Recent advancements in therapeutic biomarkers, associated challenges and considerations to overcome these challenges in prostate cancer (Review)

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Abstract. Prostate cancer is the main cause of mortality among males the global level and hold its 4th position as per Globocan data. Multiple treatment options are available for patients with low-grade prostate cancer and metastatic disease. Therapeutic biomarkers hold significant potential for identifying treatment responses either prior to commencing any therapy or following therapy. For example, the high expression of androgen receptor splice variant 7 is a well-established driver of resistance to both androgen-axis-targeted therapies and ^{223}Ra treatments in prostate cancer. Additionally, prostate-specific membrane antigen (PSMA) exhibits a significantly increased expression in prostate adenocarcinoma in comparison to normal tissue. Elevated PSMA levels are strongly associated with tumor aggressiveness. Despite the potential of biomarkers, identifying clinically actionable biomarkers remains a notable challenge due to several factors. A key obstacle is the heterogeneity of cancer cells, which can exhibit diverse molecular profiles even within the same tumor. Other challenges include the development of reliable assays, the lack of standardized methodologies for biomarker measurement and validation, sample availability, the high development cost, limited accessibility in low resource settings and stringent requirements for regulatory approval. Furthermore, the variability in tumor behavior necessitates tailored therapeutic strategies, as a one-size-fits-all approach is unlikely to be effective. Therefore, it is necessary to address the challenges associated with the currently available biomarkers in order for risk stratification and treatment efficacy to be personalized. The present review discusses recent advancements in the field of therapeutic biomarkers against prostate cancer to assess the therapeutic response, progress of therapy, associated challenges and considerations to overcome the challenges.

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Contents

1. Introduction

Prostate cancer (Pca) has emerged as the most common type of cancer affecting males worldwide, holding the 4th position among all types of cancer, with a total of 1,466,680 new cases and 396,792 related deaths worldwide in 2022 (1). A variety of modifiable and non-modifiable risk factors are linked to the development of Pca, rendering its etiology complex and challenging to understand, including age, ethnicity, family history and lifestyle management (2). Therefore, it is critical to diagnose Pca in its early stages and begin treatment as soon as possible. Multiple treatment options are available for patients with Pca, including active surveillance regimens for low-grade Pca, radiation therapy and prostatectomy surgery for localized disease, or a combination of several therapies [chemotherapy, immunotherapy, androgen deprivation therapy (ADT), or agents targeting androgen receptors] for metastatic disease (3). In this therapy, the testosterone level is reduced in the body to near castrate levels using gonadotropin hormone-releasing hormone agonists or antagonist drugs, which block the production of testosterone in the body and arrest the cell cycle of cancer cells (3). Among the aforementioned treatment options, ADT is commonly used for the treatment of metastatic Pca.

It has been demonstrated that ~30-50% of patients receiving ADT develop therapeutic resistance that leads to castrate-resistant Pca (CRPC) (4). In these patients, the levels

of prostate-specific antigen (PSA) are high even if they have low levels of testosterone. CRPC may develop due to the reactivation of androgen receptor (AR) through the upregulation of the P450 enzymes, CYP11A1 and CYP17A1 (5). Chemotherapy, along with ADT, is usually administered to patients with CRPC. For example, FDA-approved drugs, such as apalutamide and darolutamide are currently being incorporated with ADT to decrease the progression of CRPC (6,7). Both drugs competitively inhibit the binding of androgens to ARs and the transcription of genes regulated by AR signaling. However, they have been shown to have various side-effects (8). Similarly, taxanes such as docetaxel and cabazitaxel are commonly used in the treatment of metastatic CRPC (mCRPC). However, despite the improved survival rates, almost each patient eventually develops resistance, which is a critical barrier to long-term survival (9).

The varied mechanisms of action of drugs in PCa necessitate the identification of clinically valuable biomarkers to guide precision-oriented therapies. However, this remains challenging due to tumor heterogeneity at genetic, epigenetic and phenotypic levels, resulting in diverse molecular profiles even within the same tumor. Not all mutations are clinically relevant, further complicating biomarker identification. Sequential biopsies, essential for capturing tumor heterogeneity and dynamics, are often impractical due to limitations associated with both solid and liquid biopsies, as previously described (4).

Despite challenges in identifying biomarkers and characterizing PCa at the molecular level, biomarkers are invaluable for assessing treatment responses and stratifying patients into risk groups (high, intermediate or low). This stratification allows clinicians to customize treatments, opting for aggressive approaches for high-risk tumors and more conservative strategies for low-risk cases. Approved biomarkers, such as AR splice variant 7-V7 (AR-V7), have exhibited utility in predicting therapeutic outcomes. For instance, patients with high a expression of AR-V7 exhibit improved overall survival rates with taxane therapy compared to treatment with AR signaling (ARS) inhibitors (10). A number of genetic biomarkers, such as phosphatase and tensin homolog (*PTEN*), poly(ADP-ribose) polymerase (*PARP*) and homologous recombination repair (*HRR*), have emerged as potential treatment targets for Pca (11). Numerous biomarkers have surfaced that could eventually be proven reliable indicators of how different treatments would affect metastatic patients with Pca (12). However, comparisons in different studies are challenging due to differences in the type of therapy, the patient population size and prior treatments received. While biomarkers are being used to differentiate between metastatic and non-metastatic Pca, their clinical implications, for instance, and their effects on overall survival or disease-free survival, remain limited. Therefore, therapies should not only focus on the basis of the high or low expression of biomarkers, but should also be based on their effects on survival or other factors to establish genotype-phenotype associations (13,14). This will markedly improve the precision of disease treatment. It is necessary to address the challenges associated with current biomarkers in order for risk stratification and treatment efficacy to be improved for a specific patient (15).

The present discusses the advancements that have been made in therapeutic biomarkers that are used in Pca to assess the response to therapy, progress being made in therapy and associated challenges. The present review included studies assessing the efficacy of biomarker-based therapies in all types of Pca. An extensive search of the literature was performed using three online databases: PubMed, Embase and Web of Science. The search was carried out from the beginning of the databases up until August 31, 2024. To identify ongoing clinical trials for Pca treatments, a systematic search was also conducted on ClinicalTrials.gov. A combination of the following key words was used: targeted therapy, therapeutic biomarkers, inhibitors, drugs, prostate cancer, PARP inhibitors, cancer, AR-V7, PI3K/AKT, PSMA, HDAC and clinical trial. The inclusion criteria required participants with pathologically or cytologically confirmed prostate adenocarcinoma and radiographic evidence of metastases. Studies were excluded if they met any of the following criteria: i) Studies not containing sufficient data for analysis; and ii) case reports.

2. Androgen receptor splice variant 7

AR-V7 is a mutation that arises due to the truncation of the ligand-binding domain (LBD) of the AR, which allows the receptor to remain in an active state independent of androgen ligand binding (16). Wild-type ARs are 110 kDa or 919 amino acids in length. It consists of four functional domains and contains eight exons: i) The N-terminal transactivation domain (exon 1), which has an AF1 domain with two transactivation units (TAU-1 and TAU-5) that can lead to the aberrant activation of the AR in CRPC. This region is responsible for the full activation of the AR. In the presence of the ligand, 50% of AR activation is contributed by TAU-1, whereas in the absence of a ligand-binding domain, TAU-5 mediates activation. ii) The DNA-binding domain (DBD) (exons 2 and 3): It has two zinc fingers that promote receptor dimerization. iii) The hinge region (exon 4), which separates the DBD into a ligand-binding domain and has a nuclear localization signal. iv) The LBD (exon 5-8), which has an AF2 domain and is responsible for binding to the ligand. Several splice variants of AR have been identified in cell lines and xenograft models. However, due to the availability of AR-V7 specific antibody, AR-V7 is the most well-characterized AR variant (17). AR-V7 can also form dimers with full-length AR (AR-FL). The mRNA of AR-V7 includes the first three canonical exons, followed by a variant-specific cryptic exon, known as CE3. A splicing event at CE3 resulted in the production of a truncated LBD for AR-V7. Truncation occurs due to the premature termination of translation after 16 variant-specific amino acids (EKFRVGNCKHLKMTRP stop) (Fig. 1) (18). Certain splicing factors and chaperone proteins regulate the expression of AR-V7. For example, the recruitment of splicing factors hnRNPA1, U2AF65 and ASF/SF2 to AR-SV mRNA splice junctions increased. The same was not true for AR-FL. By contrast, HSP90 inhibition disrupted AR-V7 splicing. The expression of non-coding RNA PCGEM1 increases under conditions of androgen deprivation, which facilitates the interaction of this RNA with the splicing factor (U2AF565). Thus, the splicing mechanisms of AR-V7 are likely cell-context-specific and regulated by numerous factors (19).

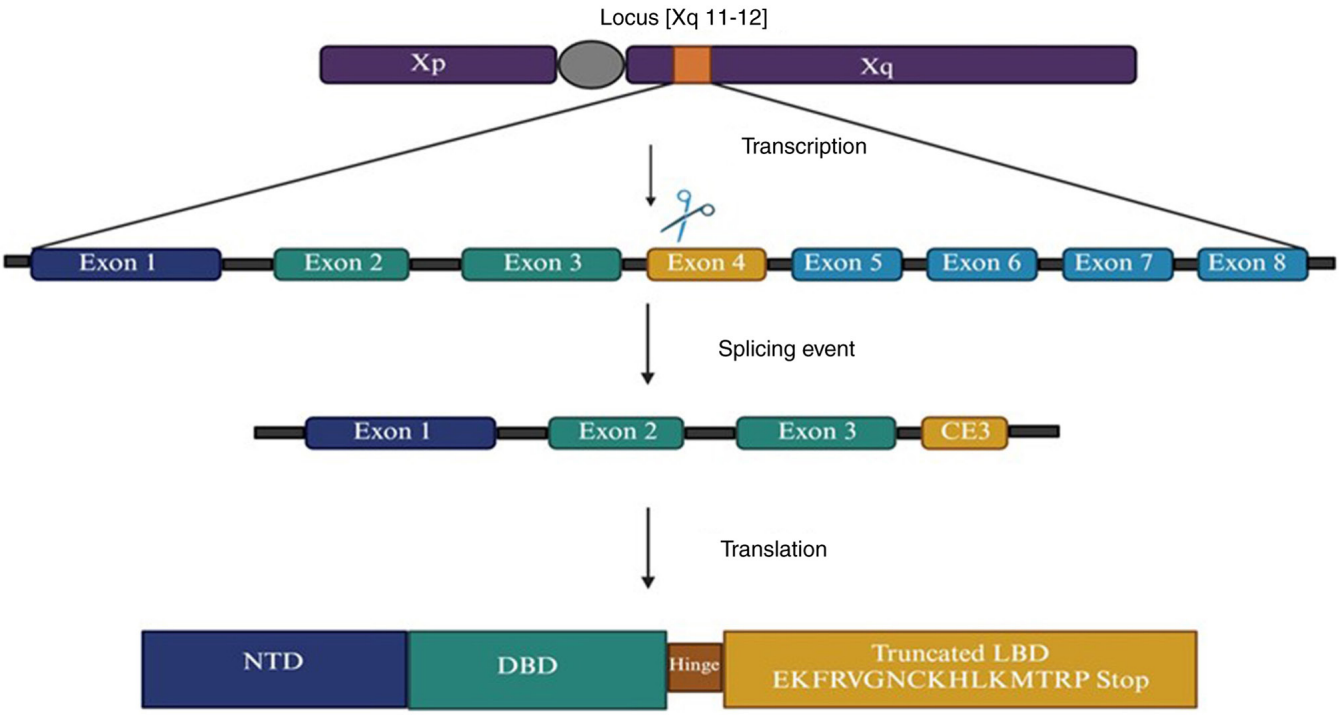

Figure 1. The *AR* gene is located on the q-arm of the X chromosome. This splicing event results in the formation of a truncated LBD, leading to the formation of AR-V7. It contains three canonical exons followed by cryptic exon 3. AR-V7 has an NTD, a DBD and a truncated LBD joined by a hinge domain. LBD, ligand-binding domain; NTD, N-terminal domain; DBD, DNA-binding domain; AR, androgen receptor; AR-V7, splice variant 7.

AR signaling has been linked to the development of certain types of cancer beyond Pca. AR-V7 can be present in primary breast cancer (BC) even in patients who have never received treatments, such as anti-estrogen therapy or drugs that block the AR. For patients with BC being considered for treatment with AR inhibitors, having certain features in the tumor (such as apocrine changes) and testing positive for AR on laboratory slides may suggest the need for further testing to test for AR-V7. It has been suggested that AR-V7 may be a helpful marker to predict how well patients with BC may respond to treatments that block the AR. This could be useful for future clinical trials testing these treatments (20). Studies using laboratory-grown cells and animal models have demonstrated that androgens and AR play a role in cancer development (21). For example, it was shown that AR signaling helps liver cancer cells grow and spread, and it also makes these cells resistant to treatments that block the AR (22). As with liver cancer and BC, AR-V7 has also been shown to play a role in bladder and kidney cancers (19). However, the role of AR-V7 in other types of cancer is not yet fully understood. Research is still in the early stages, and its effects may differ depending on the type of cancer and the specific circumstances.

AR-V7 expression in Pca. The expression of AR-V7 has been found to be markedly higher in metastatic Pca than in primary Pca specimens. For instance, in a previous study, the expression of AR-V7 in metastatic biopsy specimens was >75% following ADT, whereas its expression was only <1% in primary Pca tissues (23). In that study, 651 Pca samples (primary Pca samples, 358; metastatic biopsies, 293) were included to observe the expression of AR-V7. However, a wide heterogeneity was observed, such as the expression of AR-V7, which differed in different metastatic sites of the same patient, thus rendering it an unreliable biomarker (23). These changes necessitate the need for further biopsies on progression. However, it is challenging to collect tissue samples multiple times.

The levels of AR-V7 are generally higher in castration-resistant tumors than in androgen-dependent tumors (24). Its high expression can also be used to prognose the severity of disease. For instance, patients with AR-V7-positive hormone-sensitive Pca have been shown to have a worse prognosis following first-line hormonal therapy and prostatectomy (25). AR-V7 overexpression facilitates the development of lesions that resemble prostatic interstitial neoplasia. Of note, AR-V7 also alters the expression of genes associated with prostate stem and progenitor cells (19). These findings suggest that AR-V7 expression increases following ADT and may lead to therapeutic resistance. In mCRPC, AR-V7 is primarily nuclear, but is also present in the cytoplasm and cytoplasmic granules. A previous study on 410 patients with Pca revealed that cytoplasmic AR-V7 staining was associated with a shorter relapse-free survival (RFS), while granular cytoplasmic AR-V7 staining was linked to a longer RFS and was negatively associated with aggressive disease features, such as higher Gleason scores, tumor stage and metastasis (26). By contrast, nuclear and cytoplasmic AR-V7 staining was positively associated with Gleason scores and tumor invasiveness. Granular AR-V7 staining emerges as an independent prognostic marker for RFS, emphasizing the need to assess AR-V7 localization for accurate prognostic evaluation in prostate cancer patients (26).

The expression of AR-V7 has also been reported in circulating tumor cells (CTCs). In a previous study, immunofluorescence staining demonstrated the presence

of AR-V7-positive CTCs in 34 out of 161 samples (18%) (P<0.001). During AR-axis-targeted therapies (AATT), patients with positive AR-V7 staining demonstrate a poorer overall survival (OS) and progression-free survival (PFS) than AR-V7-negative patients. However, in patients treated with taxanes, no association was observed between AR-V7 staining and the high risk of mortality (10). That study concluded that AR-V7-positive CTCs were negatively associated with the benefits of AATT therapy and could predict therapeutic resistance in patients with mCRPC. Similarly, patients treated with AATT demonstrated worse outcomes if they had a high expression of AR-V7. These findings confirm that AR-V7 positivity is associated with adverse outcomes. These factors include a Gleason score ≥8, metastasis and prior therapeutic outcomes. Therefore, the nuclear localization of AR-V7 can help clinicians make wise decisions, such as opting for taxane therapy instead of AATT.

AR-V7 and regulated genes. It has been shown that genes induced by AR-V7 and AR-FL differ markedly in terms of tumor promoting properties. For instance, AR-V7 induces the expression of cell cycle-related genes. By contrast, AR-FL promotes the expression of genes associated with metabolism, differentiation and macromolecular synthesis (17). It has also shown that the presence of AR-FL is not required for inducing cell cycle-related genes in cells transiently transfected with AR-V7. However, the suppression of AR-FL expression using siRNA or drugs induces the expression of AR-V7-regulated genes (27). It was also previously shown that the overexpression of AR-V7 induced the expression of 407 genes and reduced the expression of 80 genes. Of these 407 positively associated genes, only 59 were found to be significantly associated with nuclear AR-V7 staining. Of these 59 genes, 33% had zinc finger domains and were associated with chromatin-binding regions proximal to the transcriptional start site. These genes included *HOXB13, ELL2, STEAP2* and *BAZ2A*. Due to the absence of the LBD in AR-V7, it can either expose or mask regions that are capable of interacting with distinct coregulators in comparison to FL-AR (28,29). These results suggest that FL-AR and AR-V7 target unique genes; however, a few genes may be common.

In Pca, specifically in phase II mCRPC trials, the levels of PSA contribute significantly to determining the response to any given therapy. It is a serum biomarker that can be measured easily. In a previous study, a ≥50% reduction in PSA level from baseline was shown to be associated with increased survival rates. That study demonstrated that patients who were AR-V7-negative had significantly better PSA response rates (100 vs. 54%; P=0.03) and a longer OS compared to AR-V7-positive patients [74 vs. 24 months; hazard ratio (HR), 0.23; P=0.02]. This suggests that AR-V7 negativity is associated with more favorable treatment outcomes in Pca (23).

AR-V7 as a predictor of the therapeutic response

Clinical relevance and challenges. The hypothalamus of the brain releases gonadotropin-releasing hormone (which stimulates the pituitary gland to produce follicle-stimulating hormone and luteinizing hormone). These hormones signal the gonads to produce testosterone, which is then converted to dihydrotestosterone (DHT) by the enzyme 5-alpha-reductase. DHT binds to the intracellular AR, forming a DHT-AR complex that migrates to the nucleus and regulates the transcription and translation of cancer-related genes. AR antagonists, such as enzalutamide, apalutamide and darolutamide (AATT) inhibit DHT from binding to the LBD of AR, which prevents the translocation of AR to the nucleus, transcription of target genes and its ability to bind to chromatin (11).

There are several mechanisms that can lead to chemoresistance following AATT: i) The upregulation of steroidogenesis, leading to the synthesis of endogenous androgens within the prostate tumor; ii) single point mutations in the LBD domain of the AR; iii) the silencing of androgen inactivation enzymes, such as HSD17B2 by methylation; and iv) the upregulation of the glucocorticoid receptor; these receptors are upregulated in metastatic Pca and provide resistance to AATT by surpassing AR blockade; v) the emergence of AR splice variants such as AR-V7; vi) the higher expression of AR due to AR gene amplification (30,31). The absence of the LBD in AR-V7 results in the constitutive activation of the AR and its target genes, even in the absence of androgens (32). Research has shown that AR-V7 can form heterodimers with AR-FL, activate canonical AR target genes such as PSA, and promote cell growth in castration-resistant conditions (33). It has been suggested that a positive correlation between AR-V7 and AR-FL expression and copy number. However, conflicting cases of AR-V7 and AR-FL suggest that AR-V7 expression following ADT is not simply a consequence of AR gene amplification (33). The inhibition of AR-V7 has also been shown to confer sensitivity to AATT. For instance, the inhibition of AR-V7 protein by the anthelmintic drug, niclosamide, inhibits Pca cell growth and restores sensitivity to enzalutamide in enzalutamide-resistant tumors (34). Notably, a higher expression of AR-V7 confers chemoresistance to AATT, but not to taxane therapy. It has been observed that the presence of AR-V7 results in significantly improved outcomes in taxane-treated patients with Pca compared to abiraterone- or enzalutamide-treated patients (35,36). These findings indicate that AR-V7 expression in CTCs serves as a marker of AATT resistance, but not taxane-based treatments. By comparison, although AR-V7 is less extensively studied in BC, its expression may contribute to resistance in hormone receptor-positive cases by circumventing estrogen receptor signaling or influencing AR activity. However, its role in BC is less well-defined and requires further research to establish its exact mechanisms and clinical relevance (37). Researchers have also explored the expression of AR-V7 in salivary gland cancers; however, the evidence is limited, largely exploratory and context-dependent (38). In another PROPHECY trial, PSA or soft tissue responses were lower in AR-V7-positive mCRPC (PSA, 0 to 11%; soft tissue response, 0 to 6%), confirming the association between AR-V7 expression, and a shorter PFS and OS with abiraterone or enzalutamide, and such patients with mCRPC should be offered alternative treatments (39). Similarly, the EXCAAPE (NCT03002220) phase IIa trial was conducted on 52 patients with mCRPC and asymptomatic bone metastases who had progressed on abiraterone acetate or enzalutamide up to six doses of ^{223}Ra (40). It was shown that AR-V7-positive patients had a shorter radiographic PFS and OS than AR-V7-negative patients (rPFS-AR-V7-negative, 5.5 months; AR-V7-positive, 2.2 months, P=0.056) (OS: AR-V7-negative, 14.8 months; AR-V7-positive, 3.5 months,

P<0.01). The incidence of grade 3/4 adverse events was lower in AR-V7-negative patients when compared to AR-V7-positive patients (5.8 and 11.5%, respectively). These results suggest that AR-V7 expression can lead to therapeutic resistance to ^{223}Ra therapy in patients with mCRPC (40).

Immune checkpoint inhibitors (nivolumab and ipilimumab) combined with androgen-axis-targeted therapies have exhibited limited efficacy in AR-V7-positive patients with mCRPC. In the STARVE-PC trial, 30 patients with progressive mCRPC and detectable AR-V7 transcripts received either nivolumab and ipilimumab (cohort 1) or the same combination with enzalutamide (cohort 2). Outcomes, including PSA response rates, PFS and overall response rates (ORR), exhibited no significant differences between cohorts. The median OS was higher in cohort 2 (14.2 vs. 8.2 months), but the results were not statistically significant. These findings highlight the need for novel immune targets and biomarkers to improve outcomes in AR-V7-positive Pca (41).

In ongoing clinical trials, a phase 3, randomized, open-label study will compare opevesostat treatment with alternative abiraterone acetate or enzalutamide in participants with mCRPC based on the LBD mutation of AR for each drug (NCT06136624 and NCT06136650). Similarly, a biomarker-driven study in patients with metastatic prostate cancer (ProBio) will treat patients based on biomarker signatures, such as androgen receptor, DNA repair deficiency, TP53, TMPRSS2-ERG gene fusion, and PI3K pathway alterations (NCT03903835).

Patients with positive AR-V7 expression should avoid AATT and ^{223}Ra therapy, as these treatments may be less effective. Instead, they should consider alternative therapeutic options.

Future research directions. Nuclear staining is a hallmark of AR-V7. The inclusion of cytoplasmic and cytoplasmic granule staining should also be included to confirm the presence of AR-V7. Taken together, AR-V7 expression in granular cytoplasmic structures is an independent prognostic factor for RFS in patients with Pca. AR-V7 binds to various co-regulators. Similarly, several genes have been found to be significantly associated with nuclear AR-V7 staining. These genes included *HOXB13, ELL2, STEAP2* and *BAZ2A*. Therefore, the inclusion of such co-regulators and genes will add to accurately dividing the patients into two groups: i) AR-V7-positive and ii) AR-V7-negative, and may ultimately help clinicians to make precise decisions. Notably, not all AR-V7-positive patients demonstrate chemoresistance to AATT. Therefore, more accuracy is required to make an accurate decision.

3. Histone deacetylases

Positively charged lysine residues are present at the N-terminal site of histone proteins. In the absence of acetylation, lysine interacts tightly with negatively charged DNA molecules, leading to chromatin condensation. This interaction prevents the access of the transcriptional machinery to the transcription site. The acetylation of lysine residues prevents this interaction and thus leads to transcription initiation. In addition to histones, non-histone proteins are capable of undergoing acetylation. Non-histone proteins (NHPs) are a diverse group of proteins found in the nucleus that are distinct from histones. These NHPs are involved in various processes, such as gene regulation, cell cycle regulation, DNA repair and differentiation. Examples of NHPs include transcription factors and DNA polymerases. The acetylation of transcription factors can either activate or repress their DNA-binding ability of transcriptional factors. If acetylation occurs in the DNA-binding region of the transfection factor, it will reduce their binding to DNA, such as FoxO1 and HMGI. On the contrary, if acetylation occurs near the DNA binding region, it will enhance the binding of TF to DNA such as p53, NF NF-κB. These findings suggest that the acetylation of both histone proteins and NHPs regulates various activities, including cell cycle, DNA repair, apoptosis, autophagy and metabolism. An imbalance in the transcription of tumor promoters and suppressor genes can lead to the development of cancer (42).

HDACs are enzymes that deacetylate histones and NHPs. To date, 18 different HDACs have been identified and are categorized into four classes based on their homology to yeast and co-factor dependencies. Class I, II and IV HDACs contain a zinc-binding domain and therefore require zinc ions for their catalytic activity, whereas class III HDACs have an NAD+ binding domain and thus require NAD+ as a co-factor (2). Class I HDACs (HDACs 1, 2, 3, and 8) have an acetylase domain located in the N-terminal region and are primarily localized in the nucleus. By contrast, class II HDACs (HDACs 4, 5, 6, 7, 9 and 10) apart from HDAC6 have an acetylase domain at the C-terminal site and are located in both the nucleus and cytoplasm. HDAC6 has two acetylase domains located in its N-terminal region. Class II HDACs are comparatively longer than the other three classes of HDACs. Class III HDACs are silent information regulator 2 proteins with seven homologues (sirtuins). These sirtuins can deacetylate many proteins such as p53 and tubulin. Therefore, class III prevents differentiation and promote tumorigenesis (43). Class IV HDAC, specifically HDAC 11, possess characteristics of both class I and class II enzymes, exhibiting HDAC activity in both its N- and C-terminal regions (44).

Research has shown that the levels of HDACs vary significantly in cancer cells, with differences depending on the cancer type. For instance, HDAC1 is found in high levels in cancers, such as prostate, stomach, lung, esophageal, colon cancers and BC. HDAC2 is more prevalent in colorectal, cervical and stomach cancers. HDAC6, HDAC8 and HDAV11 are overexpressed in breast tumors, neuroblastoma and rhabdomyosarcoma, respectively and can be used as biomarkers (45,46). These increased levels of HDACs in different cancers are caused by various factors, which may influence how effective HDAC inhibitors are in the treatment of these cancers.

HDACs expression in Pca. The regulation of ARs can be mediated at the epigenetic level. Therefore, HDAC inhibitors are also considered as therapeutic options for the treatment of mCRPC. HDACs have been shown to be upregulated in Pca. For instance, Wang *et al* (47) compared HDAC expression in primary Pca (42 cases with undetectable PSA levels), recurrent Pca (n=37), metastatic tumors (n=8) and benign prostatic tissue (n=11, did not show cancer under a microscope) using DNA microarray analysis. The expression of HDAC1, 3, 4 and 5 was significantly enhanced in benign and malignant human

prostate tissue and various PCa cell lines (47). In another study conducted on 192 patients who underwent radical prostatectomy, it was shown that HDAC1 (69.8%), 2 (74%) and 3 (94.8%) levels were significantly enhanced in the majority of cases and were found to be associated with dedifferentiation and tumor cell proliferation. Furthermore, the high expression of HDAC2 has been shown to be associated with a shorter PSA relapse time; this suggests that HDAC2 is linked to more aggressive tumor behavior and faster disease recurrence following surgery (48). The increased expression of HDAC1, HDAC2 and HDAC3 in Pca, as observed in a previous study on 192 patients, is unfavorable. Weichert *et al* (48) investigated the expression patterns of HDACs 1, 2 and 3 in a cohort of 192 patients with Pca who had undergone radical prostatectomy. Their findings revealed that HDACs 1 and 2 were positively associated with Gleason scores, with higher expression levels in high-grade tumors. Additionally, the expression of HDAC1, HDAC2 and HDAC3 was significantly associated with a positive proliferative fraction of Ki-67 in Pca cells. The probability of post-operative 7-year disease-free survival (DFS) was lower in the HDAC1 high and HDAC2 high groups than in the HDAC1 low and HDAC2 low groups, respectively (HDAC1: median DFS probability, 0.6 vs. 0.8 and in the HDAC2 high vs. HDAC2 low group) (48). The inhibition of class I HDACs mediates the re-expression of maspin, which suppresses the proliferation and migration of Pca cells (LNCaP and DU145) (49). In prostate tissues, HDAC1 is exclusively expressed in the cell nucleus and its expression is usually lower in stromal cells. Unexpectedly, HDAC8 was not detected in epithelial cells, but was uniquely expressed in the cytoplasm of stromal cells. Taken together, these findings indicate that epithelial and stromal cells exhibit distinct class I HDAC expression profiles (50). In Pca, HDACs, particularly HDAC1, HDAC2 and HDAC3, play a crucial role in driving disease progression by modifying chromatin structure and regulating gene expression to promote tumor growth, survival and metastasis (51). These changes can also lead to resistance to therapies, such as ADT and chemotherapy. The differential expression of HDACs may provide new insight for targeted therapies, such as isoform specific HDAC inhibitors, which may help overcome resistance, reduce tumor aggressiveness and improve patient outcomes by selectively modulating the epigenetic landscape. Of note however, only a limited number of studies have been conducted to determine the expression of HDACs in Pca tissues and their association with OS or PFS (47,52). However, the majority of studies have focused on reducing the uncontrolled growth of Pca cells using various HDAC inhibitors or other natural compounds. These inhibitors or compounds mediate their antitumor effects by inhibiting the expression of HDACs (53). For example, rosmarinic acid, a phenolic component of rosemary tea, induces cell cycle arrest and apoptosis by modulating HDAC2 expression in Pca cell lines (54). Similarly, apigenin inhibits HDAC and remodels chromatin to induce growth arrest and apoptosis in prostate cancer cells (55).

HDAC inhibitors and challenges. Class I, II and IV HDACs require zinc ions for their catalytic activities. Therefore, HDAC inhibitors targeting these classes of HDACs possess a zinc-binding group (ZBG). ZBGs are mainly of two types: i) Classic ZBGs, such as carboxylates, hydroxamic acids, benzamides and thiols; and ii) novel ZBGs, such as tropolone derivatives, 3-hydroxypyridin-2-thione, chelidamic derivatives, and benzoyl hydrazide scaffolds (56). Classic ZBGs are characterized by high activity, selectivity, off-target effects, potential toxicities, and instability *in vivo*. It is well known that SAHA-containing hydroxamic acid acts as a pan-HDAC inhibitor with competitive binding and fast on/off profiling. Substituting the hydroxamic acid group in SAHA with benzamide results in a class I-selective HDAC inhibitor with a fast-on/fast-off profile and competitive binding mode for HDAC1, HDAC2 and HDAC3. This differs from the properties of other benzamide inhibitors, such as MS275 and 106, described in previous studies (57). These findings indicate that the kinetic profile of benzamide-based HDAC inhibitors is influenced not only by the ZBG, but also by the cap and linker regions. Hydroxamic acid and benzamide are the most frequently utilized ZBGs among HDAC inhibitors. Hydrazide-based HDAC inhibitors show slow-binding class I selective (HDAC3) activity in competitive and non-competitive modes. The kinetic profiles of these inhibitors are not dependent on the cap or linker groups (57). HDAC inhibitors with hydrazide motifs are not dependent on zinc-binding interactions, and thus exert fewer off-target effects (56). These findings suggest that designing a variety of ZBGs can overcome the limitations of the currently available HDAC inhibitors, such as off-target effects and toxicity. HDAC inhibitors can be isoform-specific or pan inhibitors. Pan inhibitors can be used for all types of HDACs. These inhibitors are mainly divided into five classes: i) Hydroxamates, (Practinostat approved by the FDA against Pca); ii) short chain fatty acids; iii) benzamides; iv) cyclic tetrapeptides; and v) sirtuin inhibitors, including the pan-inhibitor nicotinamide and the specific SIRT1 and SIRT2 inhibitors sirtinol and cambinol, respectively. HDAC inhibitors induce various effects on cancer cells, including cell cycle arrest, stem cell renewal, differentiation and apoptosis. Additionally, they decrease angiogenesis and modulate the immune response. Several HDAC inhibitors also affect the activity of cytochrome P450 (CYP) enzymes, potentially enhancing the effects of certain anticancer drugs in combination with HDAC inhibitors (45). Several dual or multimodal HDAC inhibitors have been investigated; however, practinostat has gained FDA approval for Pca treatment. Developing newer and more specific HDAC inhibitors could provide advantages in managing drug-resistant CRPC, where earlier HDAC inhibitors have shown limited efficacy. For instance, compound 13, a class II HDAC inhibitor from the trifluoromethyl oxadiazole and hydroxamic acid series, exhibits favorable drug-like characteristics, potent anti-proliferative effects, and significant anti-migratoru activity in Pca cells (58). These advancements may improve treatment options for patients with CRPC.

HDAC inhibitors in Pca. Currently, 13 clinical trials have been reported on clinicaltrials.gov for the treatment of metastatic prostate cancer using HDAC inhibitors. Of these 13 clinical trials, seven were completed, two trials are recruiting patients, three trials were terminated and one was not recruiting any patients. Of the seven completed clinical trials, three trials were completed before 2010 and did not publish the results on Pca (NCT00045006, NCT00005634 and NCT00020579).

The results of the other four trials were not promising. For instance, a phase II study (NCT00106418) on 35 patients with metastatic CRPC revealed a partial response to romidepsin (class I) in 2 patients (DoR, >6 months; PSA level decline of >50%). A total of 11 patients discontinued the treatment due to of toxicity. The clinical trial NCT01075308 was conducted on 32 patients with CRPC using SB939 (class I, II and IV HDACs), which is an inhibitor of HDAC. SB939 did not exhibit sufficient activity based on the PSA response rate (2 patients responded, 7 patients had stable disease at 1.6-8 months) (59). Similarly, a phase II trial of intravenous panobinostat (NCT00667862) demonstrated a decrease in PSA levels in only 14% of patients with metastatic hormone refractory Pca (n=35); however, the decrease was not >50%. The major adverse effects observed were fatigue and thrombocytopenia. Furthermore, in a phase II clinical trial (NCT00330161), 27 patients with progressive metastatic Pca received vorinostat 400 mg (class I, II) daily continuously, and all patients had previously received chemotherapy. Of the 27 patients, 11 discontinued therapy due to toxicity and 13 patients had progressive disease. Stable disease was observed in 2 patients (7%), which was the optimal objective response in that trial. Data analysis indicated that elevated levels of IL-6 contributed to drug failure in patients with progressive CRPC. A >50% decline in PSMA levels was not observed in any patient. These clinical trials exhibit differences in the type of HDAC inhibitors used, the number and type of patients, and the outcome of the trials. They also suggest that PAN inhibitors are comparatively and slightly more effective than inhibitors of individual classes. Overall, the results of clinical trials suggest that HDAC inhibitors such as practinostat (SB939), panobinostat, romidepsin and vorinostat are ineffective against metastatic Pca as a monotherapeutic agents. Relatively insufficient responses have been observed in patients with mCRPC, which explains why no phase 3 studies for these drugs in patients with CRPC have been completed to date; therefore, combinatorial treatment therapies are ongoing. For example, a phase 2 trial involving 55 patients with CRPC treated with oral panobinostat and bicalutamide demonstrated improved radiographic progression-free (rPF) survival in those who had developed resistance to second-line antiandrogen therapy (60). However, adverse events were higher with a high dose of panobinostat (40 mg) (27.5%) than at a low dose of 20 mg (11.5%). These events require low-dose panobinostat for mCRPC. Notably, a low dose of the drug also reduced the PSA response from 24 weeks to 5.9 weeks (60). These results suggest that panobinostat effectively overcomes bicalutamide-induced androgen resistance in a significant number of patients with CRPC. Additionally, panobinostat is regarded to be less toxic to patients compared to other HDAC inhibitors, such as romidepsin and vorinostat (61). Similarly, trials on talazoparib in combination with belinostat against mCRPC are ongoing to observe dose-limiting toxicities in the first 10 weeks or two cycles of treatment (NCT04703920). The study will be completed in December, 2024. Overall, the results suggest that grade 3 and 4 toxicities are the main reasons for discontinuing treatment with HDAC inhibitors, either alone or in combination therapies. While HDAC inhibitors are approved for certain hematologic malignancies, their role in prostate cancer remains less well-defined.

Future research directions. A number of studies have suggested the significance of HDAC inhibition using various drugs, suggesting that less toxic HDAC inhibitors need to be developed. For instance, in a previous study, the adamantyl-capped HDAC inhibitor, CN133, was shown to have low IC50 values against HDAC1 (IC50, 0.6 nM), 2 (IC50, 2 nM) and 3 (IC50, 0.3 nM) in comparison to vorinostat (IC50, 4, 11 and 3 nM) (62). Vorinostat was more effective against HDAC6 (IC50, 2 nM) than CN133 (IC50, 4.1 nM). CN133 suppressed the proliferation, migration and invasion of 22Rv1 CRPC cells more effectively than vorinostat. CN133 also suppressed AR signaling (62). Tumor volume and weight in CN133 treated mice with tumor xenografts were also effectively reduced by 50% in comparison with the placebo group. They also developed a PET/CT imaging method to directly observe the therapeutic effects of HDAC inhibitors (62). These results suggest the following: i) Every HDAC inhibitor is not effective against all HDACs, and therefore, it is essential to first characterize HDACs inhibitors against all HDACs at the preclinical level; and ii) to then use them precisely after obtaining biomarker panel reports of patients. PAN inhibitors targeting all HDAC inhibitors may not be effective, but may rather have toxic effects.

Hybrid molecules of enzalutamide and SAHA were designed to have weak pan-anti-HDAC activity to target HSP90 and AR in enzalutamide-resistant PCa cells. The hybrid molecule was named 2-75. In comparison to enzalutamide, the hybrid molecule induced the degradation of both AR and HSP90 with more potency than SAHA in Pca cells by preventing its translocation to the nucleus. The cytoplasmic retention property allows this inhibitor to selectively inhibit HDAC6 and has a limited impact on other HDACs. In addition to targeting the AR, 2-75 also downregulated the expression of AR-V7, suggesting enhanced AR degradation in 2-75-treated cells (63). *In vivo* studies demonstrated that compared to enzalutamide, 2-75 significantly reduced tumor growth, increased apoptosis, and inhibited AR nuclear accumulation in LNCaP tumor xenograft models. These studies suggest that 2-75 can selectively inhibit HDAC6 and overcome the drug resistance and toxicity properties associated with classical AR antagonists, HDAC inhibitors, and Hsp90 inhibitors (63,64). Similarly, a CUDC-101 hybrid molecule with a HDAC inhibitory fragment and an EGFR inhibitory scaffold from erlotinib (an EGFR inhibitor) was designed. It suppressed AR, AR-V7 and HER2 expression, and upregulated p21 expression. It also significantly reduced tumor growth in xenograft mice model having aggressive 22Rv1. However, the ethinylphenyl residue of erlotinib can be oxidized by cytochrome P450 enzymes to phenol, potentially causing toxic effects. Similarly, CUDC-101 is a substrate for ABC transporters, which can contribute to drug resistance (65-67). The EGFR inhibitor, gefitinib, can reverse drug resistance to CUDC-101, leading to the development of chimeric HDAC inhibitors with gefitinib-derived cap scaffolds. The chimeric molecules 3ClQuin-SAHA and 3BrQuin-SAHA have been shown to inhibit HDAC, reduce EGFR expression similar to vorinostat and exhibit only marginal non-specific toxicity. They have also been shown to induce apoptosis and inhibit angiogenesis in DU145 cells (68). Therefore, this chimeric compound may be a more suitable candidate for anticancer therapy in Pca, as it aims to reduce

both toxicity and drug resistance. Additionally, CUDC-907 is a promising HDAC/kinase inhibitor that targets HDAC enzymes along with PI3K. Chimeric compound induced apoptosis in Pca by suppressing myc expression. Therefore, c-Myc expression should be measured before beginning any trials on patients with Pca. CUDC-907 was not found to be a substrate of ABCB1 transporters, but was a substrate for ABCB2 transporters; therefore, the drug could be combined with ABCB2 inhibitors in patients with Pca having altered myc status (69,70). These studies strongly suggest that more specific HDAC inhibitors should be developed and further characterized to reduce the toxicities associated with pan-HDAC inhibitors. Inhibitors should be tested as combinatorial treatments. It is suggested that with the development of bioinformatics tools (AdmetSAR, Osiris, molecular docking, MD simulation, MM-GBSA, C-ImmSim, etc.), it would be beneficial to design and screen multiple drugs at the bioinformatics level before conducting *in vitro* or *in vivo* experiments. Certain phytochemical drugs (caffeic acid, gallic acid, quercetin, apigenin, luteolin, resveratrol, grape seed, curcumin, etc.) also have anti-HDAC activity and, therefore, should be further characterized due to their low toxicity profile (71). The active ingredients of these phytochemicals need to be isolated and characterized to achieve improved outcomes and low IC-50 values.

4. Prostate-specific membrane antigen

PSMA is encoded by the folate hydrolase 1 (*FOLH1*) gene and has three domains: i) An extracellular domain of 707 amino acids; ii) a transmembrane domain of 24 amino acids; and iii) an intracellular cytoplasmic domain of 19 amino acids. The intracellular domain contains a motif (MWNLL) responsible for the internalization of bound PSMA via clathrin-coated pits. The deletion of this motif significantly reduced PSMA internalization. Therefore, the fusion of drugs to these 5 amino acids will result in drug-bound PSMA internalization and, thus, the presence of concentrated drug within tumor cells. FOLH1 is located on the short arm of chromosome 11. In addition to its expression in benign and malignant prostate epithelium, it is also expressed in a variety of other tissues, such as the proximal renal tubules, salivary glands, liver, esophagus, stomach, small intestine, colon, breast, fallopian tubes, testicular seminiferous tubules, hippocampal neurons and astrocytes, ependyma, cortex and medulla of the adrenal gland and ovarian stroma. The varying expression of PSMA across different types of cancer, such as lung, breast, ovarian, renal, glioblastoma and colon cancer, along with its relatively low levels in non-prostate malignancies, poses challenges for developing PSMA-targeted therapies beyond Pca (72). The amount of PSMA in cancer cells can vary depending on the type of cancer; it sometimes it can be higher, lower, or may remain the same. As PSMA is found in several types of cancer, it has become a promising target for novel cancer treatments. Scientists are developing treatments, such as specific antibodies, radioactive agents, or immune-based therapies that specifically target PSMA. These treatments are being tested in clinical trials to help attack cancer cells with PSMA, while causing less harm to healthy cells (73). Of note, >70% of primary tumors exhibited PSMA expression on the new blood vessels associated with the tumors. It was shown that medullary thyroid carcinomas and hepatocellular carcinomas were the most likely to express PSMA in their neovasculature. On the other hand, adenoid cystic carcinoma and papillary renal cell carcinoma showed PSMA expression on the neovasculature in only a few cases. Notably, while the majority of solid cancers do not exhibit PSMA expression on tumor cells, it has been observed in salivary gland tumors and, to a lesser extent, in tissues of hepatocellular carcinoma, lung cancer and BC (73). In summary, while PSMA is most well-known for its role in Pca, it is also expressed in several other types of cancer, and its presence is being studied for its potential as both a diagnostic biomarker and a therapeutic target in those cancers. In the case of Pca, PSMA is detected at a modest level, but demonstrates a 100-1,000-fold increased expression in prostate adenocarcinoma when compared to normal prostate tissues. It is positively associated with various measures of tumor aggressiveness, such as Gleason grade, tumor stage, biochemical recurrence and castration resistance. Therefore, higher levels of PSMA are associated with the worse clinical outcomes (74). PSMA is an attractive target for theragnostic purposes for several reasons, such as a selective high expression in Pca with a limited expression in benign prostate tissue, targeting of the extracellular domain by antibodies or small-molecule ligands, and the internalization of drug-bound PSMA by the specific motif present in the cytoplasmic domain. The physiological role of PSMA is to support glutamate synthesis in the brain and folate absorption in the intestines. However, its physiological role in Pca cells remains poorly understood. With the rapid advancement of PSMA-targeted therapies and imaging agents, these are currently used in prostate cancer screening, risk stratification for recurrence and ongoing therapy monitoring. Thus, it is essential to clarify the regulation and function of PSMA in Pca to enhance the precision and maximize the benefits of PSMA-targeted therapies.

PSMA as a therapeutic target and challenges

Clinical relevance. PSMA is used as a therapeutic antigenic target for antibodies and small molecule inhibitors. When combined with radionuclides, these molecules can be used in the treatment of mCRPC (Fig. 2). The radionuclides that have been used in clinical trials of mCRPC are β-particle emitters (^{177}Lu, ^{90}Y) and α-particle emitters ^{225}Ac and ^{227}Th. However, the majority of trials have used β-particle emitters linked to PSMA domains either alone or in combination with chemotherapies. Alpha particles possess significantly higher energy, meaning that they require fewer hits to kill cancer cells compared to β-emitters. The use of alpha particles may be particularly effective for treating micrometastatic disease, as a single alpha particle in close proximity to a microdeposit of prostate cancer could be sufficient to destroy cancer cells. By contrast, the amount of drug delivered to a specific metastatic site depends on the density of PSMA coverage (PSMA expression level in one cancer cell and the number of cancer cells expressing PSMA) (Fig. 2). Given the physical properties of alpha particles, an alpha emitter specifically targeting prostate cancer cells is likely to be the most effective treatment for micrometastatic disease. However, the optimal approach could be to combine different emitters with various targets, including PSMA.

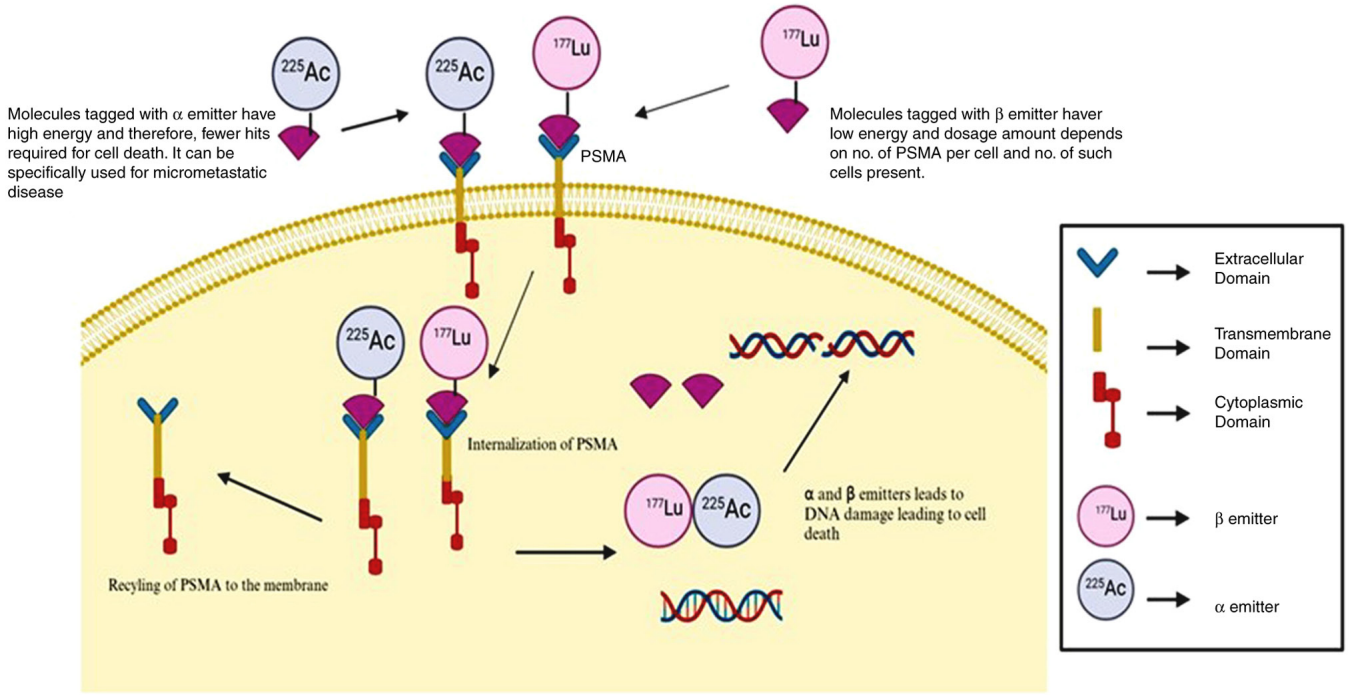

Figure 2. PSMA-targeted radiation therapy: The α and β emitter molecules are tagged with PSMA ligands, resulting in the internalization of PSMA along with the ligand tagged with α and β emitter molecules. These α- and β-emitter molecules induce double-stranded breaks in the DNA, leading to cell death. PSMA, prostate-specific membrane antigen.

Clinical trials and challenges. Combinations of α- and β-emitting agents have been developed. Clinical trials for combination therapy with the α-ray-emitting agents ^{223}Ra and ^{177}Lu-PSMA-I&T (NCT05383079, AlphaBet), 225Ac-J591 antibody, and 177Lu-PSMA-I&T (NCT04886986) are underway. In the case of Pca, there are currently no clinical trials that compare alpha and β emitters that have been linked to targeted molecules. However, in the case of colorectal carcinoma, ^{213}Bi-IMP288 (alpha emitter) was feasible and at least as effective as ^{177}Lu-IMP288 (beta emitter). IMP288 is an anti-histamine-succinyl-glycine hapten peptide. The survival rate was comparatively higher with a low dose of alpha emitters (45 days) than with the β emitter (42 days). However, higher doses of alpha emitters lead to kidney toxicity and require dose optimization (75). Clinical trial studies have utilized alpha- or β-emitting radionuclides, such as NCT03545165, NCT03805594,NCT03658447,NCT04343885,NCT03874884, NCT04419402, NCT00195039 and NCT00538668 for ^{177}Lu, NCT03276572, and NCT03724747 for alpha emitters. The results of these trials have been discussed elsewhere (76) and are not included in the present review. Overall, it was shown that β-radionucleotides or PSMA-targeted monoclonal antibodies (^{177}Lu-PSMA-617) can reduce PSMA levels by ≥50% in 59-66% of mCRPC. For ^{225}Ac-PSMA-I&T, PSA50 was observed in 50% (7/14) of patients (77). These patients received prior AR treatment. The results of 225Ac-PSMA-617 in chemotherapy-naive patients with advanced-stage Pca were more promising (78). PSA90 was observed in 14/17 (82%) patients. Of note, 1 patient developed grade 4 renal toxicity. The notable therapeutic efficacy reported in that study could be achieved with reduced toxicity to salivary glands due to de-escalation of administered activities in subsequent treatment cycles (78). Efforts have been made to reduce these effects by using several approaches, such as conjugation with an antibody to reduce salivary gland distribution (may have less exposure to tubules), dose fractionation, dose titration, and salivary gland protective measures (76), although their effectiveness remains unclear. For instance, clinical trials on alpha emitter-based therapy have been conducted using PSMA-targeted antibodies. Owing to their larger size, antibodies require a longer circulation time. In a previous study, 32 patients who were refractory to or who refused standard treatment options (including androgen receptor pathway inhibitors) and had received or been deemed ineligible for taxane chemotherapy were included. A total of 46.9% had at least 50% PSA decline at any time, with the majority of patients demonstrating hematology-based high-grade adverse events (79). Further trials are required on different types of alpha emitters (such as ^{213}Bi), and head-to-head comparisons of alpha and beta emitters (specifically^{177} Lu-PSMA-617, and 177Lu-J591 or ^{90}Y-J591) are also required.

Likewise, modifications to the PSMA-binding domains can affect the pharmacokinetic and pharmacodynamic properties, thereby influencing both antitumor activity and toxicity profile. For instance, a ^{177}Lu-labeled PSMA ligand [DOTAGA-(I-y) fk(Sub-KuE or PSMA-I&T)] leads to a PSA decline of >50% with no clinically significant hematological toxicity, nephrotoxicity, thrombocytopenia, or xerostomia. However, similar to studies involving ^{177}Lu-PSMA-617, the most frequently reported adverse events were grade 1-2 anemia, leukopenia, and transient xerostomia (80).

PSMA-directed immunotherapy can involve various approaches: i) The creation of artificial T-cell receptors containing PSMA-specific monoclonal antibodies;

and ii) the creation of bispecific T-cell engagers (BiTEs), such as targeting both PSMA and CD3 T-cell receptors to induce T-cell activation. These agents include AMG160, pasotuxizumab/AMG212/BAY2010112 (NCT03792841 and NCT01723475); iii) the infusion of dendritic cells with PSMA peptide; iv) recombinant soluble PSMA protein as a vaccine; v) targeting pathways that crosstalk with PSMA, such as the PI3K/Akt/mTOR pathway; vi) trispecific T-cell-activating constructs, such as HPN424, which includes a PSMA-targeting domain, a CD3-targeting domain, and a third domain that noncovalently binds to serum albumin to extend its half-life, is currently in phase 1 clinical development (NCT03577028).

Of note, eight studies have been reported on clinicaltrials.gov and are currently working on PSMA-directed immunotherapies. Of these eight studies, four studies have been completed, one is in an inactive state, one is withdrawn, and the remaining two are currently recruiting patients. The first completed study was a phase I trial of 177Lu-PSMA-617 and pembrolizumab in patients with mCRPC. In this trial, 37 patients were selected based on their high PSMA expression levels. These patients received prior treatment with docetaxel and an androgen receptor-targeted agent; PSA50-RR was 76%. The 12-month rPFS and OS rates were 38 and 83%, respectively. Common treatment-related adverse events were mainly grade (G) 1-2, including xerostomia, fatigue, pruritus, nausea, rash, diarrhea, anorexia, thrombocytopenia, anemia, neutropenia, elevated ALT, arthralgia and a flare in bone pain. Five patients (14%) discontinued pembrolizumab because of toxicity (81). The second completed study included 133 patients with mCRPC refractory to AR pathway inhibitor therapy and taxane-based chemotherapy received targeted acapatamab. Acapatamab (AMG160) is a prostate-specific membrane antigen (PSMA) x CD3 bispecific T-cell engager. PSA50 was observed in 30.4% of patients, and radiographic partial responses in 7.4% with a median PSA PFS of 3.3 months. The results of that study did not mention anything regarding the toxicity profile (82). The third completed study included 31 and 9 mCRPC patients were treated with subcutaneous (SC) or intravenous injections of pasotuxizumab, respectively. PSA50 was observed in 28 and 9.6% of the patients in the SC and continuous intravenous cohorts, respectively. All SC cohort patients developed anti-drug antibodies; Therefore, continuous intravenous injections were administered. The change in the sponsor terminated the continuous intravenous study (83). The completed study 4 was an open-label, phase 1/2a study of HPN424 as a monotherapy aimed to evaluate the safety, tolerability, and pharmacokinetic profile of drugs in patients with advanced-stage Pa who were refractory to androgen therapy. In comparison to other bispecific platforms, HPN424 was optimized for its small size and increased stability. That study involved 80 patients who received targeted doses ranging from 1.3 to 160 ng/kg as a fixed dose and up to 300 ng/kg with step dosing following an initial priming dose. The most frequently observed grade >3 treatment-emergent adverse events were increased AST levels, anemia and ALT levels. Dose-limiting toxicities included grade 3 cytokine release syndrome, elevated lipase levels and seizures. However, these events did not hinder the dose escalation. A total of 63 patients continued the treatment for >24 weeks; 3 of these 63 patients had PSA50 responses. That study was terminated early (84). These studies vary significantly in several aspects, including the type of therapy, patient population size, prior treatments received and PSA50 responses, making direct outcome comparisons challenging.

As regards the recruiting studies, the first recruiting study evaluated the safety and tolerability of AMG 509 in adult mCRPC participants (NCT04221542). The second recruiting study was a phase 1 trial of 177Lu-PSMA-617 and olaparib in patients with mCRPC. In that study, 48 patients were recruited and received prior treatment with docetaxel and an androgen receptor pathway inhibitor. The PSA50-RR was 62% (18/29) and PSA90-RR was 48% (14/29). Of note, 5 of the 7 patients (71%) with measurable RECIST disease exhibited a partial response. Common treatment-related adverse events included xerostomia, anemia, thrombocytopenia, neutropenia, nausea, fatigue, constipation, anorexia, vomiting and diarrhea. These toxicities were transient and without clinical sequelae (85).

PSMA-directed immunotherapies. The results of PSMA-directed immunotherapies were not as significant as those of radionucleotide-based therapies. For instance, PSA50 is in the range of 59-66% in the case of radionucleotide-based therapies (76), while for PSMA-directed immunotherapies, it is 28-30.4%. The 62-76% response was achieved when 177Lu-PSMA-617 was combined with pembrolizumab or olaparib. Additionally, adverse outcomes were not mentioned in the majority of studies. Toxicities were manageable in the trispecific T-cell-activating construct (HPN424). However, PSA50 was only observed in 3 out of 63 patients. These patients had already received two prior systemic therapies. However, a description of these therapies has not yet been provided. PSMA-directed immunotherapies indirectly target prostate tumor cells, which can also affect nearby cells that have low levels of PSMA. Even a small amount of PSMA can activate T-cell-mediated immunotherapies. Therefore, clinical trials have not selected patients based on PSMA expression levels.

Future research directions. i) Alpha and beta emitters have different properties; therefore, head-to-head comparisons of these emitters would be beneficial in the treatment of patients with mCRPC. Therefore, additional alpha and beta emitters should be characterized for mCRPC. Alpha emitters will require lower doses. In the case of lutetium PSMA, the primary sites of overexpression in healthy tissues include the salivary and lacrimal glands, which account for the symptoms of dry eyes and mouth. Due to its ability to target cancer cells directly, the lutetium has the potential to induce cytotoxic effects in various tissues. Therefore, combinatorial therapies, including both alpha and beta emitters with dose optimization or fractionation, may benefit mCRPC. A small number of trials are underway, and the results will shed light on this issue. The PSMA-targeted alpha emitter (^{225}Ac) has shown promising results in chemotherapy-naïve mCRPC patients (n=17). The results suggest that alpha emitters could be more effective in treating chemotherapy-naïve patients, and thus, more recruitment of patients is required at the global level. ii) Additionally, linking these radionuclides with different PSMA domains should also be attempted at preclinical and clinical levels. iii) The results of PSMA-directed immunotherapies suggest that they should be combined with other therapies such as AR blockade, PARP inhibitors, and other

cross-talk chemotherapies. iv) Patients with bone metastases should receive anti-resorptive therapy. A balance between toxicity and efficacy is a requirement for clinical studies. Patients should be divided into two cohorts: a) They have not received prior treatments; and b) have received prior standard treatments. This should be the minimal requirement, as radionuclide therapy shows promising results in naïve patients. Therefore, a collaborative approach should be used to include a greater number of patients. The collaborative approach will provide broad spectrum and early outcome of the studies.

5. Immune checkpoint proteins

Cytotoxic T-lymphocyte associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1) are among the most widely studied immune checkpoints. CTLA-4 functions early in the immune response, whereas PD-1 is expressed later on mature T-effector cells in peripheral tissues, including tumors. PD-1 binds to its ligands, PD-L1 and PD-L2, inhibiting T cell-mediated tumor cell killing. Blocking PD-1/PD-L1 interaction enhances the ability of T-cells to destroy tumor cells. T-cell activation occurs when CD28 on T-cells interacts with CD80 and CD86 on antigen-presenting cells. Once activated, T-cells begin to express CTLA-4, which binds to CD80 and CD86 with greater affinity than CD28. This competition between CD28 and CTLA-4 exerts an inhibitory effect on T-cell activation, helping to prevent over-activation and autoimmunity (Fig. 3). Consequently, various immune checkpoint inhibitor therapies targeting the PD-1/PD-L1 and CTLA-4 interactions have been developed for cancer. The role of ICIs is generally to suppress the immune response, but how it affects cancer outcomes is not straightforward. For example, higher levels of CTLA-4 and PD-1 in tumor samples have been linked to worse survival in nasopharyngeal, liver cancer, but interestingly, better survival in non-small cell lung cancer (86-88). In summary, both CTLA-4 and PD-1 can suppress the immune system in the tumor environment; however, their roles and effects can vary depending on the type of cancer and the cells they are acting on.

Clinical trials. Immune checkpoint inhibitors enhance the life expectancy of patients with several solid tumors. However, CTLA-4 and PD-1 as monotherapy or in combination therapy achieve only limited benefits in terms of biochemical and radiological responses in advanced-stage Pca. For instance, in monotherapies, overall survival ranges between 7.9-28.7 months while for combinatorial therapies, it ranges between 8.5-23 months. However, it is very difficult to compare clinical trials due to the different characteristics of the patients, different drugs used, number of patients and different primary endpoints. Overall, the results suggest that patients with mCRPC should be treated after evaluating certain parameters and biomarkers. For example, it was previously shown that patients with mCRPC with alkaline phosphatase concentration <1.5 ULN, a hemoglobin concentration ≥110 g/l and no visceral metastases (n=146) had a significantly higher mOS in the treatment group (22.7 months) when compared with placebo (15.8 months) (P=0.0038) (89). Similarly, another study demonstrated that patients with high a expression of PD-L1 IC2/3, CD8, CXCL9 and TAP1 experienced a longer PFS in the atezolizumab and enzalutamide arm. This was also observed alongside other potentially relevant biomarkers, including alterations in phosphatase and tensin homolog (PTEN) (90). In another study, the ORR was higher in chemotherapy-naïve patients treated with nivolumab plus ipilimumab with PD-L1 ≥1%, homologous recombination deficiency, or above-median tumor mutational burden (91). All three studies (89-91) included patients with different characteristics who were treated with different drugs (Table I) (89-100). In a single arm, phase II trial, it was shown that the efficacy of ICIs, including PD-1 and CTLA-4 inhibitors, varied across subgroups of patients with Pca on the basis of mismatch repair deficiency (dMMR), non-synonymous tumor mutational burden (hTMB), a BRCA2 mutation (BRCAm), or biallelic CDK12 inactivation (CDK12i). It was shown that dual ICIs exhibited modest responses in the hTMB, BRCAm,and CDK12i subgroups, but demonstrated exceptional efficacy in dMMR (101). Thus, careful patient selection based on biomarkers may be essential for the effective use of immune checkpoint inhibitors, helping to identify specific subgroups of patients who are likely to benefit from these treatment strategies. Heterogeneity in Pca encompasses genetic, phenotypic and microenvironmental variations, posing challenges to treatment and contributing to resistance. Addressing this complexity is crucial for advancing precision therapies, enhancing patient outcomes, and overcoming resistance in advanced stages of the disease. For example, significant heterogeneity was observed in the ipilimumab treated group. No significant heterogeneity was found in the pembrolizumab and nivolumab plus ipilimumab subgroups (102). Aligning patients with the most suitable treatment is essential, especially in overcoming the challenges of inter- and intra-patient heterogeneity and tumor evolution. Advanced tools, such as imaging technologies, next-generation sequencing, circulating tumor DNA, CTCs and artificial intelligence provide promising avenues to enhance patient selection and optimize treatment outcomes.

Vaccines or cryotherapy, in combination with immunotherapies, have shown promising results (Table I) (103,104). However, the number of patients included in these studies was small; therefore, the inclusion of more patients in subsequent trials is necessary. BiTEs are designed to bind to both T-cells and tumor cells, thereby facilitating tumor cell destruction by activated T-cells. One such BiTE, AMG 160, targets PSMA on tumor cells and CD3 on T-cells. The role of AMG-160 has been studied in combination with the PD-1 monoclonal antibody AMG 404 (NCT04631601) and with pembrolizumab (NCT03792841). The NCT04631601 trial has been terminated, and trial NCT03792841 has only been completed for a monotherapy arm that involves 43 PSMA-positive patients. In a trial involving 43 PSMA-positive patients, 27.6% had a confirmed PSA response, 13.3% achieved a confirmed partial response (PR), and 53.3% experienced stable disease (42). Additionally, XmAb®22841 is a bispecific antibody that targets both CTLA-4 and LAG-3, aiming to enhance tumor-selective T cell activation to improve therapeutic outcomes. This clinical trial was designed to assess the maximum tolerated dose and/or recommended dose of XmAb22841, both as a standalone treatment and in combination with pembrolizumab. The study will focus on evaluating the safety, tolerability, pharmacokinetics, immunogenicity, and antitumor activity in patients with selected

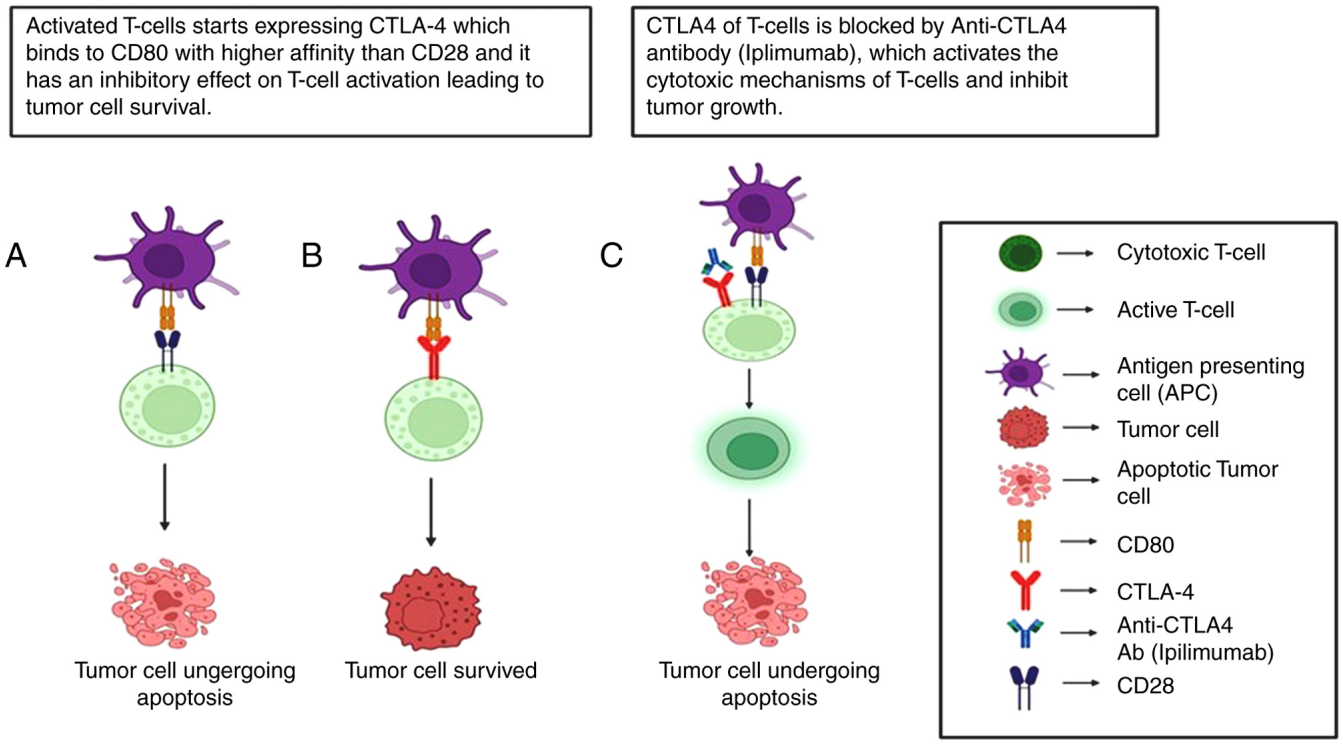

Figure 3. (A) The interaction of CD28 on T-cells and CD80 on APCs results in the activation of T-cells, leading to apoptosis of tumor cells. (B) Activated T-cells begin expressing CTLA-4, which has a higher affinity for CD80 than for CD28, thus inducing tumor cell survival by inhibiting T-cell activation. (C) The binding of anti CTLA-4 antibody to CTLA-4 on T-cells removes the blockage between CD80 and CD28, which facilitates cytotoxic T-cell activation. These cytotoxic T cells induce apoptosis in tumor cells. CTLA-4, cytotoxic T-lymphocyte associated protein 4.

advanced solid tumors (NCT03849469). To the best of our knowledge, the results of that study have not been reported.

Overall, immune checkpoint inhibitor monotherapy for Pca has not demonstrated a significant survival benefit. The possible reasons could be innate resistance, the activation of immune suppressive mechanisms by tumor cells, the response by both PD-1-positive or -negative patients (105,106), and the lack of characterization of tumor-infiltrating lymphocytes (TILs). For instance, $CD8^+$ TILs can suppress tumor growth in early-stage Pca. However, the presence of both $CD8^+$ TILs and PD-L1 in node-positive PCa cases has been linked to disease progression (107-109). However, the results remain controversial. Similarly, immune checkpoint inhibitor therapeutic responses in bone metastases were lower than those in soft-tissue metastases due to TGF-β-mediated signaling that favored Th17 and restrained Th1 lineage expansion. As patients with mCRPC have bone metastasis, the optimization of immunotherapies is warranted (110). Despite the FDA approval of pembrolizumab for microsatellite-high tumors, the response rate of microsatellite-high prostate cancer patients to anti-PD-1 therapy has not been well documented (111).

Future research directions. i) The efficacy of immunotherapy can be enhanced by selecting patients on the basis of biomarker panels, such as mutations in *BRCA1* and/or *BRCA2, ATM, and CHEK2*. ii) The subgrouping of patients should be mandatory in every clinical trial to provide high benefits to patients with mCRPC. iii) Additionally, patient selection should be performed not only on the basis of one biomarker, but also on the basis of treatment-associated biomarker panels. For example, both PD-1-positive and -negative patients may benefit from treatment, as shown in previous studies (105,106). Therefore, it would be beneficial to perform sequencing-based experiments at a preclinical level. iv) The differentiation of therapy-resistant patients from therapy-sensitive patients may prevent the side-effects of therapies. v) Combined therapies including vaccines along with immune checkpoint inhibitors should be performed at a larger scale due to their beneficial effects on advanced-stage Pca. vi) Collaborative clinical trials should be conducted using the same drugs and similar types of patients. vii) Combined therapies involving cross-reactive targets should also be evaluated. viii) The evaluation of the vaccines with T-cell amplifiers and anti PD-1 therapy. Currently, one study is evaluating the effect of SLT-10 and GX-17 either alone or in combination with pembrolizumab in patients with mCRPC. SLT-10 was developed by VaxiGen and is a DNA vaccine, whereas GX-17 is a T-cell immunity amplifier by interleukin-7(IL-7) fused to hyFc (developed by Genexine) (NCT06344715).

6. PARP

PARP inhibitors have emerged as promising cancer therapies. Single-strand breaks (SSB) are repaired by PARP enzymes in the DNA. However, PARP inhibitors bind to PARP enzymes and prevent SSB repair, leading to the generation of double-strand breaks. Patients with mutations in homologous recombination repair genes such as *BRCA* are unable to repair these breaks, resulting in cell death through apoptosis. In addition to *BRCA*, other DNA repair genes, such as *ATM,*

Table I. Clinical trials data of immune checkpoint inhibitors in prostate cancer.

A, Monotherapy

Serial no.	Drug	Trial	No. of patients	Eligibility of patients	Outcome
1.	Bone-directed radiotherapy + ipilimumab 10 mg/kg	Phase III NCT00861614	399: T 400: PC	Bone metastasis from castration-resistant prostate cancer that had progressed after docetaxel treatment	Patients with alkaline phosphatase concentration (<1.5 ULN), haemoglobin concentration ≥110 g/l, and no visceral metastases (n=146) had significantly higher mOS in the treatment group (22.7 months) when compared with placebo (15.8 months); P=0·0038. Overall mOS, PSA reductions, and mPFS 11·2 months, 13.1, and 30.7% for ipilimumab and 10·0 months, 5.2, and 18.1% for the placebo
2.	Ipilimumab 10 mg/kg vs. placebo	Phase III NCT01057810	399: T 199: PC	-Asymptomatic or minimally symptomatic-Chemotherapy-naive -No visceralmetastases mCRPC	Treatment group mOS:28.7 months, mPFS: 5.6 months, PSA response rate: 23%; deaths: 9 (2%), immune-related grade 3 to 4 AEs: 31% Placebo arm mOS, 29.7 months; mPFS, 3.8 months; PSA response rate, 8%; deaths: 0 arm. Immune-related grade 3 to 4 AEs, 2%
3.	Pembrolizumab 10 mg/kg	Phase II NCT02054806	23	-Advanced prostate adenocarcinoma -unsuccessful standard therapy -measurabledisease (RECIST v1.1) -PD-L1 expression: ≥1% of tumor or stromal cells.	mOS, 7.9 months; mPFS, 3.5 months; DoR, 13.5 months; R, 4/23 patients; stable disease, 8/23; AEs, 14 (60.9%) patients with no pembrolizumab-related deaths or discontinuations occurred
4.	Pembrolizumab 200 mg	Phase II NCT02787005	258; 252 discontinued due to progression C1 group: 133 patients, PD-L1-positive C2 group: 67 patients, PD-L1-negative C3: bone-predominant disease, irrespective of PD-L1	Patients previously received ≥1 next generation hormonal agents and 1 or 2 chemotherapies, including docetaxel.	C1: Median follow up, 9.5 months; ORR, 6%; DCR, 11%; PSA response, 6%; mOS, 10 months; OS at 24 months, 22%; AEs, 57% C2: Median follow up, 7.9 months; ORR, 3%; DCR, 6%; PSA response, 8%; mOS. 8 months; OS at 24 months. 16%; AEs, 60% C3: Median follow up, 14.2 months; DCR, 21%; PSA response, 0%; mOS, 14 months; OS at 24 months, 21%; AEs, 71%

B, Combined therapy

Serial no.	Drug	Trial	No. of patients	Eligibility of patients	Outcome
1.	Pembrolizumab plus enzalutamide	II NCT02787005	n=126 C4: RECIST-measurable (n=81) C5: bone-predominant (n=45)	-Previously receive abiraterone and enzalutamide resistant	C4: mOS, not reached; rPFS rates, 17%; AEs, 75% 12-month OS rate: 70% C5: mOS, 19 months; rPFS rates, 23%; AEs, 69%; 12-month OS rate, 75%

Table I. Continued.

B, Combined therapy

Serial no.	Drug	Trial	No. of patients	Eligibility of patients	Outcome
1.	Pembrolizumab plus enzalutamide	II NCT02787005	n=126 C4: RECIST-measurable (n=81) C5: bone-predominant (n=45)	-Previously receive abiraterone and enzalutamide resistant	C4: mOS, not reached; rPFS rates, 17%; AEs, 75% 12-month OS rate: 70% C5: mOS, 19 months; rPFS rates, 23%; AEs, 69%; 12-month OS rate, 75%
2.	Pembrolizumab plus olaparib	Phase 1b/2 NCT02861573	n=84; 42 patients discontinued due to progression; 57% of patients had measurable disease	-Docetaxel-pre-treated, molecularly unselected patients with mCRPC with progression within 6 months of screening per PSA or radiologic bone/soft tissue progression enrolled.-may have received 1 other chemotherapy and ≤2 2nd-generation hormone therapy (HT)	OS: 14 months PSA response rate, 7/82; rPFS, 4 months; ORR in measurable disease (24/42 patients), 2/24
3.	Pembrolizumab plus enzalutamide	Phase 1b/2 NCT02861573	n=102; 73 patients discontinued due to progression 39% patients measurable disease	-Patients who failed or became intolerant to ≥4 weeks of abiraterone in prechemotherapy mCRPC state -whose disease progressed within 6 months of screening per PSA progression or radiologic bone or soft tissue progression enrolled	Median follow-up: 13 months OS: 20.4 months PSA response rate, 22/101; rPFS, 6.1 months; ORR in measurable disease, 3/25 (12%)
4.	Atezolizumab + enzalutamide vs. enzalutamide	Phase III NCT03016312	759	mCRPC whose disease progressed on abiraterone	-Longer progression-free survival was seen with atezolizumab arm in patients with high PD-L1 IC2/3, CD8 expression and established immune gene signatures. -Exploratory analysis linked progression-free survival in the atezolizumab arm with immune genes such as CXCL9 and TAP1, together with other potentially relevant biomarkers including phosphatase and tensin homolog alterations. -No role for atezolizumab incombination with enzalutamide in unselected patients with CRPC -Careful patient selection may be required for immune checkpoint inhibitors to identify subgroups of patients who may benefit from this treatment approach.

Table I. Continued.

B, Combined therapy

Serial no.	Drug	Trial	No. of patients	Eligibility of patients	Outcome
5.	Nivolumab (NIVO) plus ipilimumab	II NCT02985957	78	Cohort 1: Asymptomatic/minimally symptomatic patients who progressed after 2nd-generation hormone therapy and have not received chemotherapy for mCRPC Cohort 2: same as cohort 1 and patients who progressed after taxane-based chemotherapy	Cohort I: ORR, 26%; PSA, 21%; Cohort 2: ORR, 10%; PSA, 13% ORR was higher in patients with PD-L1 ≥1%, DNA damage repair (DDR), homologous recombination deficiency (HRD), or above-median tumor mutational burden.
6.	ADXS-PSA with pembrolizumab vs. ADXS-PSA	Phase I/II NCT02325557	n=50 mCRPC, ≥18 years who received ≤2 prior chemo-/targeted-/immunotherapies or ≤1 prior chemotherapy in a metastatic setting Part A (PA; n=13): ADXS-PSA every 3 weeks Part B (PB; n=37) ADXS-PSA, 200 mg pembrolizumab every 3 weeks with a 4th pembrolizumab dose 3 weeks later (in 12 weeks cycles), for up to 2 years or until progression/toxicity	mCRPC, ≥18 years who received ≤2 prior chemo-/targeted-/immunotherapies or ≤1 prior chemotherapy in a metastatic setting Part A (PA; n=13): ADXS-PSA every 3 weeks Part B (PB; n=37) ADXS-PSA, 200 mg pembrolizumab every 3 weeks with a 4th pembrolizumab dose 3 weeks later (in 12 weeks cycles), for up to 2 years or until progression/ toxicity	PA: PSA reduction, 14%; mOS, 8.5 months PB: PSA reduction, 43%; PSA 50, 7/15 patients mOS, 23 months ADXS-PSA + pembro reduced PSA ≥50% and prolonged OS
7.	DNA vaccine encoding prostatic acid phosphatase (PAP) + pembrolizumab	Pilot Trial	n=26	Group 1: receive vaccine and pembrolizumab together (n=13) Group 2: receive vaccine for 12 weeks and then pembrolizumab for 12 weeks (n=13)	Group 1: PSA decline, 62% Group 2: PSA decline, 8% PSA declines were associated with the development of PAP-specific Th1-biased T cell immunity and $CD8^+$ T-cell infiltration in metastatic tumor biopsy specimens.

Table I. Continued.

B, Combined therapy

Serial no.	Drug	Trial	No. of patients	Eligibility of patients	Outcome
8.	Ipilimumab and a poxviral vaccine targeting prostate-specific antigen	Phase I dose-escalation trial, NCT00113984	30; 24 of whom had not been previously treated with chemotherapy	mCRPC	Of the 24 patients who were chemotherapy-naive, 14 (58%) had PSA declines from baseline, of which six were greater than 50%. Of the remaining 6 patients, PSA decline was observed in 1 patient. The use of a vaccine targeting PSA that also enhances co-stimulation of the immune system did not seem to exacerbate the immune-related adverse events associated with ipilimumab.
8.	Granulocyte-macrophage colony-stimulating factor-transduced allogeneic prostate cancer cells vaccine (GVAX) + ipilimumab	Phase 1 dose-escalation trial NCT01510288	28: 16 patients with 3·0 mg/kg level of GVAX	Eligible patients had documented mCRPC and had not been previously treated with chemotherapy.	GVAX combined with 3·0 mg/kg ipilimumab is tolerable and safe for patients with mCRPC. PSA50: 25% (7/28)
9.	Pembrolizumab plus prostatic cryotherapy	Pilot Trial study	12	Newly diagnosed oligometastatic prostate cancer between 2015 and 2016	PSA decline: 42% (5/12); mPFS:14 months, and mSTFS: 17.5 months. PD-L1 expression: Not detectable by IHC. AEs: grade ≤2, and there were no apparent complications from cryotherapy.

OS, overall survival; mOS, median overall survival; PSA, prostate-specific antigen; mPFS, median progression-free survival; rPFS, radiographic progression-free survival; mCRPC, metastatic castration-resistant prostate cancer; AEs, adverse events; ORR, overall response rate; DCR, disease control rate.

FANCA, RAD51B, RAD51C, MLH1, and *MSH2* can also be used as markers in the selection of patients with prostate cancer for PARP inhibitor-based therapy. Of note, two PARP inhibitors, olaparib (Lynparza) and rucaparib (Rubraca), have received FDA approval for the treatment of mCRPC. These agents have slightly different indications, dosages (olaparib, 300 mg twice daily; rucaparib, 600 mg twice daily) and adverse effect (AE) profiles. For example, olaparib was selected for patients with mCRPC who showed resistance to enzalutamide and abiraterone. However, rucaparib has been selected for patients with mCRPC who have been treated with AR-directed or taxane therapy. FDA breakthrough therapy designation has been provided to expedite drug development. Niraparib (Zejula) has received this designation by FDA for mCRPC. In addition to niraparib, talazoparib (Talzenna) has emerged as a PARP inhibitor for mCRPC treatment. The pharmacokinetic properties of these inhibitors differ, as discussed elsewhere, and are not included in this review (112). Approximately 18 drugs have been approved for the treatment of HER2-positive BC by the FDA. However, the development of novel small-molecule drugs that specifically target chemoresistance in triple-negative BC (TNBC) remains challenging. PARP inhibitors are particularly effective in treating TNBC, BCs and ovarian cancers that carry BRCA1 or BRCA2 mutations. Emerging evidences also suggest their role in ovarian and oral cancer (113). However, the development of novel small-molecule drugs that specifically target chemoresistance in various types of cancer remains challenging.

Clinical relevance. After the release of data from the phase 3 PROfound study (NCT02987543), olaparib received FDA approval for the treatment of mCRPC in 2020. PROfound study (NCT02987543). The PROfound study was conducted on patients with mCRPC who had disease progression while receiving a new hormonal agent (e.g., enzalutamide or abiraterone). Patients received olaparib, enzalutamide, or abiraterone plus prednisone. Patients were divided into two cohorts: i) Cohort A included genetic mutations in *the BRCA1, BRCA2,* or *ATM* genes, ii) and cohort B included mutations in 12 other prespecified genes. In both cohorts, patients were treated with either olaparib or AR-therapy of the physician's choice (cohort A: Olaparib-162 patients and AR-therapy-83 patients; cohort B: Olaparib-94 patients and AR-therapy-48 patients). The median OS in cohort A was higher than that in cohort B (19.1 months vs. 14.7 months). Additionally, in the olaparib arm of cohort B, the median OS was higher than that in the AR arm (14.1 vs. 11.5 months). Anemia and nausea were the main toxic effects in patients who received olaparib. Recently, a PROfound study also showed the superior effect of olaparib on improving quality of life by reducing pain compared with either enzalutamide or abiraterone (114). In the olaparib arm of cohort A, pain progression was not reached, while it was 9.92 months in the control arm. The findings of that study indicate that in patients with mCRPC who experienced disease progression while on enzalutamide or abiraterone and had alterations in homologous recombination repair, olaparib was associated with longer progression-free survival and improved response measures as well as improved patient-reported outcomes compared to either enzalutamide or abiraterone (115).

In the phase 3 PROpel study, patients with mCRPC were randomly assigned to receive three drugs, namely: i) abiraterone, ii) prednisone/prednisolone, and iii) olaparib (300 mg) twice daily or the placebo. In that study, patients did not receive any prior chemotherapy and were not selected based on HRR mutation status. That study was conducted at 126 centers in 17 countries. Patients were divided into two groups: Group 1 received abiraterone, prednisone/prednisolone, and olaparib, whereas group 2 received abiraterone, prednisone/prednisolone, and the placebo. The median rPFS was higher in group 1 than in group 2 (24.8 vs. 16.6 months, $P<0.0001$). The most frequent adverse event of anemia in cohort 1 was managed by applying two strategies: i) Dose reduction or ii) temporary cessation of treatment (116,117). These findings suggest that, irrespective of the HRR markers, olaparib had superior benefits in cohort 1. However, other studies have shown the benefits of PARP inhibitors in the presence of HRR mutations and therefore, these findings need to be validated in subsequent studies (115,118). These studies suggest that it would be beneficial to identify the importance of HRR biomarkers in the selection of patients for PARP therapy.

Based on the results of the phase 2 TRITON2 trial (NCT02952534) on mCRPC, rucaparib has received accelerated FDA approval. In that study, the eligibility criteria for patients were the presence of somatic or germline alterations in the HRR genes. The patients received 600 mg of rucaparib twice daily. That study included 115 patients with deleterious BRCA alterations. The confirmed PSA response rate was 54.8% (95% CI, 45.2-64.1%) (119). ORRs were similar for patients with a germline or somatic BRCA alteration and for patients with a BRCA1 or BRCA2 mutation, whereas a higher PSA response rate was observed in patients with a BRCA2 mutation. The results revealed a beneficial effect of the drug on patients with Pca. Two ongoing phase 3 trials will assess the effect of this drug either alone (TRITON3 study: NCT02975934) or in combination with enzalutamide as first-line treatment for mCRPC (CASPAR study: NCT04455750) (120). The results from the previous phase 1b CASPAR trial demonstrated that 4 out of 8 patients (50%) had a confirmed PSA response (≥50%). Additionally, 1 patient with measurable disease achieved a confirmed complete radiographic response following treatment with a combination of rucaparib 600 mg twice daily and enzalutamide 160 mg once daily, which was observed even in the presence of AR alterations and absence of DNA damage repair gene alterations (121). The breakthrough therapy designation of niraparib was based on data from the phase 2 GALAHAD study (NCT02854436) (122). In that study, 223 patients were divided into the BRCA (n=142) and non-BRCA (n=81) cohorts. In the final analysis, the ORR in the measurable BRCA cohort (n=76) was 34.2%. The median follow-up duration was 10.0 months, and the median duration of response was 5.55 months (95% CI, 3.91-7.20). The phase 3 MAGNITUDE trial (NCT03748641) is currently evaluating the combinatorial effect of niraparib with abiraterone acetate and prednisone in men with mCRPC. Patients with or without HRR gene mutations were eligible for enrollment in this trial. To date, patients with HRR gene mutations have shown survival benefits such as enhanced rPFS, ORR, time to initiation of cytotoxic chemotherapy, symptomatic progression, and PSA progression (122,123). Talazoparib is also being evaluated for

mCRPC in the phase 3 TALAPRO-2 study (NCT03395197). TALAPRO-2 compares rPFS in two arms: arm 1, talazoparib plus enzalutamide (with and without HRR mutations), and arm 2, enzalutamide plus placebo (with and without HRR mutations). A total of 20 secondary outcome measures, including OS and ORR, were also included (124). In a non-randomized phase I/II clinical trial, 17 patients had previously received enzalutamide and/or abiraterone. Patients received durvalumab 1,500 mg every 28 days and olaparib 300 mg tablets every 12 h until disease progression or unacceptable toxicity was observed. An rPFS of 16.1 months was achieved in patients with mCRPC treated with olaparib and durvalumab, with an rPFS rate of 51.5% within 1 year. Furthermore, patients with HRR mutations exhibited a high rPFS probability (rPFS rate of 83.3% within 1 year) compared to patients with no mutations in HRR (36.4%) (125). The combinatorial therapeutic regimen of olaparib and pembrolizumab also improved the response in patients with mCRPC (regardless of HRR state; arm I) when compared with olaparib (arm 2) or pembrolizumab (arm 3) monotherapy (phase Ib/II KEYNOTE-365). The ORRs and PSA response rates in arm 1 were higher in the patients with BRCA mutations than in the BRCA-non-mutated cohorts (BRCA-mutated patients: ORR, 33%; PSA response rate, 50%; BRCA-non-mutated cohort, ORR: 6%; PSA response rate: 14%) (126). The ORR for pembrolizumab and olaparib were 5 and 6%, respectively. Overall, these results suggest that pembrolizumab + olaparib may improve the PSA response rate regardless of HRR mutation status.

Overall, the results of clinical trials suggest that PARP inhibitors can exert beneficial effects with or without HRR mutation status in patients. Therefore, an important question that remains is whether biomarkers proofing is important in patients treated with PARP inhibitors. PARP inhibitors also impact downstream expression of antigen-regulated genes. Thus, combinatorial therapies with PARP inhibitors may be advantageous in certain populations. However, further studies are necessary.

Future research directions. Notably, the combination of PARP inhibitors with novel hormonal therapies is expected to provide advantages to a broader population of patients with prostate cancer. The following considerations specific to PARP inhibitors can be advantageous for patients with Pca: i) Identifying treatment-specific biomarkers to PARP inhibitors both before and after beginning therapy; ii) using a combination of various PARP inhibitors in combination therapies; iii) identifying resistance-related biomarkers and targeting these biomarkers in combination with PARP inhibitors; and iv) the selection of patients on the basis of biomarker profiling. These considerations could lead to improved survival rates for patients with mCRPC.

7. PI3K/AKT/mTOR

The PI3K/AKT/mTOR signaling pathway is essential for regulating cell growth and survival. It begins with activation of the PI3K enzyme, which recruits and activates the AKT subfamily (AKT1, AKT2 and AKT3). Once activated, AKT phosphorylates various downstream effectors, including mTOR. In normal cells, the PI3K pathway is controlled by negative feedback mechanisms, such as the dephosphorylation of signaling molecules by PTEN. However, the hyperactivation of this pathway has been observed in 49% of patients with mCRPC (127). Of these patients, 41% have loss-of-function aberrations in PTEN and activating alterations in PIK3CA and AKT1. Moreover, the PI3K pathway is activated by the inhibition of the AR pathway in PTEN-deficient Pca, and can thus function as a resistance mechanism to ARS inhibitors (128). The PI3K/AKT/mTOR pathway is essential for regulating cell growth, survival and metabolism, thus rendering it a critical target for cancer therapy across several cancer types beyond pancreatic cancer, including breast, gastric, ovarian, colorectal, glioblastoma and endometrial cancers. The hyperactivation of the PI3K/AKT/mTOR pathway can lead to treatment resistance in cancer cells. Consequently, this pathway and its associated components have emerged as promising targets for the development of anticancer therapeutics aimed at overcoming resistance and improving treatment efficacy (129). Therefore, co-targeting the AR axis and PI3K pathways may overcome resistance to AR-mediated therapy.

This concept was evaluated in the phase 3 IPATential150 trial, which randomized 1,101 patients with mCRPC to receive first-line treatment with ipatasertib (400 mg once daily orally) in combination with abiraterone (1,000 mg once daily orally) and prednisolone (5 mg twice daily orally) or a placebo alongside the same regimen of abiraterone and prednisolone. Ipatasertib is a selective inhibitor of all three AKT isoforms (130). The trial was conducted at 200 sites across 26 countries or regions. In this trial, 554 (50%) patients were assigned to the placebo-abiraterone group and 547 (50%) to the ipatasertib-abiraterone group. All patients had asymptomatic or mildly symptomatic mCRPC with progressive disease and an ECOG score of 0 or 1. The median follow-up period was 19 months. The treatment group demonstrated a higher median PFS and ORR compared with the placebo group (PFS, 19.2 months vs. 16.6 months; ORR, 61 vs. 44%). Immunohistochemical analysis indicated that 521 patients experienced a loss of PTEN. Among these patients, the median PFS was 18.5 months for the ipatasertib-abiraterone group compared to 16.5 months for the placebo-abiraterone group (HR, 0.77; P=0.034), with ORRs of 61 vs. 39%, respectively (130). These data indicate that a combinatorial approach targeting both the AKT and AR signaling pathways with ipatasertib and abiraterone may serve as a promising treatment for males with mCRPC and a loss of PTEN, a group that typically has a poor prognosis. Other inhibitors of the PI3K pathway, both as monotherapy and in combination, have also been explored in early phase trials (Table II) (131-140). The majority of studies have completed phase 1 trials to determine the recommended dose for phase 2 trials. One phase 2 trial revealed a comparative beneficial effect on naïve patients with CRPC (NCT00814788), whereas in another study, the presence of AKT was considered a detrimental factor for disease progression (NCT01051570) (Table II). In patients with advanced-stage CRPC with abiraterone resistance, samotolisib (LY3023414), along with the absence of AR-V7, may be a better strategy (NCT02407054). By contrast, in patients with CRPC with abiraterone resistance, apalutamide (ARN509) and everolimus were not found to exhibit any beneficial effects (NCT02106507) (Table II).

Table II. Clinical trials data of PI3K/AKT/mTOR inhibitors in prostate cancer.

A, Completed trials

Clinical trials ID/ no. of patients	AKT/PI3K/mTOR inhibitor	Phase	Treatment plan	Prior therapy	Outcomes
NCT02215096/n=36	PIK3 inhibitor GSK2636771 in combination with enzalutamide	I	Fixed dose of enzalutamide (160 mg) once daily received GSK2636771 in a dose escalation manner to evaluate the long-term safety of the combination as well as the 12-week non-PD rate- 200 mg (n=22) 300 mg (n=12) 400 mg (n=2)	Enzalutamide	Dose-limiting toxicities (DLTs) occurred least at the dose of 200 mg and therefore, this was considered the recommended dose -(DLTs: 200 mg: n=1; 300 mg: n=2,400 mg: n=2). -PSA50: 4/34 (12%) -radiographic partialresponse: 1/34 (3%) -12-week non-progressive disease rate: 50% -limited antitumor efficacy but adequate safety and tolerability with no new or unexpected AEs.
NCT02064608/n=20	mTOR inhibitor AZ12729279	I	This is a single arm study whereby a cohort of 20 patients with early high risk prostate cancer were treated with a 15-day course of AZD2014 (mTOR inhibitor) treatment prior to radical prostatectomy.	Information not provided	-PSA decline: 3/20 (15%) over two-week dosing period. -Mean fasting plasma glucose before treatment: 4.88±0.56 mmol/l -Mean fasting plasma glucose after treatment: 6.16±0.78 mmol/l -*mTOR1/2 (pS6, 4EBP1* and NDRG1 were inhibited
NCT02091531/n=9	mTOR inhibitor MLN0128	II	Patients were treated with the established phase II dose of MLN0128 (4 mg p.o. daily continuously; 1 cycle=4 weeks) to assess mechanisms of sensitivity and enzalutamide and/or abiraterone resistance	Abiraterone acetate and/or enzalutamide	-All patients had a rise in PSA on treatment, with a median 159% increase from baseline (range: 12-620%). -Eight patients discontinued treatment early because of radiographic progression, grade 3 toxicity, or investigator discretion. -AR activation result from mTOR inhibition, and poor inhibition of mTOR signaling targets (*pAKT, 4EBP1* phosphorylation, and *eIF4E* activity)
NCT03618355/n=14; 2 patients withdraw due to dose limiting toxicity	eRapamycin (encapsulated rapamycin)	I	Experimental: Cohort 1: 0.5 mg weekly (n=3), Cohort 2; 1 mg weekly (n=3); Cohort 3: 05 mg daily (n=6) oral administration of encapsulated rapamycin Patients were treated for 3 months and followed for 6 months to assess safety,	Immunosuppressed and ADT by CYP3A4 a cytochrome inhibitor	-Central memory CD8 T-cells and $CD3^+/CD56^+$ NK cells were more prevalent in cohort 3 than other cohorts at 1month and 3 months. -dose of 0.5 mg daily produced stable serum rapamycin through the duration of treatment and resulted in a positive immune impact. -no significant change in PSA level -no patients clinicallyprogressed on therapy

Table II. Continued.

A, Completed trials

Clinical trials ID/ no. of patients	AKT/PI3K/mTOR inhibitor	Phase	Treatment plan	Prior therapy	Outcomes
			PSA, pharmacokinetics, immune response, and quality of life (QOL)		
NCT01548807/n=16: 1 withdraw	mTOR inhibitor everolimus and radiation therapy	I	14 days everolimus daily therapy (5,7.5 or 10 mg/daily) followed by prostate bed irradiation (15 IMRT, 1 proton) with daily cone beam CT localization in 37 fractions of 1.8 Gy each (total dose 66.6 Gy) for the treatment of BCR following prostatectomy. Median follow-up time: 20.1 months	Prostatectomy and salvage radiotherapy	-No dose-limiting toxicities were observed. -five patients(33%) developed secondary biochemical recurrence (sBCR). sBCR was defined as a rise in PSA of >0.5 ng/ml above nadir following the completion of protocol therapy. -Median time to sBCR: 33.8 months
NCT02407054/ n=129	Samotolisib (LY3023414)	II	Participants received 200 mg of LY3023414 BID in combination with 160 mg of enzalutamide QD beginning Cycle 1 day 1. A treatment cycle was defined as 28 days	Advanced CRPC with progression on prior abiraterone	-Median rPFS in samotolisib/enzalutamide vs. placebo/ enzalutamide arm: 10.2 vs. 5.5 months; P=0.03 -rPFS inpatients without AR-V7 in the samotolisib/enzalutamide vs. placebo/enzalutamide arm: 13.2 months vs. 5.3 months; P=0.03
NCT00459186/n=12	Everolimus and docetaxel	I	Everolimus 10 mg daily for 2 weeks, followed by Everolimus + docetaxel at one of three doses: 5 mg everolimus and docetaxel at 60 mg/m^2, 10 mg everolimus and docetaxel at 60 mg/m^2, and 10 mg everolimus and docetaxel at 70 mg/m^2. everolimus was given daily. Docetaxel was given every 3 weeks by intravenous infusion. Patients also received prednisone 5 mg by mouth twice daily.	Eligible patients had progressive, metastatic, chemotherapy-naive CRPC	-The combination of everolimus 10 mg/day and docetaxel 60 mg/m^2 was comparatively well tolerated. -PSA50: 5/12 -Four patients had partial metabolic response (PMR) and PSA50 was observed in 3 of 4 patients.

Table II. Continued.

A, Completed trials

Clinical trials ID/ no. of patients	AKT/PI3K/mTOR inhibitor	Phase	Treatment plan	Prior therapy	Outcomes
NCT00814788/n=24	Bicalutamide (an mTOR) inhibitor	II	Patients receive oral Bicalutamide (50 mg) and oral Everolimus (10 mg) once daily on days 1-28. Courses repeat every 28 days in the absence of disease progression or unacceptable toxicity. -Meanlength of treatment: 8 cycles	Eligible patients should have progressive CRPC with serum testosterone <50 ng/dl	-PSA response: 18/24 (75%) -PSA50: 15/24 (62.5%) -mOS: 28 months -Grade 3 (13 patients) or Grade 4 (1 patient with sepsis): 14 (54%)
NCT01051570/n=26	Carboplatin, everolimus, and Prednisone (RAD 001)	II	Carboplatin: AUC=4 by Calvert's formula (max dose 600 mg)*IV over 30-60 min, Day 1 of a 21 day cycle everolimus: 10 mg Orally daily, starting from Day 2 continuously Prednisone 5 mg Orally twice daily, continuously	-Docetaxel	-Median time to progression: 2.5 months -mOS:12.5 months -Disease progression in AKT +ve patients within 9 weeks -Minimal clinical efficacy
NCT02106507/n=9	Apalutamide (ARN509) and everolimus	I	Apalutamide, 240 mg/day Cohort 1: everolimus 5 mg (n=3) Cohort 2: everolimus 10 mg (n=6). -The median time on treatment was 17 weeks	Abiraterone acetate and prednisone (AAP)	-SD: 9/9 patients. Patients came off study for: progression (n=3), investigator choice (n=3) and toxicity unrelated to treatment (n=2). -The treatment responsewas similar to historical data of AAP followed by apalutamide alone -Study closed in favour of evaluating novel AR/PI3K pathway combinations in patients who have not yet been exposed to AAP

B, Ongoing clinical trials

Clinical trials ID/ no. of patients	AKT/PI3K/mTOR inhibitor	Phase	Treatment plan	Outcomes
NCT05593497	Capivasertib in combination with abiraterone and leuprolide	II	Four-week intensified androgen deprivation drugs (iADT; abiraterone and leuprolide), 16-week treatment with Capivasertib in combination with iADT	Prior to radical prostatectomy and resistant to standard treatments

Table II. Continued.

B, Ongoing clinical trials

Clinical trials ID/ no. of patients	AKT/PI3K/mTOR inhibitor	Phase	Treatment plan	Outcomes
NCT05593497	Capivasertib in combination with abiraterone and leuprolide	II	Four-week intensified androgen deprivation drugs (iADT; abiraterone and leuprolide), 16-week treatment with Capivasertib in combination with iADT	Prior to radical prostatectomy and resistant to standard treatments
NCT03903835	Treatments will be given on the basis of biomarker profile of patients	III	Biomarker signatures will include: Androgen receptor DNA-repair deficiency TP53 TMPRSS2-ERG gene fusion PI3K pathway alterations	Information not provided
NCT06190899	Gedatolisib (inhibitor of PIK3/mTOR) in combination with darolutamide (AR-inhibitor)	I/II	Experimental: Phase 1 Arm 1 Arm 1-120 mg of Gedatolisib (administered once weekly for 3 weeks on/1 week off) in combination with darolutamide 600 mg orally administered twice daily (equivalent to a total daily dose of 1200 mg on Days 1-28 of each cycle)	Chemotherapy plus ADT for castration-sensitive disease, including docetaxel plus darolutamide

PSA, prostate-specific antigen.

Ongoing clinical trials are listed in Table II. It is suggested that careful patient selection is necessary to understand their treatment-related genomic profiles.

Future research directions. Several molecules within the PI3K/AKT/mTOR signaling pathway are involved in the pro-survival activity in prostate cancer. The inhibition of these molecules may be advantageous in terms of bypassing the pro-survival pathways associated with drug resistance. Therefore, combination treatment with AR inhibitors and PI3K/AKT/mTOR signaling pathway inhibitors may be beneficial in overcoming AR-mediated resistance. However, biomarker analysis may play an indispensable role in the identification of a suitable inhibitor. For instance, if a clinician knows about the biomarker that will eventually be enhanced owing to drug resistance, then they can wisely select the inhibitor that will decrease the expression of drug resistance-associated biomarkers. This would allow for specific personalized therapy for patients. Additionally, the development of isoform-specific AKT inhibitors could avoid some of the toxicities of pan-AKT inhibitors and improve the side-effect profile in patients (141).

8. Implications and future directions

Although multiple treatment options with varying mechanisms are available, careful patient selection should be performed to maximize the treatment response with minimal toxicity. Effective biomarkers could revolutionize clinical care by making treatments more personalized and reducing unnecessary treatments. Biomarkers can identify specific molecular and genetic traits to classify patients by their risk levels. This helps provide aggressive treatments to those who need them most while avoiding side effects in others. Emerging technologies, such as liquid biopsies, offer non-invasive alternatives to traditional tissue sampling, allowing for real-time monitoring of tumor dynamics and treatment responses. These advancements could lead to the earlier detection of resistance mechanisms and more adaptive treatment strategies. With advancements in the field of whole-genome sequencing and next-generation sequencing, the detection of circulating tumor cells and circulating tumor DNA is possible, resulting in the identification of predictive biomarkers before starting therapies. For example, plasma circulating free DNA levels have been shown to accurately predict patients suitable for proton vs. photon radiotherapy and may serve as a minimally invasive predictive biomarker for acute and late gastrointestinal toxicity reported by patients (142). In the future, biomarker-based screening may further enhance precision medicine, improving outcomes, multi-cancer detection by common biomarkers, while reducing the financial and emotional burdens associated with ineffective or excessive treatments. In addition, integration with artificial intelligence will optimize the analysis of complex biomarker datasets. Since biomarkers also change following the treatment response, biomarkers other than PSMA should also be evaluated at preclinical and clinical level to observe response to therapy. Translating research findings into clinical applications requires rigorous validation through clinical trials to ensure biomarkers are robust and reproducible across diverse populations.

Using biomarkers in healthcare also comes with ethical, practical and technical challenges, particularly in areas with limited resources. A key issue is the high cost of developing, testing and using biomarker-based treatments, which can make these difficult to access and increase health inequalities. Advanced tools, such as liquid biopsies and next-generation sequencing require special equipment and trained experts, which may not be available in low-resource areas. Additionally, the need for regular testing or real-time monitoring could overwhelm healthcare systems that already face staff shortages or limited funding. Ethically, focusing on costly personalized treatments could leave out people who can't afford them, creating concerns about equitable care. Additionally, technical challenges, such as the standardization of biomarker assays and the interpretation of complex data, complicate their widespread adoption. Addressing these barriers requires innovative, cost-effective solutions and global collaborations to ensure biomarker-driven therapies benefit all patients, regardless of socioeconomic status. Making biomarker development more efficient with affordable tests and ensuring they have practical use in clinics can help connect research with real-world applications. This will promote precision medicine while keeping healthcare sustainable. Owing to the high cost of NGS, the majority of biomarker-driven clinical trials are being conducted in developed countries. More collaboration between developed and developing countries should be explored to overcome this financial issue. In summary, therapeutic biomarkers should be evaluated to provide precision in personalized therapies for prostate cancer.

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Authors' contributions

LG was involved in the conceptualization of the study, as well as in the writing and preparation of the original draft of the manuscript. DB and CJ prepared the tables. RG supervised the study and edited the manuscript. All the authors have read and approved the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

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Competing interests

The authors declare that they have no competing interests.

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