(8):702–15. doi: 10.1016/j.trecan.2020.04.010

5. Achard V, Putora PM, Omlin A, Zilli T, Fischer S. Metastatic prostate cancer: treatment options. *Oncology* (2022) 100(1):48–59. doi: 10.1159/000519861

6. Barata PC, Sartor AO. Metastatic castration-sensitive prostate cancer: Abiraterone, docetaxel, or…. *Cancer* (2019) 125(11):1777–88. doi: 10.1002/cncr.32039

7. Kyriakopoulos CE, Chen YH, Carducci MA, Liu G, Jarrard DF, Hahn NM, et al. Chemohormonal therapy in metastatic hormone-sensitive prostate cancer: long-term survival analysis of the randomized phase III E3805 CHAARTED trial. *J Clin Oncol* (2018) 36(11):1080–7. doi: 10.1200/JCO.2017.75.3657

8. von Amsberg G, Merseburger AS. Therapie des metastasierten kastrationsresistenten Prostatakarzinoms [Treatment of metastatic, castration-resistant prostate cancer]. *Urologe A* (2020) 59(6):673–9. doi: 10.1007/s00120-020-01187-9

9. Tucci M, Caffo O, Buttigliero C, Cavaliere C, D'aniello C, Di Maio M, et al. Therapeutic options for first-line metastatic castration-resistant prostate cancer: Suggestions for clinical practise in the CHAARTED and LATITUDE era. *Cancer Treat Rev* (2019) 74:35–42. doi: 10.1016/j.ctrv.2019.01.002

10. Byeon S, Kim H, Kim J, Kwon M, Hur JY, Jeon HG, et al. Docetaxel rechallenge in metastatic castration-resistant prostate cancer: A retrospective, single-center study. *Investig Clin Urol* (2020) 61(6):588–93. doi: 10.4111/icu.20200214

11. Heidenreich A, Bastian PJ, Bellmunt J, Bolla M, Joniau S, van der Kwast T. EAU guidelines on prostate cancer. Part II: treatment of advanced, relapsing, and castration-resistant prostate cancer. *Eur Urol* (2014) 65:467–79. doi: 10.1016/j.eururo.2013.11.002

12. Vullierme MP, Ruszniewski P, de Mestier L. Are recist criteria adequate in assessing the response to therapy in metastatic NEN? *Rev Endocr Metab Disord* (2021) 22(3):637–45. doi: 10.1007/s11154-021-09645-1

13. Sweeney CJ, Chen YH, Carducci M, Liu G, Jarrard DF, Eisenberger M, et al. Chemohormonal therapy in metastatic hormone-sensitive prostate cancer. *N Engl J Med* (2015) 373(8):737–46. doi: 10.1056/NEJMoa1503747

14. Thomas C, Brandt MP, Baldauf S, Tsaur I, Frees S, Borgmann H, et al. Docetaxel-rechallenge in castration-resistant prostate cancer: defining clinical factors for successful treatment response and improvement in overall survival. *Int Urol Nephrol* (2018) 50(10):1821–7. doi: 10.1007/s11255-018-1963-1

15. Hamid AA, Huang HC, Wang V, Chen YH, Feng F, Den R, et al. Transcriptional profiling of primary prostate tumor in metastatic hormone-sensitive prostate cancer and association with clinical outcomes: correlative analysis of the E3805 CHAARTED trial. *Ann Oncol* (2021) 32(9):1157–66. doi: 10.1016/j.annonc.2021.06.003

16. Oudard S, Kramer G, Caffo O, Creppy L, Loriot Y, Hansen S, et al. Docetaxel rechallenge after an initial good response in patients with metastatic castration-resistant prostate cancer. *BJU Int* (2015) 115(5):744–52. doi: 10.1111/bju.12845

17. Assi T, Rassy E, Farhat F, Kattan C, Kattan J. Docetaxel rechallenge in patients with metastatic prostate cancer: A comprehensive review. *Oncol Res Treat* (2020) 43(6):299–306. doi: 10.1159/000506693

18. Lavaud P, Gravis G, Foulon S, Joly F, Oudard S, Priou F, et al. Anticancer activity and tolerance of treatments received beyond progression in men treated upfront with androgen deprivation therapy with or without docetaxel for metastatic castration-naïve prostate cancer in the GETUG-AFU 15 phase 3 trial. *Eur Urol* (2018) 73(5):696–703. doi: 10.1016/j.eururo.2017.09.022

19. Zhu S, Chen J, Ni Y, Zhang H, Liu Z, Shen P, et al. Dynamic multidisciplinary team discussions can improve the prognosis of metastatic castration-resistant prostate cancer patients. *Prostate* (2021) 81(11):721–7. doi: 10.1002/pros.24167

20. Pandya D, Shah M, Kaplan F, Martino C, Levy G, Kazanjian M, et al. Treatment-emergent neuroendocrine prostate cancer with a germline BRCA2 mutation: identification of a candidate reversion mutation associated with platinum/PARP-inhibitor resistance. *Cold Spring Harb Mol Case Stud* (2021) 7(1):a005801. doi: 10.1101/mcs.a005801

21. Oudard S, Fizazi K, Sengeløv L, Daugaard G, Saad F, Hansen S, et al. Cabazitaxel versus docetaxel as first-line therapy for patients with metastatic castration-resistant prostate cancer: A randomized phase III trial-FIRSTANA. *J Clin Oncol* (2017) 35(28):3189–97. doi: 10.1200/JCO.2016.72.1068

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 12 October 2023
DOI 10.3389/fonc.2023.1142976

OPEN ACCESS

EDITED BY
Ran Xu,
Central South University, China

REVIEWED BY
Fabio Grizzi,
Humanitas Research Hospital, Italy
Chen Yang,
Fudan University, China

*CORRESPONDENCE
Hengqing An
13201226586@163.com
Ning Tao
38515412@qq.com

RECEIVED 01 February 2023
ACCEPTED 26 September 2023
PUBLISHED 12 October 2023

CITATION
Abudoubari S, Bu K, Mei Y, Maimaitiyiming A, An H and Tao N (2023) Prostate cancer epidemiology and prognostic factors in the United States. *Front. Oncol.* 13:1142976. doi: 10.3389/fonc.2023.1142976

Prostate cancer epidemiology and prognostic factors in the United States

Saimaitikari Abudoubari[1,2], Ke Bu[2], Yujie Mei[2], Abudukeyoumu Maimaitiyiming[3], Hengqing An[3,4]* and Ning Tao[2,4]*

[1]Department of Radiology, The First People's Hospital of Kashi Prefecture, Kashi, Xinjiang, China, [2]College of Public Health, Xinjiang Medical University, Urumqi, Xinjiang, China, [3]The First Affiliated Hospital, Xinjiang Medical University, Urumqi, Xinjiang, China, [4]Xinjiang Clinical Research Center for Genitouriary System, Urumqi, Xinjiang, China

Objective: Using the latest cohort study of prostate cancer patients, explore the epidemiological trend and prognostic factors, and develop a new nomogram to predict the specific survival rate of prostate cancer patients.

Methods: Patients with prostate cancer diagnosed from January 1, 1975 to December 31, 2019 in the Surveillance, Epidemiology, and End Results Program (SEER) database were extracted by SEER stat software for epidemiological trend analysis. General clinical information and follow-up data were also collected from 105 135 patients with pathologically diagnosed prostate cancer from January 1, 2010 to December 1, 2019. The factors affecting patient-specific survival were analyzed by Cox regression, and the factors with the greatest influence on specific survival were selected by stepwise regression method, and nomogram was constructed. The model was evaluated by calibration plots, ROC curves, Decision Curve Analysis and C-index.

Results: There was no significant change in the age-adjusted incidence of prostate cancer from 1975 to 2019, with an average annual percentage change (AAPC) of 0.45 (95% CI:-0.87~1.80). Among the tumor grade, the most significant increase in the incidence of G2 prostate cancer was observed, with an AAPC of 2.99 (95% CI:1.47~4.54); the most significant decrease in the incidence of G4 prostate cancer was observed, with an AAPC of -10.39 (95% CI:-13.86~-6.77). Among the different tumor stages, the most significant reduction in the incidence of localized prostate cancer was observed with an AAPC of -1.83 (95% CI:-2.76~-0.90). Among different races, the incidence of prostate cancer was significantly reduced in American Indian or Alaska Native and Asian or Pacific Islander, with an AAPC of -3.40 (95% CI:-3.97~-2.82) and -2.74 (95% CI:-4.14~-1.32), respectively. Among the different age groups, the incidence rate was significantly increased in 15-54 and 55-64 age groups with AAPC of 4.03 (95% CI:2.73~5.34) and 2.50 (95% CI:0.96~4.05), respectively, and significantly decreased in ≥85 age group with AAPC of -2.50 (95% CI:-3.43~-1.57). In addition, age, tumor stage, race, PSA and gleason score were found to be independent risk factors affecting prostate cancer patient-specific survival. Age, tumor stage, PSA and gleason score were most strongly associated with prostate cancer patient-specific survival by stepwise regression screening, and

nomogram prediction model was constructed using these factors. The Concordance indexes are 0.845 (95% CI:0.818~0.872) and 0.835 (95% CI:0.798~0.872) for the training and validation sets, respectively, and the area under the ROC curves (AUC) at 3, 6, and 9 years was 0.7 or more for both the training and validation set samples. The calibration plots indicated a good agreement between the predicted and actual values of the model.

Conclusions: Although there was no significant change in the overall incidence of prostate cancer in this study, significant changes occurred in the incidence of prostate cancer with different characteristics. In addition, the nomogram prediction model of prostate cancer-specific survival rate constructed based on four factors has a high reference value, which helps physicians to correctly assess the patient-specific survival rate and provides a reference basis for patient diagnosis and prognosis evaluation.

KEYWORDS

prostate cancer, epidemiologic trends, specific survival, predictive models, nomogram

1 Introduction

Prostate cancer (PCa) is one of the leading causes of cancer-related deaths (1) and currently the second most common male malignancy worldwide (2). 375 304 deaths from prostate cancer were reported worldwide in 2020 (3). The incidence and mortality rates of prostate cancer vary greatly from country to country, and even within a single country, the incidence and mortality rates of prostate cancer vary greatly in different regions (4). Studies have reported the highest incidence of prostate cancer in Western and Northern Europe, North America and Australia/New Zealand, with intermediate incidence in Eastern Europe, South America, South Africa and Western Asia, and the lowest incidence in South and East Asia and other parts of Africa. Southern Africa, the Caribbean, and South America had the highest mortality rates. Europe, North and Central America, and Australia/New Zealand have intermediate mortality rates, and Asia had the lowest mortality rates (5).

In addition, the increasing number of articles published each year on prostate cancer is evidence that the global interest in prostate cancer has been increasing. Although the incidence and prevalence of prostate cancer are thought to have increased over the last few decades, there is a lack of recent data on the epidemiological characteristics and survival analysis of prostate cancer patients. On the other hand, most studies on prostate cancer are based on a small number of cases in a single institution and lack reliability. Therefore, in this study, we conducted a population-based study using information from the Surveillance, Epidemiology, and End Results (SEER) of the American Institute for Cancer Research to systematically analyze the epidemiologic, clinical, and prognostic characteristics of prostate cancer.

The prognosis of prostate cancer patients remains difficult to assess, although there is an increasing focus on the prognosis and survival of prostate cancer patients. The current prognostic analysis of prostate cancer is still mainly based on the American Joint Committee on Cancer tumor TNM staging system (6). This system assesses the prognosis of patients based on tumor volume (T), regional lymph node tumor invasion (N), and distant metastases (M). However, the TNM staging system is not yet able to adequately assess patient-specific survival, and more reliable predictive evaluation indicators need to be explored (7). Among the currently available predictive tools, nomogram is considered to be the most accurate and characteristic method for predicting prognosis of cancer patients (8). To our knowledge, few studies have used nomogram to predict the prognosis of prostate cancer patients. In this study, a more detailed nomogram was developed based on a relatively large cohort of prostate cancer patients in the SEER database to predict the 3, 6, and 9 year specific survival rates of prostate cancer patients to provide a reference for patient treatment and prognostic evaluation.

2 Materials and methods

2.1 data sources

The SEER database used for this study is an authoritative source of information on cancer epidemiology (incidence and prevalence) and clinical characteristics (primary tumor site, tumor morphologic features and stage of diagnosis, first course of treatment, and life-state follow-up) in the United States. Patients aged 15 years and older with prostate cancer diagnosed from January 1, 1975 to December 31, 2019 were obtained through SEER*Stat 8.4.1 software and analyzed for epidemiological trends in prostate cancer. General clinical data and follow-up data of 105 135 patients with prostate cancer diagnosed by pathology from January 1, 2010 to December 1, 2019 were also collected for analysis of prognostic influencing factors. Inclusion criteria: (1)

patients with prostate cancer clearly diagnosed by pathology; (2) complete general clinical and follow-up data; (3) age ≥ 15 years. Exclusion criteria: (1) those with unclear pathological findings; (2) those with unclear general clinical information, etc. The data used in this study were freely available and publicly available. Therefore, review and informed consent were exempted.

2.2 Collection of clinical data related to prognostic analysis

Clinical data with serious missing wsa excluded from the database, and finally age, race, PSA, bone metastases, lung metastases, tumor grade, tumor stage, gleason score, and follow-up-related information were included. Follow-up-related information included specific survival time and follow-up outcome. The specific survival time was defined as the time interval from the date of diagnosis to the date of death due to tumor recurrence of the patient, and the follow-up outcomes included death from tumor-related causes during follow-up or the end of follow-up (survival or death from other causes). All of the above information is described in detail in the SEER database.

2.3 Tumor stage, tumor grade and race of study subjects

We used the SEER staging system in our study. Tumor stage was divided into different metastatic conditions such as localized, regional and distant metastasis. Localized prostate cancer was defined as a tumor that was completely confined to the organ of origin. Regional prostate cancer was defined as beyond the boundaries of the organ of origin, directly into surrounding organs or tissues, through the lymphatic system into regional lymph nodes, or through a combination of extension and regional lymph nodes. Finally, distant metastasis was defined as the appearance of metastatic lesions in organs or tissues relatively distant from the site of the primary cancer. Since tumor stage-related data in the SEER database was only recorded from 1998 to 2017, only data between 1998 and 2017 was analyzed for tumor stage-related data. For the tumor grade, the SEER classification scheme systematically classified cases into 4 classes: G1: highly differentiated; G2: moderately differentiated; G3: poorly differentiated; and G4: undifferentiated. Patients were classified into the following 4 racial categories: white people, black people, Asian and Pacific Islander, American Indian and Alaska Native.

2.4 Statistical analysis

SEER*Stat 8.4.1 software was used to calculate age-adjusted incidence, limited persistence prevalence (10 and 20 year prevalence), and mortality from 1975 to 2019. The Joinpoint 4.9.1 software was used to characterize incidence trends by combining annual percentage change (APC) and average annual percentage change (AAPC) calculated by point regression. The logarithm of the age-adjusted rates for each year were first regressed over time and then the annual percentage change were calculated using a slope transformation. APC and AAPC were comparable at different scales, allowing comparison of other incidence rates between malignancy cohorts. The entire sample set collected from January 1, 2010 to December 1, 2019 was also randomly divided 2:1 into training and validation sets (random number seed = 105 135), training set (n = 70 090), and validation set (n = 35 045). SPSS 25.0 software was used to statistically analyze the collected data, and the count data was described using percentages (%). One-way Cox regression was used to analyze the influential factors associated with prostate cancer-specific survival. Factors that were statistically significant in the one-way Cox regression analysis were included in the multi-factor Cox regression to analyze the independent risk factors associated with prostate cancer-specific survival. Eviews 12.0 software was used to calculate the Akaike Information Criterion value (AIC) of each independent risk factor, and a larger AIC indicated that the factor was more important to the model, and all independent risk factors were ranked according to the AIC value, and the factors were gradually included in the model according to the ranking, while R4.1.2 software (car, rms, pROC, timeROC, ggDCA, survival packages), The larger the C index is, the more accurate the model prediction is, and evaluate whether the newly added factors make the C index of the model improve. The reliability of the model was assessed by plotting the ROC curve and calculating the AUC. Calibration curves (using 1000 bootstrap auto-sampling method) were plotted to validate the model. The test level was 0.05.

3 Results

3.1 Patient characteristics

From the SEER database, a total of 1 366 129 prostate cancer patients (mean age at diagnosis, 67.90 ± 9.31 years; median age 68.00 (61.00,75.00) years were identified from 1975 to 2015. Among these, 264 450 (19.36%) were under 60, 511 638 (37.45%) were 60 to 69, 424 216 (31.05%) were 70 to 79, and 165 825 (12.14%) were 80 years and above. 1 091 443 (79.89%) were white people, 173 154 (12.67%) were black people, 63 014 (4.61%) were Asian and Pacific Islander patients, 4 896 (0.36%) were American Indian and Alaska Native patients, and 33 622 (2.46%) were patients of unknown race. In addition, of the 1 145 591 (83.86%) prostate cancers with known tumor grade, 125 356 (9.18%) were G1, 593 294 (43.43%) were G2, 421 653 (30.86%) were G3, and 5 288 (0.39%) were G4. Of the 1 041 770 (76.25%) prostate cancers with known tumor stage, 845 925 (61.92%) were Localized, 134 484 (9.84%) were regional, and 61 361 (4.49%) were distant metastases (Table 1).

3.2 Annual incidence rate

Using population data from the SEER database, we calculated the annual age-adjusted incidence rate of prostate cancer per 100 000 persons with reference to the standard 2000 US population. The

TABLE 1 Baseline characteristics of prostate cancer patients in SEER database.

Characteristic	1975-1991 (n)	1992-1999 (n)	2000-2019 (n)	Overall [n(%)]
	128 873	173 826	1 063 430	1 366 129(100.00%)
Age(Y)				
<60	9 450	23 811	231 189	264 450(19.36%)
60~69	38 786	59 082	413 770	511 638(37.45%)
70~79	52 588	65 957	305 671	424 216(31.05%)
≥80	28 049	24 976	112 800	165 825(12.14%)
Tumor grade				
G1	33 770	20 416	71 170	125 356(9.18%)
G2	44 085	102 205	447 004	593 294(43.43%)
G3	25 421	34 900	361 332	421 653(30.86%)
G4	2 113	983	2 192	5 288(0.39%)
Unknown	23 484	15 322	181 732	220 538(16.14%)
Race				
White people	115 139	143 822	832 482	1 091 443(79.89%)
Black people	8 214	17 237	147 703	173 154(12.67%)
AI/AN	–	703	4 193	4 896(0.36%)
Asian/P Islander	–	10 427	52 587	63 014(4.61%)
Unknown	5 520	1 637	26 465	33 622(2.46%)
Tumor stage				
Localized	–	32 649	813 276	845 925(61.92%)
Regional	–	6 332	128 152	134 484(9.84%)
Distant	–	2 259	59 102	61 361(4.49%)
Unstaged	128 873	132 586	62 900	324 359(23.74%)

age-adjusted incidence rate of prostate cancer was 121.65 cases per 100 000 persons in 1975 and 147.86 cases per 100 000 persons in 2019, with an AAPC (95% CI) of 0.45 (- 0.87~1.80), and detailed incidence data were presented in Table 2; Figure 1, and Supplementary Table 1. Using data from the SEER database, long-term trends in prostate cancer incidence among different races can be explored. Incidence rates for different races did not change significantly between 1975 and 2019, with AAPC (95% CI) of 0.31 (-1.01~1.65), 0.61 (-1.10~2.35) and 0.65 (-0.51~1.83) for white people, black people, and other races, respectively. Since more detailed information on race was recorded in SEER 12 (1992-2019), we could explore the incidence trends of these races in further detail. Among White people, Black people, American Indians and Alaska Natives, Asian and Pacific Islanders, the incidence of prostate cancer decreased between 1992 and 2019, with AAPC (95% CI) of -2.65 (-4.17~-1.10), -2.09 (-3.36~-0.81), -3.40 (-3.97~-2.82), and -2.74 (-4.14~-1.32) (Figure 2; Supplementary Table 1). Among the different ages, the incidence rate increased significantly in 15-54 and 55-64 age groups, with AAPC(95% CI) of 4.03 (2.73~5.34) and 2.50 (0.96~4.05), respectively; the incidence rate decreased significantly in ≥85 age group, with AAPC(95% CI) of -2.50 (-3.43~-1.57); in 65- 74 and 75-84 age group remained unchanged, with AAPC(95% CI) of 1.21 (-0.11~2.54) and -0.79 (-1.95~0.38), respectively (Figure 3; Supplementary Table 1).

The incidence of prostate cancer among white people increased in the 15-54 and 55-64 age groups, with AAPC (95% CI) of 3.61 (1.92~5.32) and 2.24 (0.96~3.53), respectively; decreased in and ≥85 age group, with AAPC (95% CI) of -2.54 (-3.56~-1.50); and remained unchanged in the 65-74 and 75- 84 age groups remained unchanged, with AAPC (95% CI) of 1.08 (-0.22~2.40) and -0.89 (-2.06~0.29), respectively (Supplementary Figure 1; Supplementary Table 1). The incidence of prostate cancer among black people increased in the 15-54 age group with an AAPC (95% CI) of 4.73 (2.20~7.31); decreased in the ≥85 age group with an AAPC (95% CI) of -2.46 (-3.30~-1.62); and remained unchanged in the 55-64, 65-74, and 75-84 age groups with an AAPC (95% CI) of 1.97 (-0.76~4.78), 1.00 (-0.84~2.87), and -0.97 (-3.19~1.31), respectively (Supplementary Figure 2; Supplementary Table 1). The incidence of prostate cancer among American Indians and Alaska Natives remained unchanged in the 15-54 age group with an

10.3389/fonc.2023.1142976

TABLE 2 Incidence of prostate cancer over time.

Registry	Year	Rate (per 100 000 persons)	Lower CI	Upper CI	Number of PC cases (n)	Number at risk (n)
SEER8	1975	121.65	118.03	125.35	4 771	5 940 293
	1976	124.24	120.62	127.93	4 997	6 066 708
	1977	127.86	124.26	131.54	5 305	6 183 531
	1978	125.83	122.29	129.43	5 349	6 304 757
	1979	131.26	127.69	134.90	5 691	6 434 477
	1980	133.75	130.19	137.38	5 949	6 562 981
	1981	135.13	131.58	138.74	6 123	6 661 878
	1982	134.33	130.85	137.87	6 282	6 741 691
	1983	137.68	134.21	141.22	6 596	6 832 059
	1984	138.57	135.13	142.08	6 777	6 929 434
	1985	145.33	141.85	148.87	7 310	7 027 803
	1986	149.27	145.81	152.80	7 711	7 129 499
	1987	168.00	164.38	171.67	8 902	7 216 079
	1988	173.84	170.21	177.54	9 417	7 308 895
	1989	182.31	178.65	186.03	10 168	7 387 431
	1990	212.91	208.99	216.88	12 071	7 488 078
	1991	262.79	258.53	267.09	15 454	7 602 113
SEER12	1992	285.32	281.73	288.94	25 512	12 087 125
	1993	249.75	246.46	253.08	23 029	12 209 948
	1994	216.48	213.46	219.53	20 453	12 308 371
	1995	204.18	201.28	207.11	19 680	12 433 844
	1996	204.01	201.15	206.90	20 053	12 577 090
	1997	210.64	207.77	213.55	21 096	12 779 857
	1998	205.83	203.02	208.67	21 039	12 984 930
	1999	219.84	216.97	222.74	22 964	13 166 050
SEER17	2000	218.53	216.62	220.45	51 294	28 494 316
	2001	222.51	220.60	224.43	53 328	28 912 565
	2002	223.11	221.22	225.00	54 904	29 275 401
	2003	201.61	199.84	203.40	50 863	29 590 069
	2004	200.65	198.90	202.42	51 824	29 966 562
	2005	189.98	188.30	191.68	50 252	30 276 193
	2006	204.24	202.51	205.97	55 561	30 614 378
	2007	210.93	209.20	212.67	59 087	30 968 322
	2008	193.46	191.83	195.10	56 135	31 353 874
	2009	188.99	187.41	190.58	56 767	31 735 085
	2010	180.10	178.57	181.63	55 618	32 080 579
	2011	177.21	175.72	178.71	56 408	32 406 115
	2012	148.02	146.68	149.36	48 781	32 731 169

(Continued)

TABLE 2 Continued

Registry	Year	Rate (per 100 000 persons)	Lower CI	Upper CI	Number of PC cases (n)	Number at risk (n)
	2013	141.04	139.75	142.33	47 902	33 029 532
	2014	129.14	127.92	130.36	45 135	33 343 112
	2015	134.64	133.42	135.87	48 430	33 670 052
	2016	139.17	137.95	140.41	51 436	33 969 818
	2017	145.23	143.99	146.47	55 049	34 223 179
	2018	144.73	143.51	145.96	56 010	34 428 001
	2019	147.86	146.64	149.08	58 646	34 599 429

AAPC (95% CI) of -0.87 (-2.45~0.74); it decreased in the 55-64, 65-74, 75-84 and ≥85 age groups with an AAPC (95% CI) of -2.92 (-3.83~-2.00), -3.31 (-4.03~-2.59), -3.57 (-4.53~-2.61), and -5.05 (-6.77~-3.29), respectively (Supplementary Figure 3; Supplementary Table 1). The incidence of prostate cancer among Asian and Pacific Islanders increased in the 15-54 age group with an AAPC (95% CI) of 1.61 (0.04~3.21); remained unchanged in the 55-64 age group with an AAPC (95% CI) of 0.66 (-0.71~2.05); decreased in the 65-74, 75-84 and ≥80 age groups with an AAPC (95% CI) of -1.99 (-3.34~-0.62), -4.05 (-6.08~-1.98), and -6.03 (-6.58~-5.49), respectively (Supplementary Figure 4; Supplementary Table 1).

3.3 Incidence and prevalence of prostate cancer by tumor stage and tumor grade

Among different tumor stages, the incidence of localized prostate cancer decreased from 149.90 cases per 100 000 in 1998 to 102.29 cases per 100 000 in 2019, with an AAPC (95% CI) of -1.83 (-2.76~-0.90). The incidence of regional and distant metastatic prostate cancer remained unchanged, with an AAPC (95% CI) of -1.77 (-3.91~0.43) and 0.57 (-0.80~1.96), respectively (Figure 4; Supplementary Table 1). For different tumor grade, the incidence of G2 prostate cancer increased the most, from 16.20 cases per 100 000 in 1975 to 56.93 cases per 100 000 in 2017, with

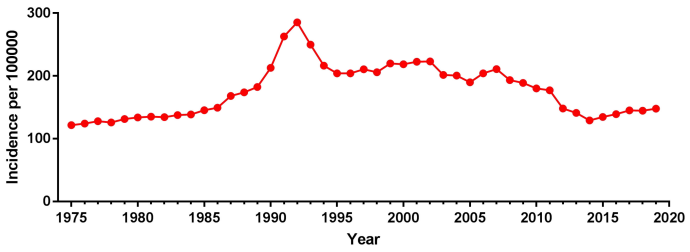


FIGURE 1
Incidence of prostate cancer over time.

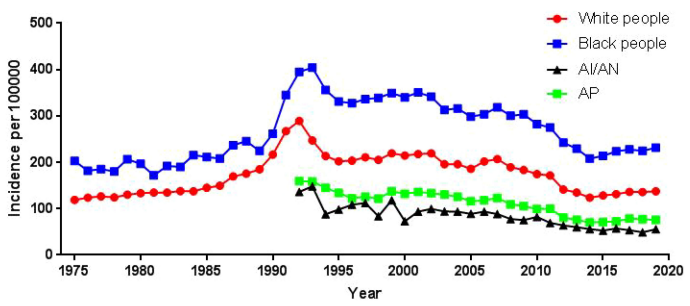


FIGURE 2
Incidence of prostate cancer over time by race.

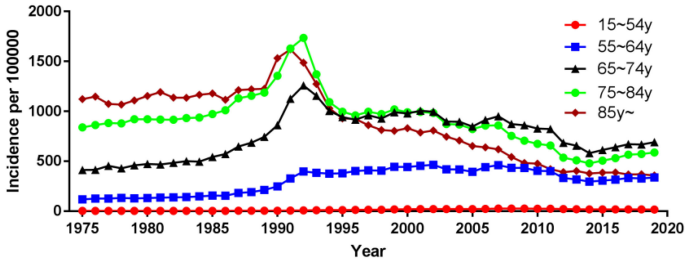


FIGURE 3
Incidence of prostate cancer over time by age.

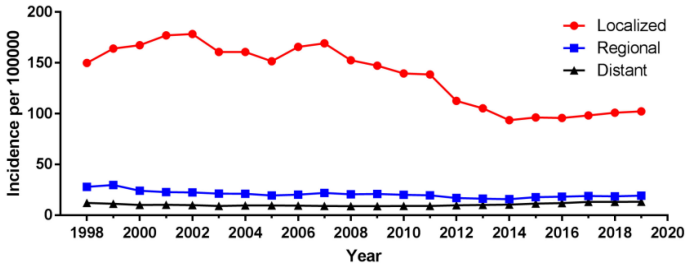


FIGURE 4
Incidence of prostate cancer over time by tumor stage.

an AAPC (95% CI) of 2.99 (1.47~4.54), followed by G3 prostate cancer with an AAPC (95% CI) of 1.77 (0.08~3.48). The incidence of G4 prostate cancer decreased, with an AAPC (95% CI) of -10.39 (-13.86~-6.77). The incidence of G1 prostate cancer remained unchanged, with an AAPC (95% CI) of 0.47 (-1.95~2.95); (Figure 5; Supplementary Table 1).

In addition, the 20-year limited-duration prevalence of prostate cancer increased significantly from 0.20918% in 2000 to 1.87472% in 2019 (Supplementary Figure 5). Detailed 20-year and 10-year limited-duration prevalence and absolute counts are presented in Table 3. Among prostate cancers with different tumor stage, the greatest increase in prevalence was seen for localized prostate cancer (from 0.07393% in 1998 to 1.5752% in 2017), followed by regional prostate cancer (from 0.01404% in 1998 to 0.27791% in 2017) (Supplementary Figure 6). For the different tumor grade, the greatest increase in the prevalence was observed in G2 prostate cancer (from 0.12456% in 1998 to 1.01298% in 2017) (Supplementary Figure 7).

3.4 Trends in age at diagnosis

We calculated the mean age at diagnosis for prostate cancer patients by tumor stage for each year from 1998 to 2019 (Supplementary Figure 8). The mean age at diagnosis for prostate cancer patients with different tumor stages remained constant over the 22-year study period. There were significant differences between the mean ages of patients with different tumor stages, the mean age of patients with localized prostate cancer was 3.10 (95% CI:2.54~3.65) years higher than the mean age of patients with regional prostate cancer. The mean age of patients with localized prostate cancer was 4.72 (95% CI:4.28~5.17) years lower than the

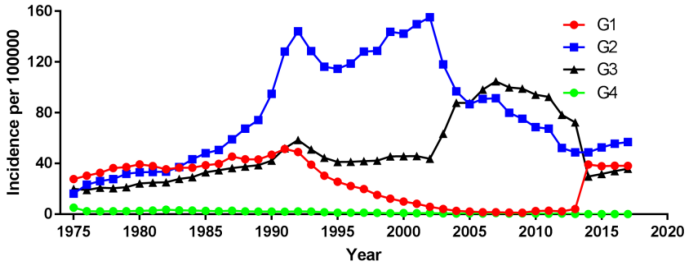


FIGURE 5
Incidence of prostate cancer over time by tumor grade.

TABLE 3 10-year and 20-year prevalence of prostate cancer.

Year	20-year duration Prevalence (%)	20-year Count (n)	10-year duration Prevalence (%)	10-year Count (n)
2000	0.20918	22079		
2001	0.41265	51308		
2002	0.60290	101463		
2003	0.77411	148580		
2004	0.93140	191490		
2005	1.07618	233094		
2006	1.21475	274007		
2007	1.35672	320617		
2008	1.47347	362921		
2009	1.57995	403756		
2010	1.66610	441745	0.17401	53964
2011	1.74986	480462	0.34321	108665
2012	1.79398	512183	0.47873	155697
2013	1.82431	538811	0.59424	198064
2014	1.83763	560611	0.68965	235364
2015	1.84864	582321	0.77976	272445
2016	1.86587	606070	0.87225	311511
2017	1.88303	632081	0.96536	352969
2018	1.89545	657413	1.05328	393784
2019	1.91003	683806	1.13858	435122

mean age of patients with distant metastases; The mean age of patients with regional prostate cancer was 7.82 (95% CI:7.39~8.25) years lower than that of patients with distant metastases.

3.5 Survival

The median (95% CI) survival time (months) for all patients was 157.00 (156.63~157.37). For the different age groups, patients in the ≥80 age group had the shortest survival time with a median (95% CI) of 49.00 (48.63~49.37), while patients < 60 years had the longest survival time with a median (95% CI) of 304.00 (301.79~306.21). the median (95% CI) survival time for patients in the 60~69, 70~79 age groups were 202.00 (201.33~202.70) and 119.00 (118.59~119.42), respectively (Supplementary Figure 9).

Among prostate cancers with different tumor stages, patients with distant metastatic prostate cancer had the shortest survival time with a median (95% CI) of 26.00 (25.66~26.34). The median (95% CI) survival times for patients with localized and regional prostate cancer were 187.00 (186.44~187.56) and 217.00 (215.16~218.84) (Supplementary Figure 10). For different tumor grade, patients with G4 prostate cancer had the shortest survival time with a median (95% CI) of 48.00 (45.49~50.51). Patients with G2 prostate cancer had the longest survival time with a median (95% CI) of 185.00 (184.46~185.54). Patients with G1 and G3 prostate cancer had a median (95% CI) survival time with 140.00 (138.91~141.09) and 143.00 (142.40~143.60), respectively (Supplementary Figure 11). All these survival analyses were statistically significant (P<0.05).

We further evaluated 3-year, 6-year and 9-year survival patterns according to tumor stage and tumor grade. The 3-year, 6-year and 9-year survival rates for patients with localized and regional prostate cancer were mostly higher than 80%, and the survival rates were relatively high. The 9-year survival rates of patients with localized G1 and G3 prostate cancer were (77.75 ± 0.48%) and (71.12 ± 0.15%), The 6-year and 9-year survival rates of patients with localized G4 prostate cancer (66.21 ± 2.19%) and (50.76 ± 2.36%) were relatively low; The 9-year survival rates of patients with regional G3 prostate cancer were (77.14 ± 0.25%), the 3-year, 6-year and 9-year survival rates of patients with regional G4 prostate cancer (70.69 ± 3.29%), (57.87 ± 3.59%) and (50.23 ± 3.69%) were relatively low; In distant metastatic prostate cancer survival rates were low for all tumor grades, among which the survival rate of G4 prostate cancer is the worst, the 3-year, 6-year and 9-year survival rate were (19.44 ± 2.84%), (10.24 ± 2.21%) and (4.98 ± 1.64%), respectively (Table 4).

Overall, survival rates for localized, regional and distant metastatic prostate cancer improved from 3-year survival rates in 1998 (91.50 ± 0.24%), (92.56 ± 0.49%) and (38.04 ± 1.51%) to 3-year survival rates in 2016 (95.34 ± 0.12%), (95.04 ± 0.27%) and (44.79 ±

TABLE 4 Survival analysis of patients with prostate cancer: actuarial survival of prostate cancer patients by tumor stage and tumor grade.

Tumor grade	Localized				Regional				Distant			
	Median Survival (months)	Survival Rate (%)			Median Survival (months)	Survival Rate (%)			Median Survival (months)	Survival Rate (%)		
		3Year	6Year	9Year		3Year	6Year	9Year		3Year	6Year	9Year
Overall	187	94.05	85.86	76.45	217	94.79	87.78	80.08	26	40.97	21.00	12.78
G1	179	95.79	88.98	77.75	240	95.91	90.45	83.07	54	64.86	36.48	24.32
G2	201	95.38	88.52	80.12	245	97.31	92.85	87.18	48	63.47	40.26	28.69
G3	163	92.08	81.88	71.12	198	94.07	85.90	77.14	30	45.93	23.75	14.09
G4	104	82.98	66.21	50.76	94	70.69	57.87	50.23	17	19.44	10.24	4.98

0.85%); from 6-year survival rates in 1998 (80.40 ± 0.34%), (83.47 ± 0.70%) and (20.52 ± 1.26%) to 6-year survival rates in 2013 (87.90 ± 0.18%), (88.03 ± 0.45%) and (17.29 ± 0.73%), respectively; from 9-year survival rates in 1998 (68.97 ± 0.39%), (73.85 ± 0.83%) and (12.71 ± 1.04%) to 9-year survival rates (79.14 ± 0.21%), (80.89 ± 0.51%) and (11.49 ± 0.68%) in 2010, respectively (Supplementary Figures 12–14).

3.6 General clinical information and univariate and multivariate cox regression

General clinical information is shown in Table 5. median follow-up was 69 months and 4 261 cases of specific death. Univariate and multifactorial Cox regression analysis revealed that tumor stage, race, PSA, age and gleason score were independent risk factors for specific survival in patients with prostate cancer (P<0.05) (Table 6).

3.7 Development and validation of a nomogram model for patient-specific survival in prostate cancer

To ensure the accuracy of the model, the factors that had the greatest influence on the specific survival rate of prostate cancer patients were screened based on the AIC and C index. It was found that the model constructed based on four factors: tumor stage, PSA, age and gleason score, had the highest C-index (Table 7), indicating that the nomogram built based on the above four factors could accurately assess the specific survival rate of prostate cancer patients at 3, 6 and 9 years (Figure 6). Finally, four indicators/variables, including Tumor stage, Gleason score, PSA, and Age were retained in the regression equation. Cox regression model: h (t, x)=h0 (t) exp ($1.049X_1$ + 1.064X2 + $0.540X_3$ + $0.453X_4$), with independent variables: X_1 = Tumor stage, X_2 = Gleason score, X_3 = PSA, and X_4 = Age (Table 8). The areas under the ROC curves were 0.806, 0.784, and 0. 774 for the training set at 3, 6, and 9 years, respectively. The areas under the ROC curves were 0.747, 0.749, and 0. 737 for the validation set at 3, 6, and 9 years, respectively (Figure 7), which had good reference value. The calibration curve was used for internal validation, with the X-axis representing the predicted mortality rate and the Y-axis representing the actual mortality rate. Both sets of data are seen to fit close to the diagonal line, indicating that the actual curve fits well with the ideal curve, and there was good agreement between the model-predicted overall survival rates and the true values at 3, 6, and 9 years (Figure 8).

After evaluating the accuracy of the model, reevaluate whether the inclusion of four factors can benefit prostate cancer patients in clinical practice. Using Decision Curve Analysis (DCA) to evaluate the net benefits of patients, calculate the clinical value of the model and its impact on actual decision-making. The Y-axis represents the calculated benefits, the X-axis represents the risk threshold, and the wavy line of the nomogram is further away from the intersection of the line, closer to the upper right, indicating greater clinical benefits. The results indicate that, the prediction model constructed based on four factors had more clinical benefits for patients compared to individual prediction models for each factor (Figure 9).

Application of nomogram: First, number each patient, then select any ID number to view the patient's information and calculate the patient's survival rate. For patient number 10035, Tumur stage=Regional, Gleason score=7, PSA=6.2ng/ml, Age=65 year. Its score is: 2.5 (Tumur stage=Regional)+47.5 (Gleason score=7)+32.5 (PSA=6.2ng/ml)+27.5 (Age=65 year)=110. The corresponding 3-year survival rate, 6-year survival rate, and 9-year survival rate of prostate cancer patients with a total score of 110 are 93.25%, 48.00%, and 1.30%.

4 Discussion

In this population-based study, we analyzed prostate cancer epidemiology and prognostic factors using data from a large number of prostate cancer patients reported in the SEER database from 1975 to 2019. The overall incidence of prostate cancer remained constant over 45 years, which is consistent with trends found in earlier epidemiological studies (9). We have analyzed many details of prostate cancer incidence trends and found a decrease in incidence trends across tumor stages, with the greatest decrease in localized prostate cancer, which may be due to the impact of effective preventive measures on prostate cancer incidence or the decreasing rate of patients undergoing PSA testing in the last decade or the prevalence of preventive measures for prostate cancer (10). Among the different tumor grade, except

TABLE 5 General clinical data of training set and validation set samples of prostate cancer patients in SEER Database[*n*(%)].

Characteristic	All patients (*n*=105135) [n(%)]	Training set (*n*=70090) [n(%)]	Validation set (*n*=35045) [n(%)]	X^2	*P*
Tumor grade				62.694	<0.001
G1	9722(9.2)	6424(9.2)	3298(9.4)		
G2	41757(39.7)	27302(39.0)	14455(41.2)		
G3	53567(51.0)	36300(51.7)	17267(49.3)		
G4	89(0.1)	64(0.1)	25(0.1)		
Tumor stage				25.590	<0.001
Localized	85669(81.5)	56816(81.1)	28853(82.3)		
Regional	16625(15.8)	11355(16.2)	5270(15.1)		
Distant	2841(2.7)	1919(2.7)	922(2.6)		
Race				1513.487	<0.001
White people	81188(77.2)	56424(80.5)	24764(70.7)		
Black people	16234(15.4)	8750(12.5)	7484(21.3)		
Others	7713(7.4)	4916(7.0)	2797(8.0)		
Bone metastasis				0.837	0.371
No	102465(97.5)	68288(97.4)	34177(97.5)		
Yes	2670(2.5)	1802(2.6)	868(2.5)		
Lung metastasis				1.349	0.276
No	105040(99.9)	70032(99.9)	35008(99.9)		
Yes	95(0.1)	58(0.1)	37(0.1)		
PSA(ng/ml)				21.975	<0.001
<4	13460(12.8)	9153(13.1)	4307(12.3)		
4.1~10	64215(61.1)	42566(60.7)	21649(61.8)		
10.1~20	16587(15.8)	11193(16.0)	5394(15.4)		
>20	10873(10.3)	7178(10.2)	3695(10.5)		
Age(Y)				151.174	<0.001
<60	26736(25.4)	17223(24.6)	9513(27.1)		
60~69	46760(44.5)	31019(44.3)	15741(44.9)		
70~79	25837(24.6)	17756(25.3)	8081(23.1)		
≥80	5802(5.5)	4092(5.8)	1710(4.9)		
Gleason score				273.069	<0.001
≤6	41530(39.5)	26486(37.8)	15044(42.9)		
7	43772(41.6)	30220(43.1)	13552(38.7)		
8~10	19833(18.9)	13384(19.1)	6449(18.4)		

for G4 prostate cancer for which there has been a decrease, the incidence trend of prostate cancer has increased in all tumor grades, with the greatest increase in G2 prostate cancer.

Changes in patient management and disease related regulations during the period from 1975 to 2020 affect the incidence rate, prevalence, survival rate and other patient outcomes of prostate cancer. Several screening studies from the late 1980s to the early 1990s showed that, compared with the assessment of palpable tumors by digital rectal examination, PSA detection could identify more prostate cancer in the clinical local stage of organ limitation, especially in the United States, which led to a rapid rise in the incidence rate of prostate cancer (11–16).

This study shows that the incidence rate of prostate cancer increased sharply from 1988 to 1992, and reached the peak in

TABLE 6 Univariate and multivariate analysis of prognostic factors related to specific survival in patients with prostate cancer.

Characteristic	Univariate analysis		Multivariate analysis	
	HR (95%*CI*)	*P*	*HR* (95%*CI*)	*P*
Tumor grade				
G1	Reference value			
G2	1.792(1.301~2.468)	<0.001		
G3	11.453(8.415~15.588)	<0.001		
G4	34.352(19.731~59.807)	<0.001		
Tumor stage				
Localized	Reference value		Reference value	
Regional	2.223(2.048~2.412)	<0.001	1.427(1.310~1.554)	<0.001
Distant	44.854(41.878~48.042)	<0.001	8.582(7.900~9.323)	<0.001
Race				
White people	Reference value		Reference value	
Black people	1.185(1.094~1.283)	<0.001	1.221(1.126~1.325)	<0.001
Others	0.948(0.840~1.069)	0.385	0.650(0.576~0.733)	<0.001
Bone metastasis				
No	Reference value			
Yes	36.943(34.618~39.425)	<0.001		
Lung metastasis				
No	Reference value			
Yes	23.791(17.997~31.451)	<0.001		
PSA(ng/ml)				
<4	Reference value		Reference value	
4.1~10	1.007(0.875~1.158)	0.926	0.868(0.754~0.999)	0.048
10.1~20	3.058(2.646~3.534)	<0.001	1.517(1.310~1.758)	<0.001
>20	14.555(12.727~16.645)	<0.001	2.579(2.233~2.977)	<0.001
Age(Y)				
<60	Reference value		Reference value	
60~69	1.214(1.104~1.334)	<0.001	1.067(0.970~1.173)	0.180
70~79	2.398(2.182~2.634)	<0.001	1.671(1.518~1.839)	<0.001
≥80	8.985(8.122~9.939)	<0.001	2.984(2.683~3.318)	<0.001
Gleason score				
≤6	Reference value		Reference value	
7	2.970(2.619~3.368)	<0.001	2.210(1.945~2.512)	<0.001
8~10	23.949(21.358~26.854)	<0.001	7.843(6.915~8.895)	<0.001

incidence rate. This may be due to the extensive introduction of PSA monitoring (officially approved by FDA in 1986), which increased the detection of asymptomatic diseases. After that, the incidence rate of prostate cancer began to decline, which may be related to the recommendation of the United States Preventive Services Task Force (USPSTF) in 2008 to screen men ≥ 75 years old. Around 2012, the incidence rate of prostate cancer began to stabilize slowly, which may be due to concerns about over diagnosis and over treatment of prostate cancer. The U.S. Preventive Services Working Group recommended changing PSA to routine testing (16, 17). Therefore, after years of "excitement", clinical doctors are starting to test fewer and fewer patients. It is

TABLE 7 Consistency index of clinical factors in training set and validation set and *AIC* value of each factor.

Variable	*AIC*	Training set		Validation set	
		C index	*95%CI*	*C index*	*95%CI*
Gleason score	316515	0.789	0.760~0.818	0.752	0.709~0.795
PSA(ng/ml)	316401	0.693	0.654~0.732	0.696	0.645~0.747
Age(Y)	316366	0.641	0.602~0.680	0.632	0.581~0.683
Tumor stage	316342	0.736	0.701~0.771	0.738	0.691~0.785
Race	316342	0.513	0.484~0.542	0.520	0.479~0.561
Gleason score/PSA		0.820	0.791~0.849	0.801	0.769~0.851
Gleason score/PSA/Age		0.828	0.799~0.857	0.811	0.774~0.848
Gleason score/PSA/Age/Tumor stage		0.845	0.818~0.872	0.835	0.798~0.872
Gleason score/PSA/Age/Race		0.826	0.800~0.855	0.811	0.774~0.848
Gleason score/PSA/Age/Tumor stage/Race		0.844	0.817~0.871	0.834	0.795~0.873

worth noting that the change trend of incidence rate is parallel to the acceptance of PSA screening in some regions such as the United States, Europe and Australia. incidence rate is greatly affected by PSA testing and related screening plans (18). It can be considered that as long as there is screening, the incidence rate will increase.

In addition, these changes in patient management and disease-related regulations may also affect patient survival and other prognostic factors. In the early 1990s, the emergence of PSA screening also led to a shift in the diagnosis stage of prostate cancer, with an increase in the proportion of men diagnosed with localized diseases. Early detection and treatment of prostate cancer improved patient survival and other prognostic factors. Since 2008, the decrease in PSA testing has led to an increase in the number of late stage prostate cancer patients and a decrease in the number of early stage prostate cancer patients, which has led to poor treatment outcomes and a decrease in patient survival rates for most late stage patients.

In addition to PSA, medical imaging has always been a key component of early detection of prostate cancer (19). Medical imaging and other examination methods will also affect the incidence rate, prevalence, survival rate and other prognosis of prostate cancer. Hricak et al (20) published the first application of mpMRI in the prostate in 1983. Since then, mpMRI has been increasingly used for the diagnosis of prostate cancer (21). Many studies have confirmed the diagnostic reliability of mpMRI in detecting prostate cancer (22, 23). In the past, the lack of consistency in the diagnostic criteria of mpMRI led to differences in the number of patients diagnosed with prostate cancer in different regions, affecting the accuracy of the incidence rate of prostate cancer (24). In addition, different medical imaging equipment and quality applied in different regions will also affect the number of prostate cancer patients in different regions. For example, imaging examinations with high sensitivity may diagnose more patients, leading to an increase in the incidence rate of prostate cancer; Imaging examination with low sensitivity may diagnose a small number of patients, leading to a decline in the incidence rate of prostate cancer, and the incidence rate may be underestimated. In addition, the study found that mpMRI has less diagnosis of low-risk diseases and more diagnosis of high-risk diseases, which may lead to the underestimation of the incidence rate of low-risk prostate cancer and the overestimation of the incidence rate of high-risk prostate cancer (25). In order to standardize the evaluation of prostate imaging examination results, the European Society of Urogenital Radiology (eSUR)

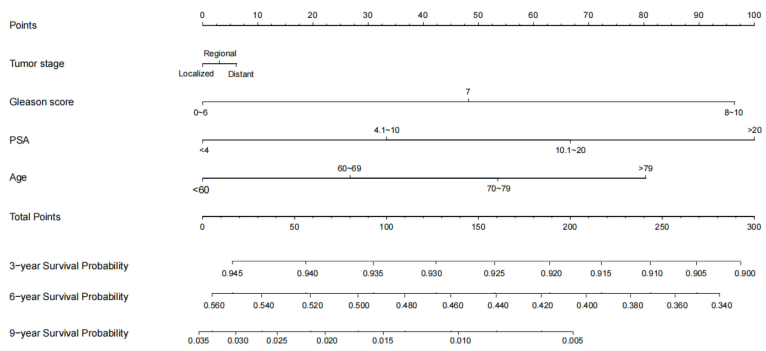


FIGURE 6
Nomogram of 3, 6, 9-year specific survival prediction of prostate cancer patients.

TABLE 8 Variable evaluation table.

Variable	evaluation
X_1=Tumor stage	0=Localized, 1=Regional, 2=Distant
X_2=Gleason score	0 = 0~7, 1 = 7, 2 = 8~10
X_3=PSA	0 = 0~4, 1 = 4.1~10, 2 = 10.1~20, 3= ≥20.1
X_4=Age	0=<60, 1 = 60~69, 2 = 70~79, 3= ≥80

released an expert consensus based guideline in 2012: Prostate Imaging Report and Data System (PiraS). In 2015, the American College of Radiologists published a revised version. These guidelines provide clear diagnostic criteria for the Likert score of multi parameter series, and further correct the accuracy of the incidence rate of prostate cancer (26).

In addition, the improvement of mpMRI technology has generated more information about tumor characteristics, which may help improve surgical planning and patient prognosis. For example, mpMRI has good sensitivity in identifying multifocal, seminal vesicle invasion, and extracapsular dilation (27–30). The high sensitivity of imaging examination methods allows doctors to grasp the important disease conditions of patients and choose the best treatment method. In addition, mastering more disease information during the surgical process can ensure the accuracy of doctors' surgical operations, avoid various medical accidents caused by unfamiliarity with the condition, thereby improving patient survival rate, improving patient prognosis, and prolonging patient life.

Imaging examination methods can not only affect the diagnosis of prostate cancer, but also affect the positive rate of surgical margins. The positive rate of surgical margins affects patients' later tumor recurrence and metastasis, thereby affecting their prognosis and survival time. Research has shown that there is a statistically significant correlation between the probability of receiving MPMRI before surgery and the lower probability of positive surgical margins (31). Cole et al[31] found that the mpMRI group had a lower probability of positive surgical margins, their propensity score weighted sensitivity analysis also found that the probability of surgical margin positivity was lower in males who underwent MRI examination. Another report from Stockholm stated that the positive margin rate in the mpMRI group (26.7%) was significantly lower than that in the non MRI group (33%) (32). Cole et al (31) found that the proportion of men who underwent MRI examination before surgery increased from 2.9% in 2004 to 28.2% in 2015. An increase in the proportion of men who underwent MRI examination before surgery may reduce the positive rate of surgical resection, improve the patient's condition and prognosis, and prolong their lifespan. The contents discussed above may lead to certain inevitable differences in the incidence rate, prevalence and survival rate of prostate cancer. However, as the most authoritative and representative database in the United States, SEER database can represent the epidemiological characteristics of local prostate cancer in this study.

Because our study found that the incidence and prevalence of prostate cancer remain at a high level, and more relevant studies are needed to evaluate the best treatment for these patients. So in this study, we performed a survival analysis using the SEER database and confirmed the significance of early diagnosis of age, tumor stage and tumor grade in prognosis. Our findings are consistent with other studies in that patients over 80 years of age had a poor prognosis, whereas patients under 60 years of age had the best prognosis; patients with G4 prostate cancer had a poor prognosis, whereas patients with G2 prostate cancer had the best prognosis. In our analysis, patients with localized and regional prostate cancer at the time of diagnosis had a better prognosis than patients with distant metastatic prostate cancer. This result highlights the importance of early detection and treatment of prostate cancer. This is consistent with the results of Gandaglia's (33) studies. For the entire cohort, survival rates improved over time, and this improvement may be related to advances in anticancer therapy, including the availability and use of targeted therapies.

Currently, the main evaluation index for prostate cancer survival is TNM staging, but TNM staging is not able to accurately assess patient survival (6), and more reliable evaluation indexes or prediction tools need to be explored, and nomograms are currently more commonly used tools for cancer prognosis evaluation, which can more accurately estimate the probability of

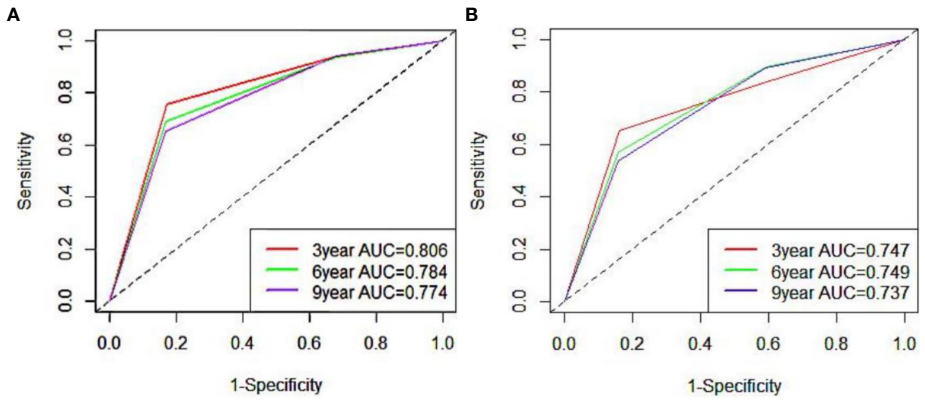


FIGURE 7
ROC curve of 3, 6 and 9 years of nomogram prediction model (**A**:training set; **B**:validation set).

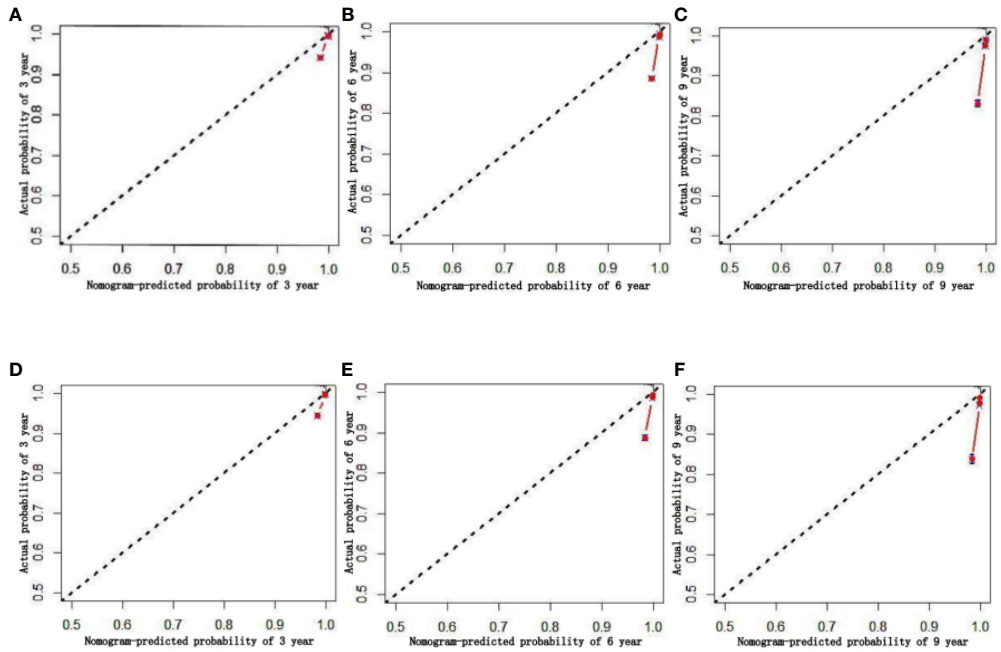


FIGURE 8
Calibration chart of 3、6、9-year specific survival probability (**A**–**C**:training set; **D**–**F**:validation set).

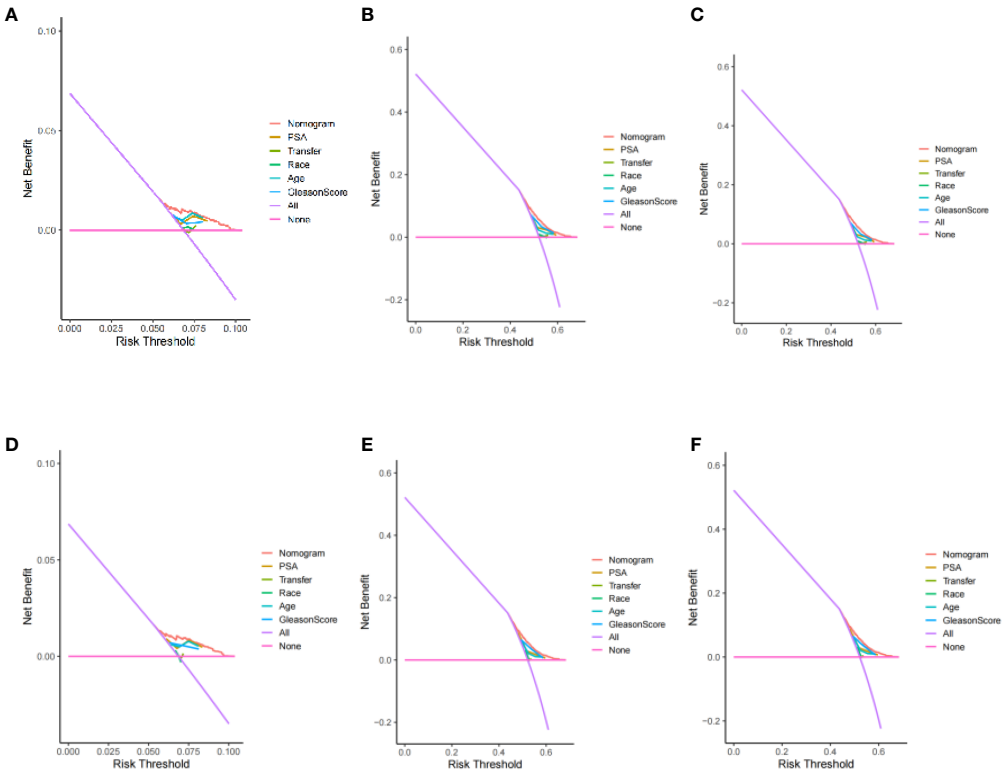


FIGURE 9
Decision curve analysis of 3、6、9-year specific survival probability (training set: **A**: 3 year; **B**: 6 years; **C**: 9 years) (validation set: **D**: 3 year; **E**: 6 years; **F**: 9 years).

a specific event for each individual by incorporating multiple risk factors compared to a single evaluation index (34, 35). However, there is no prognostic prediction model for prostate cancer constructed based on large sample data. Therefore, in this study, we screened prostate cancer prognostic influencing factors and built a prognostic model based on the information of prostate cancer patients in the SEER database to provide a reference basis for the assessment of patient prognosis.

We performed a multivariate survival analysis using a Cox regression. We found that age, race, tumor stage, PSA, and gleason score were associated with patient-specific survival in prostate cancer patients. Further analysis revealed that gleason score, tumor stage, age and PSA had the greatest impact on patient-specific survival in prostate cancer. gleason score is the main indicator for treatment selection and assessment of prognosis, and as gleason score increases, patient survival decreases along with gleason score (36). Previous studies have indicated that patients with prostate cancer with a pathological gleason score ≥8 have a high rate of positive seminal vesicle invasion、 cut margins、earlier biochemical recurrence、shorter survival time, therefore should be more aware of prognostic monitoring and follow-up (37). The results of this study suggest that gleason score is an important influential factor in the prognosis of prostate cancer patients, and patients with high gleason score have a lower specific survival rate. This is consistent with the findings of Ohtaka (38). Studies have shown that tumor metastasis can cause deterioration in the function of other tissues and organs, in addition, embolization of tumors in blood vessels may even cause vascular embolism, all of which can lead to poor physical condition and shortened survival. The results of this study showed that patients with metastatic prostate cancer had a lower survival rate. This is consistent with the results of studies by Hao (39) and DeSantis (40).

Age is closely related to the prognosis of prostate cancer, and the results of this study showed that the older the age, the worse the prognosis of the patients, with the best prognosis in patients <60 years old. This is consistent with the findings of Matsushita (41). Therefore, knowledge about urological and prostate cancer-related diseases and regular physical examinations are needed for prevention or early detection of urological-related diseases in the higher age groups. Previous studies have shown that PSA is an independent risk factor for the prognosis of prostate cancer patients, and high PSA levels are associated with a high risk of cancer death. However, recent studies found no relationship between PSA levels and prognosis in mPCa patients (42). the effect of PSA on the prognosis of prostate cancer is still controversial. Therefore, PSA must be combined with other factors when determining prognosis. The results of this study showed that patients with PSA (4.1-10 ng/ml) had a better prognosis than other groups of patients. This is consistent with the results of the study by Zijian Tian (43).

With the results of survival analysis, our nomogram including 4 important prognostic parameters (age, PSA, tumor stage and gleason score) can provide simple and accurate prognostic prediction for prostate cancer patients. For example, according to our nomogram, a patient with prostate cancer aged 65 years (26 points), PSA 13.0 ng/ml (67 points), gleason score 7 (48 points) and regional (3 points) had a 3-year survival rate of 92.7% (144 points), a 6-year survival rate of 45.0% (144 points) and a 9-year survival rate of 0.9% (144 points). Overall, this simple and effective tool can more accurately evaluate the survival of patients through various parameters of prostate cancer patients, thus facilitating clinical decision making and communication with patients and their families.

4.1 Limitations and advantages

Our study has several limitations. First, the SEER database may not capture all prostate cancer cases; therefore, we may actually underestimate the true incidence and prevalence of prostate cancer. Although the SEER database has a large sample size, it lacks important treatment information such as perioperative chemotherapy and postoperative chemotherapy, and the database includes patients over a large time span, and with the gradual development of medical technology, treatment varies from period to period, and this study is not yet able to correct for these possible confounding factors. In addition, novel targeted therapies have been used to treat patients with localized advanced or distant metastases with good survival benefits in selected cohorts over the past decades, and this information may confound the results of the survival analysis and may lead to differences in survival benefits in patients from different time periods. In addition, after establishing a nomogram model in this study, we only conducted internal validation using our data from this database to verify the accuracy of the model. We found that the model had good predictive accuracy, but the internal validation was not convincing and required external validation using other datasets. However, due to the inability to find suitable data other than the database, external validation was not conducted. Therefore, in the later stage, we need to find a suitable dataset for external validation. In addition, our hospital has also started collecting relevant data for further external validation to improve the prediction accuracy of the model and expand its application scope.

However, our research also has several advantages. To our knowledge, this study is one of the largest and latest explorations in cancer, the SEER database used in this study is the most authoritative and representative database in the United States, its data size and long-term follow-up data largely compensate for the shortcomings, and provide comprehensive epidemiological and survival data related to cancer, which can represent the epidemiological characteristics of local prostate cancer.

5 Conclusion

In this study, the incidence of prostate cancer remained unchanged over 45 years, but the incidence of prostate cancer with different characteristics changed significantly. In terms of survival, there were differences in survival rates by tumor stage and tumor grade. However, outcomes generally improved with advances in diagnosis and treatment. In addition, a new nomogram was established and validated in this study that can

effectively predict 3-, 6-, and 9-year survival rates in prostate cancer patients. It can provide accurate and useful information for physicians and patients and guide treatment strategies for prostate cancer patients.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: Surveillance, Epidemiology, and End Results Program (SEER) database.

Author contributions

Conception and design: SA, NT, and HN. Collection and assembly of data: SA and KB. Data analysis and interpretation: SA, YM, and AM. Manuscript writing: All authors. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the Key Projects of Xinjiang Uyghur Autonomous Region [grant number 2022D01D39]. The National Natural Science Foundation of China [grant number 82360476]. The Xinjiang Uygur Autonomous Region Natural Science Foundation [grant number 2022TSYCCX0026].

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1142976/full#supplementary-material

References

1. Adamaki M, Zoumpourlis V. Prostate Cancer Biomarkers: From diagnosis to prognosis and precision-guided therapeutics. *Pharmacol Ther* (2021) 228:107932. doi: 10.1016/j.pharmthera.2021.107932

2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* (2018) 68(6):394–424. doi: 10.3322/caac.21492

3. WHO. *Global health estimates 2020:deaths by cause, age, sex, by Country and by region, 2000-2019* . Available at: https://www.who.int/data/gho/data/themes/mortality-and-global-health-estimates/ghe-leading-causes-of-death.

4. Kensler KH, Rebbeck TR. Cancer progress and priorities: prostate cancer. *Cancer Epidemiol Biomarkers Prev* (2020) 29(2):267–77. doi: 10.1158/1055-9965.EPI-19-0412

5. Trabzonlu L, Kulac I, Zheng Q, Hicks JL, Haffner MC, Nelson WG, et al. Molecular pathology of high-grade prostatic intraepithelial neoplasia: challenges and opportunities. *Cold Spring Harb Perspect Med* (2019) 9(4):a030403. doi: 10.1101/cshperspect.a030403

6. Kim TH, Jeon HG, Jeong BC, Seo SI, Jeon SS, Choi HY, et al. Development of a new nomogram to predict insignificant prostate cancer in patients undergoing radical prostatectomy. *Scandinavian J Urol* (2017) 51(1):27–32. doi: 10.1080/21681805.2016.1266384

7. Wijk YV, Halilaj I, van Limbergen E, Walsh S, Lutgens L, Lambin P, et al. Decision support systems in prostate cancer treatment: an overview. *BioMed Res Int* (2019) 2019:1–10. doi: 10.1155/2019/4961768

8. Shariat SF, Karakiewicz PI, Suardi N, Kattan MW. Comparison of nomograms with other methods for predicting outcomes in prostate cancer: a critical analysis of the literature. *Clin Cancer Res* (2008) 14(14):4400–7. doi: 10.1158/1078-0432.CCR-07-4713

9. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin* (2022) 72(1):7–33. doi: 10.3322/caac.21708

10. Li J, Berkowitz Z, Hall IJ. Decrease in prostate cancer testing following the US preventive services task force (USPSTF) recommendations. *J Am Board Fam Med* (2015) 28(4):491–3. doi: 10.3122/jabfm.2015.04.150062

11. Stamey TA, Yang N, Hay AR, McNeal JE, Freiha FS, Redwine E. Prostate-specific antigen as a serum marker for adenocarcinoma of the prostate. *N Engl J Med* (1987) 317(15):909–16. doi: 10.1056/NEJM198710083171501

12. Catalona WJ, Smith DS, Ratliff TL, Dodds KM, Coplen DE, Yuan JJ, et al. Measurement of prostate-specific antigen in serum as a screening test for prostate cancer. *N Engl J Med* (1991) 324(17):1156–61. doi: 10.1056/NEJM199104253241702

13. Wang TY, Kawaguchi TP. Preliminary evaluation of measurement of serum prostate-specific antigen level in detection of prostate cancer. *Ann Clin Lab Sci* (1986) 16(6):461–6.

14. Cooner WH, Mosley BR, Rutherford CL Jr, Beard JH, Pond HS, Terry WJ, et al. Prostate cancer detection in a clinical urological practice by ultrasonography, digital rectal examination and prostate specific antigen. *J Urol* (1990) 143(6):1146–52. doi: 10.1016/S0022-5347(17)40211-4

15. Brawer MK, Chetner MP, Beatie J, Buchner DM, Vessella RL, Lange PH. Screening for prostatic carcinoma with prostate specific antigen. *J Urol* (1992) 147(3 Pt 2):841–5. doi: 10.1016/S0022-5347(17)37401-3

16. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin* (2019) 69(1):7–34. doi: 10.3322/caac.21551

17. Moyer VAU.S. Preventive Services Task Force. Screening for prostate cancer: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med* (2012) 157(2):120–34. doi: 10.7326/0003-4819-157-2-201207170-00459

18. Pernar CH, Ebot EM, Wilson KM, Mucci LA. Cold spring harb perspect med. *Cold Spring Harb Perspect Med* (2018) 8(12):a030361. doi: 10.1101/cshperspect.a030361

19. Gillies RJ, Schabath MB. Radiomics improves cancer screening and early detection. *Cancer Epidemiol Biomarkers Prev* (2020) 29(12):2556–67. doi: 10.1158/1055-9965.EPI-20-0075

20. Hricak H, Williams RD, Spring DB, Moon KL Jr, Hedgcock MW, Watson RA, et al. Anatomy and pathology of the male pelvis by magnetic resonance imaging. *AJR Am J Roentgenol* (1983) 141(6):1101–10. doi: 10.2214/ajr.141.6.1101

21. Hamoen EHJ, de Rooij M, Witjes JA, Barentsz JO, Rovers MM. Use of the prostate imaging reporting and data system (PI-RADS) for prostate cancer detection with multiparametric magnetic resonance imaging: A diagnostic meta-analysis. *Eur Urol* (2015) 67(6):1112–21. doi: 10.1016/j.eururo.2014.10.033

22. Siddiqui MM, Rais-Bahrami S, Truong H, Stamatakis L, Vourganti S, Nix J, et al. Magnetic resonance imaging/ultrasound-fusion biopsy significantly upgrades prostate

cancer versus systematic 12-core transrectal ultrasound biopsy. *Eur Urol* (2013) 64 (5):713–9. doi: 10.1016/j.eururo.2013.05.059

23. Delongchamps NB, Rouanne M, Flam T, Beuvon F, Liberatore M, Zerbib M, et al. Multiparametric magnetic resonance imaging for the detection and localization of prostate cancer: combination of T2-weighted, dynamic contrast-enhanced and diffusion-weighted imaging. *BJU Int* (2011) 107(9):1411–8. doi: 10.1111/j.1464-410X.2010.09808.x

24. Dickinson L, Ahmed HU, Allen C, Barentsz JO, Carey B, Futterer JJ, et al. Scoring systems used for the interpretation and reporting of multiparametric MRI for prostate cancer detection, localization, and characterization: could standardization lead to improved utilization of imaging within the diagnostic pathway? *J Magn Reson Imaging* (2013) 37(1):48–58. doi: 10.1002/jmri.23689

25. Kasivisvanathan V, Rannikko AS, Borghi M, Panebianco V, Mynderse LA, Vaarala MH, et al. PRECISION study group collaborators. MRI-targeted or standard biopsy for prostate-cancer diagnosis. *N Engl J Med* (2018) 378(19):1767–77. doi: 10.1056/NEJMoa1801993

26. Barentsz JO, Richenberg J, Clements R, Choyke P, Verma S, Villeirs G, et al. European Society of Urogenital Radiology. ESUR prostate MR guidelines 2012. *Eur Radiol* (2012) 22(4):746–57. doi: 10.1007/s00330-011-2377-y

27. Bloch BN, Furman-Haran E, Helbich TH, Lenkinski RE, Degani H, Kratzik C, et al. Prostate cancer: accurate determination of extracapsular extension with high-spatial-resolution dynamic contrast-enhanced and T2-weighted MR imaging–initial results. *Radiology* (2007) 245(1):176–85. doi: 10.1148/radiol.2451061502

28. Feng TS, Sharif-Afshar AR, Smith SC, Miller J, Nguyen C, Li Q, et al. Multiparametric magnetic resonance imaging localizes established extracapsular extension of prostate cancer. *Urol Oncol* (2015) 33(3):109.e15–22. doi: 10.1016/j.urolonc.2014.11.007

29. Hegde JV, Chen MH, Mulkern RV, Fennessy FM, D'Amico AV, Tempany CM. Preoperative 3-Tesla multiparametric endorectal magnetic resonance imaging findings and the odds of upgrading and upstaging at radical prostatectomy in men with clinically localized prostate cancer. *Int J Radiat Oncol Biol Phys* (2013) 85(2):e101–7. doi: 10.1016/j.ijrobp.2012.08.032

30. Giganti F, Coppola A, Ambrosi A, Ravelli S, Esposito A, Freschi M, et al. Apparent diffusion coefficient in the evaluation of side-specific extracapsular extension in prostate cancer: Development and external validation of a nomogram of clinical use. *Urol Oncol* (2016) 34(7):291.e9–291.e17. doi: 10.1016/j.urolonc.2016.02.015

31. Cole AP, Chen X, Langbein BJ, Giganti F, Kasivisvanathan V, Emberton M, et al. Geographic variability, time trends and association of preoperative magnetic resonance imaging with surgical outcomes for elderly United States men with prostate cancer: A surveillance, epidemiology, and end results-medicare analysis. *J Urol* (2022) 208 (3):609–17. doi: 10.1097/JU.0000000000002736

32. Jäderling F, Akre O, Aly M, Björklund J, Olsson M, Adding C, et al. Preoperative staging using magnetic resonance imaging and risk of positive surgical margins after prostate-cancer surgery. *Prostate Cancer Prostatic Dis* (2019) 22(3):391–8. doi: 10.1038/s41391-018-0116-z

33. Gandaglia G, Leni R, Bray F, Fleshner N, Freedland SJ, Kibel A, et al. Epidemiology and prevention of prostate cancer. *Eur Urol Oncol* (2021) 4(6):877–92. doi: 10.1016/j.euo.2021.09.006

34. Dong D, Zhao D, Li S, Liu W, Du F, Xu X, et al. Nomogram to predict overall survival for patients with non-metastatic cervical esophageal cancer: a SEER-based population study. *Ann Transl Med* (2020) 8:1588. doi: 10.21037/atm-20-2505

35. Zhao F, Lu RX, Liu JY, Fan J, Lin HR, Yang XY, et al. Development and validation of nomograms to intraoperatively predict metastatic patterns in regional lymph nodes in patients diagnosed with esophageal cancer. *BMC Cancer* (2021) 21:22. doi: 10.1186/s12885-020-07738-9

36. Kim J, Park JS, Ham WS. The role of metastasis-directed therapy and local therapy of the primary tumor in the management of oligometastatic prostate cancer. *Invest Clin Urol* (2017) 58(5):307–16. doi: 10.4111/icu.2017.58.5.307

37. Krimphove MJ, Cole AP, Fletcher SA, Harmouch SS, Berg S, Lipsitz SR, et al. Evaluation of the contribution of demographics, access to health care, treatment, and tumor characteristics to racial differences in survival of advanced prostate cancer. *Prostate Cancer Prostatic Dis* (2019) 22(1):125–36. doi: 10.1038/s41391-018-0083-4

38. Ohtaka M, Miyoshi Y, Kawahara T, Ohtake S, Yasui M, Uemura K, et al. Low-molecular-weight protein tyrosine phosphatase expression as a prognostic factor for men with metastatic hormone-naïve prostate cancer. *Urologic Oncol* (2017) 35 (10):607.e9–607.e14. doi: 10.1016/j.urolonc.2017.05.019

39. Hao Y, Bjerke GA, Pietrzak K, Melhuish TA, Han Y, Turner SD, et al. TGFβ signaling limits lineage plasticity in prostate cancer. *PloS Genet* (2018) 14(5):e1007409. doi: 10.1371/journal.pgen.1007409

40. DeSantis CE, Lin CC, Mariotto AB, Siegel RL, Stein KD, Kramer JL, et al. Cancer treatment and survivorship statistics, 2014. *CA Cancer J Clin* (2014) 64(4):252–71. doi: 10.3322/caac.21235

41. Miyake H, Matsushita Y, Watanabe H, Tamura K, Motoyama D, Ito T, et al. Prognostic significance of time to castration resistance in patients with metastatic castration-sensitive prostate cancer. *Anticancer Research: Int J Cancer Res Treat* (2019) 39(3):1391–6. doi: 10.21873/anticanres.13253

42. Boevé LMS, Hulshof MCCM, Vis AN, Zwinderman AH, Twisk JWR, Witjes WPJ, et al. Effect on survival of androgen deprivation therapy alone compared to androgen deprivation therapy combined with concurrent radiation therapy to the prostate in patients with primary bone metastatic prostate cancer in a prospective randomised clinical trial: data from the HORRAD trial. *Eur Urol* (2019) 75(3):410–8. doi: 10.1016/j.eururo.2018.09.008

43. Tian Z, Meng L, Wang X, Wang X, Ma T, Wang M, et al. Survival in patients with metastatic prostate cancer undergoing radiotherapy: the importance of prostate-specific antigen-based stratification. *Front Oncol* (2021) 11:706236. doi: 10.3389/fonc.2021.706236

Frontiers in Oncology

Advances knowledge of carcinogenesis and tumor progression for better treatment and management

The third most-cited oncology journal, which highlights research in carcinogenesis and tumor progression, bridging the gap between basic research and applications to imrpove diagnosis, therapeutics and management strategies.

Discover the latest Research Topics

See more →

Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact