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# The Past, Present, and Future of PSMA-Targeted Therapies in Prostate Cancer

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## ABSTRACT

Prostate-specific membrane antigen (PSMA) is a well-characterized cell-surface antigen with 100- to 1000-fold increased expression in prostate cancer relative to normal prostate tissue, establishing it as a compelling therapeutic target in metastatic castration-resistant prostate cancer (mCRPC). Decades of clinical investigation across radioligand therapy, antibody-drug conjugates (ADCs), chimeric antigen receptor T-cell (CAR-T) therapy, and bispecific T-cell engagers (BiTEs) have validated PSMA as a clinically actionable target. However, across these modalities, antigen expression heterogeneity, the immunosuppressive tumor microenvironment, and construct-level limitations have consistently emerged as the primary determinants of therapeutic outcome. The approval of $^{177}$Lu-PSMA-617 (lutetium-177 vipivotide tetraxetan; Pluvicto) established proof-of-concept for PSMA-directed therapy in mCRPC, yet a substantial proportion of patients derive limited or no durable benefit, and early immunotherapeutic platforms encountered recurring challenges including cytokine release syndrome, immunogenicity, and insufficient T-cell persistence. Next-generation bispecific constructs incorporating tumor-conditional activation via molecular masking, exemplified by VIR-5500, have demonstrated markedly improved tolerability and encouraging anti-tumor activity in Phase 1 evaluation. This review traces the clinical and biological insights that have guided successive generations of PSMA-targeted therapeutic design and discusses the combination strategies and molecular profiling tools that will be essential for extending durable benefit across the broader mCRPC population.

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## Introduction

Prostate-specific membrane antigen (PSMA) was first identified in the 1980s by Horoszewicz and colleagues at the Roswell Park Memorial Institute, where the development of the 7E11-C5 monoclonal antibody against the LNCaP prostate cancer cell line established PSMA as a prostate-restricted antigenic marker with significant clinical potential [1]. Since its discovery, PSMA has been extensively investigated as a therapeutic target, spurring the development of a broad range of PSMA-directed strategies including radioligand therapeutics, antibody-drug conjugates (ADCs), chimeric antigen receptor T-cell (CAR-T) therapy, and bispecific T-cell engagers (BiTEs) [2–4]. While several of these approaches have demonstrated meaningful anti-tumor activity, the field has encountered recurring challenges related to the immunosuppressive tumor microenvironment, antigen heterogeneity, and construct-level limitations that have informed successive generations of therapeutic design. Most recently, VIR-5500, a novel dual-masked PSMA-directed bispecific T-cell engager currently in Phase 1 clinical evaluation, has shown encouraging results in heavily pretreated mCRPC [5]. The development of VIR-5500 reflects decades of iterative progress in PSMA-directed therapeutic design, and its early clinical profile provides an opportunity to examine how construct-level inno-

vation is shaping the future of PSMA-targeted immunotherapy in mCRPC. This review traces the history of PSMA-targeted therapeutic development in mCRPC, spanning radioligand therapy and early immunotherapeutic platforms including ADCs, CAR-T cells, and BiTEs through to next-generation bispecific constructs. Throughout, it examines the biological and clinical insights that have guided each successive wave of design and the opportunities that remain.

## PSMA as a Therapeutic Target: Biology, Expression, and Clinical Rationale

PSMA is a type II transmembrane glycoprotein encoded by the FOLH1 gene, consisting of a short intracellular domain, a single transmembrane domain, and a large extracellular domain that houses the catalytic active site [6]. The protein functions as a zinc-dependent metallopeptidase with two principal enzymatic activities: folate hydrolase activity, which cleaves gamma-linked glutamates from polyglutamated dietary folates to facilitate intestinal folate absorption, and N-acetylated alpha-linked acidic dipeptidase (NAALADase) activity, which hydrolyzes the neuropeptide N-acetyl-L-aspartyl-L-glutamate (NAAG) in the central nervous system [7,8]. The designation of FOLH1 as the official gene name reflects PSMA's primary characterized role as a folate hydrolase, though its precise physiological function within the prostate epithelium remains incompletely understood [9]. PSMA expression is specific to prostate tissue and has 100- to 1000-fold increased expression in prostate cancer (PCa) tissues across patients [10,11]. Due to its prevalence and abundant expression in mCRPC, PSMA has established itself as a well-characterized and extensively pursued druggable target, motivating decades of therapeutic development [10,11].

## PSMA Radioligand Therapy: From 177Lu to Next-Generation Alpha Emitters

The most clinically advanced PSMA-targeted therapeutic to date is $^{177}$Lu-PSMA-617 (lutetium-177 vipivotide tetraxetan; Pluvicto, Novartis), a radioligand therapeutic consisting of two components: PSMA-617, a small-molecule glutamate-urea-lysine ligand that binds with high affinity and specificity to the extracellular domain of PSMA on prostate cancer cells, and lutetium-177 ($^{177}$Lu), a beta-particle-emitting radioisotope chelated to the ligand via a DOTA moiety [12]. Following receptor-mediated binding and internalization, the conjugate delivers targeted beta-particle radiation directly to PSMA-expressing tumor cells and the surrounding microenvironment, inducing DNA double-strand breaks, cell cycle arrest, and apoptosis [4,12]. The pivotal phase III VISION trial [4] demonstrated that $^{177}$Lu-PSMA-617 plus best standard of care significantly improved overall survival compared to standard of care alone (median 15.3 vs. 11.3 months; HR 0.62, 95% CI 0.52–0.74; $p < 0.001$) and radiographic progression-free survival (8.7 vs. 3.4 months; HR 0.40; $p < 0.001$), establishing PSMA as a clinically actionable target in mCRPC. Phase II data from the LuPSMA trial [13] further supported this, with 64% of treated patients achieving a ≥50% PSA decline. While these results established $^{177}$Lu-PSMA-617 as a significant therapeutic advance, the challenge of heterogeneous PSMA expression has motivated investigation of alpha-particle-emitting radioligand strategies with distinct biophysical properties.

Unlike beta emitters such as $^{177}$Lu, alpha particles deliver substantially higher linear energy transfer with a shorter tissue path length, producing densely ionizing, difficult-to-repair double-stranded DNA breaks [14,15]. Critically, alpha emitters also exert a crossfire bystander effect that can irradiate neighboring tumor cells with low or absent PSMA expression, an advantage in heterogeneous disease [14,15]. Early clinical experience with $^{225}$Ac-PSMA-617 (actinium-225 PSMA-617) in heavily pretreated mCRPC patients, including those refractory to prior $^{177}$Lu-PSMA-617, demonstrated $PSA_{50}$ responses in approximately 67% of patients across retrospective series, with complete PSA remissions observed even in patients with extensive prior treatment [14,15]. The clinical development of $^{225}$Ac-PSMA-617 is now advancing into prospective randomized evaluation. The AcTFirst trial [16] is an ongoing phase III study evaluating $^{225}$Ac-PSMA-617 in combination with an androgen receptor pathway inhibitor (ARPI) versus investigator's choice of standard of care in ARPI-pretreated, taxane-naive, RLT-naive patients with PSMA-positive mCRPC. Standard of care options include ARPI change, taxane-based chemotherapy, or $^{177}$Lu-PSMA-617 (ClinicalTrials.gov, NCT06855277). The PSMAcTION [17] trial is a complementary phase II/III randomized study similarly evaluating $^{225}$Ac-PSMA-617 versus standard of care in patients who have progressed on

or after prior $^{177}$Lu-PSMA therapy, with enrollment now underway across 13 sites in 7 countries (clinicaltrials.gov, NCT06780670).

Despite these advances in radioligand design, marked PSMA expression heterogeneity and variable treatment response remain consistent features of the mCRPC landscape. The extent to which radioligand therapy can achieve uniform therapeutic benefit across the broader patient population continues to be an important area of investigation. A key contributor to this variability is the dynamic regulation of PSMA expression at the tumor cell surface. Membranous PSMA expression in mCRPC exhibits significant inter- and intra-patient variability and is closely linked to DNA damage repair (DDR) gene aberrations [18]. Consequently, tumors lacking DDR defects may express substantially lower PSMA levels, limiting their susceptibility to radioligand-mediated cytotoxicity [18]. This biological heterogeneity translates directly into differential clinical outcomes. In a cohort of 85 mCRPC patients, low average PSMA expression on pre-therapeutic $^{68}$Ga-PSMA-PET/CT was identified as an independent negative prognosticator of overall survival (5.3 vs. 15.1 months; HR 3.74; $p < 0.001$) [19]. The presence of even a single low-PSMA-expressing metastasis was independently associated with markedly different outcomes (7.9 *vs*. 21.3 months; HR 4.30; $p = 0.006$), illustrating how intra-patient tumoral antigen heterogeneity rather than peak expression governs therapeutic efficacy [19]. Higher baseline PSMA-PET SUVmean has been shown to predict superior response to $^{177}$Lu-PSMA-617 [20]. Conversely, patients with high-volume FDG-avid, PSMA-discordant disease, representing a dedifferentiated PSMA-low phenotype, showed notably different outcomes regardless of treatment assignment [20]. Collectively, these data affirm PSMA as a clinically validated therapeutic target while demonstrating that its heterogeneous expression landscape, shaped by epigenetic regulation, DDR gene status, and tumor dedifferentiation, underscores the need for improved patient stratification and combination approaches that extend therapeutic coverage beyond PSMA expression alone [18,19,21,22].

## Antibody-Drug Conjugates: Payload Refinement and Dual-Target Strategies

Substantial effort has also been invested in PSMA-targeted antibody-drug conjugates (ADCs), which deliver cytotoxic payloads directly to PSMA-expressing tumor cells via receptor-mediated internalization [3,23]. These agents have provided important insights into the construct requirements for effective PSMA-targeted cytotoxic delivery, and their clinical development has directly informed next-generation design. MLN2704 was the first PSMA-directed ADC to enter clinical evaluation, consisting of the maytansinoid cytotoxin DM1 conjugated to the humanized J591 anti-PSMA antibody [3]. The phase I trial demonstrated targeted delivery to PSMA-expressing cells, though dose-limiting peripheral neuropathy and modest antitumor activity indicated the need for continued construct refinement. A subsequent PSMA-directed ADC employing monomethyl auristatin E (MMAE) as its cytotoxic payload demonstrated limited efficacy, with a $PSA_{50}$ decline rate of only 14% in second-line or beyond mCRPC, and was similarly constrained by cumulative toxicity [23]. Notably, these early findings were attributable to construct-level considerations rather than absence of target engagement, and PSMA internalization was confirmed in both trials, an important distinction that has guided next-generation ADC design.

Next-generation PSMA ADC development has focused on refining payload design and conjugation chemistry to overcome the construct-level barriers identified in earlier generations. ARX517, composed of a fully humanized anti-PSMA monoclonal antibody linked to the microtubule inhibitor amberstatin-269 *via* site-specific conjugation chemistry, is currently under evaluation in the Phase I/II APEX-01 trial [24]. ARX517 is designed to achieve a homogeneous drug-to-antibody ratio and reduce the off-target instability-related toxicity observed in earlier-generation PSMA ADCs [24]. The ADC received FDA Fast Track Designation in July 2023, and early data from APEX-01 have demonstrated a favorable safety profile alongside encouraging antitumor activity. Deep PSA and ctDNA reductions as well as confirmed RECIST v1.1 responses were observed in heavily pretreated mCRPC patients, with full peer-reviewed efficacy data anticipated [24].

Beyond payload optimization, next-generation ADC development has also sought to address the antigen heterogeneity that limits the reach of single-target approaches. ABBV-969 is a first-in-class bispecific ADC targeting both PSMA and STEAP1 via a dual variable domain immunoglobulin format with a topoisomerase-1 inhibitor payload [25]. It was spe-

cifically designed to capture tumor cells expressing either or both antigens, broadening the therapeutic coverage of PSMA-low tumor populations. Phase I data from the first-in-human study reported a confirmed objective response rate of 45% among 29 RECIST-evaluable heavily pretreated mCRPC patients, with $PSA_{50}$ and $PSA_{90}$ responses in 67% and 28% of patients, respectively, at active dose levels, representing a notable early ADC efficacy signal in this disease [25].

The progression from early PSMA ADC generations to next-generation constructs reflects a deliberate evolution in both payload design and antigen targeting strategy. These advances demonstrate that the construct-level limitations of earlier ADC generations were surmountable, and that PSMA internalization and target engagement remain valid and exploitable features of prostate cancer biology [3,23].

## CAR-T Cell Therapy: TME Resistance and Emerging Engineering Strategies

PSMA-directed CAR-T therapy faces a distinct set of challenges in mCRPC, where the immunosuppressive tumor microenvironment presents a more direct obstacle to sustained immune engagement than to cytotoxic payload delivery. mCRPC tumors are immunologically cold, characterized by low MHC-I antigen presentation and a profoundly immunosuppressive TME [26]. This TME is characterized by heterogeneous antigen expression, an abundance of inhibitory cytokines, immunosuppressive stromal populations, and regulatory T cells that collectively suppress effector immune-cell function and contribute to disease progression [27,28]. These localized features render mCRPC largely refractory to immune checkpoint blockade, and have similarly influenced the activity of T-cell-based immune therapeutic strategies, including PSMA-directed chimeric antigen receptor T-cell (CAR-T) therapy [2,29]. Despite the compelling rationale for PSMA as a therapeutic target in this setting, the clinical translation of PSMA-directed CAR-T therapy has been shaped by a convergent set of obstacles: inadequate T-cell persistence, an inability to sustain engagement within the immune-suppressive TME, dose-limiting toxicity, and the emergence of antigen heterogeneity-driven resistance [2]. These observations have motivated substantial re-engineering efforts aimed at overcoming the specific biological barriers imposed by the mCRPC TME on adoptive cellular immunotherapy.

Early clinical experience with PSMA-directed CAR-T therapy illustrated these barriers in detail. The prostate TME is enriched in transforming growth factor-beta (TGF-β) and populated by tumor-associated macrophages and myeloid-derived suppressor cells. These immune-suppressive populations drive T-cell exhaustion and limit effector persistence, posing a fundamental obstacle to PSMA-directed CAR-T therapy [28]. The first-in-human phase I trial of CART-PSMA-TGFβRDN evaluated a CAR-T construct incorporating a dominant-negative TGF-β receptor to render the CAR-T cells resistant to TGF-β-driven immunosuppression [30]. Transient antitumor activity was observed, with one patient achieving a greater than 98% PSA reduction [30]. The trial also provided important insights into TME complexity: 5 of 13 treated patients developed grade ≥2 cytokine release syndrome (CRS), including a grade 4 CRS event with concurrent sepsis [30]. Additionally, in patients who initially responded, CAR-T cell activity was accompanied by the upregulation of multiple alternative TME-localized inhibitory molecules following adoptive cell transfer [30]. This finding highlighted that neutralizing a single immunosuppressive signal may be insufficient to sustain durable anti-tumor activity when the TME retains the capacity to engage in compensatory adaptive mechanisms.

A subsequent phase I trial of P-PSMA-101, an autologous stem cell memory T-cell (T~SCM~)-enriched construct specifically engineered to improve in vivo engraftment and persistence, sought to address the T-cell fitness limitation directly [31]. In this trial, CRS occurred in 61% of 33 treated patients, dose-limiting toxicities were observed in 18%, and activation of an inducible caspase-9 safety switch was required in 24% of patients [31]. These findings illustrate the ongoing tension between the immune activation necessary for anti-tumor efficacy and the systemic toxicity that shapes deliverable dose intensity in this patient population.

Building on these insights, the next generation of PSMA-directed CAR-T design has incorporated multi-layered engineering strategies designed to simultaneously address antigen heterogeneity, TME suppression, and T-cell persistence. AB-3028 (ArsenalBio) exemplifies this approach [32]. The AB-3028 construct employs a dual-antigen logic gate for tumor-selective recognition, limiting activity to cells co-expressing both target antigens. To resist TGF-β-mediated suppression within the TME,

an shRNA module targeting TGFBR2 is also incorporated. Additionally, a synthetic pathway activator enhances T-cell persistence through JAK/STAT signaling. Preclinical data demonstrated selective anti-tumor activity, superior functional persistence relative to earlier single-module constructs, and a survival benefit supporting the initiation of a Phase I/II clinical trial now underway in mCRPC [32,33].

## Bispecific T-Cell Engagers: From First-Generation Limitations to Tumor-Conditional Activation

The microenvironmental and immunogenic challenges encountered in PSMA-directed CAR-T development have been similarly observed in bispecific T-cell engager (BiTE) therapy [34]. Unlike CAR-T cells, BiTEs redirect endogenous T cells to PSMA-expressing tumor cells through simultaneous CD3 and PSMA engagement and have yet to encounter a comparable pattern of clinical obstacles. Pasotuxizumab, the first PSMA×CD3 BiTE to enter clinical testing, was associated with the development of neutralizing anti-drug antibodies (ADAs) in all patients receiving subcutaneous administration, directly curtailing drug exposure and necessitating early termination of that dosing arm [35]. JNJ-63898081, a subsequent PSMA×CD3 bispecific antibody, yielded a $PSA_{50}$ response rate of only 7.7% across 39 patients, with every patient experiencing at least one treatment-emergent adverse event and 19 patients developing treatment-emergent ADAs, informing early discontinuation [36]. Acapatamab, a half-life-extended BiTE designed to improve pharmacokinetics and prolong T-cell engagement, achieved confirmed $PSA_{50}$ responses in 30.4% of dose-expansion patients, representing a significant BiTE efficacy signal in the class. However, CRS was observed in over 97% of patients across cohorts; treatment-emergent ADAs were detected in 55% and impacted serum drug exposure in 36% [37]. Durable antitumor activity was not sustained, leading to discontinuation of its clinical development [37]. Across all three BiTE programs, the recurring themes of immunogenicity, CRS-imposed dose constraints, and transient rather than sustained T-cell engagement within an immune-cold, heterogeneously PSMA-expressing tumor have pointed to a common underlying biology and directly informed next-generation construct design [35–37].

These clinical insights from first-generation BiTE studies have driven a fundamental evolution in construct design. The recurring obstacles were mechanistically consistent: uncontrolled systemic T-cell activation producing prohibitive cytokine release syndrome (CRS), immunogenicity-driven pharmacokinetic challenges, and an inability to sustain T-cell engagement within an immunosuppressive TME [35–37]. The next generation of PSMA-targeting bispecific platforms has directly addressed these considerations through two converging engineering strategies. The first is tumor-conditional activation via molecular masking, and the second is co-stimulatory rather than direct CD3-activating T-cell engagement. Each approach is designed to decouple anti-tumor efficacy from the systemic toxicity observed in previous approaches.

The most compelling evidence of this design evolution comes from VIR-5500, a dual-masked PSMA×CD3 bispecific T-cell engager incorporating the PRO-XTEN® masking technology developed by Vir Biotechnology [5]. The foundational principle of VIR-5500 is tumor-selective activation: the bispecific molecule is rendered systemically inert by dual peptide masks that are specifically cleaved by proteases dysregulated within the tumor microenvironment, concentrating T-cell-activating activity at the tumor site while minimizing off-tumor CD3 engagement in healthy tissues [5]. This mechanistic refinement has produced a markedly improved clinical profile relative to first-generation PSMA BiTEs. In updated Phase 1 dose-escalation data reported at the 2026 ASCO Genitourinary Cancers Symposium, 58 heavily pretreated mCRPC patients received VIR-5500 monotherapy across dose levels ranging from 30 to 4,000 μg/kg. No dose-limiting toxicities were observed; CRS was largely abrogated and restricted to grade 1 events only, and there was a low rate of grade ≥3 treatment-related adverse events without the need for prophylactic corticosteroids or anti-IL-6 agents at doses up to 3,500 μg/kg Q3W5. In contrast to the CRS rates exceeding 97% observed with acapatamab [5,37], this tolerability profile is consistent with the tumor-selective activation mechanism conferred by PRO-XTEN masking. Importantly, the improved safety margin did not come at the cost of efficacy: at doses ≥3,000 μg/kg Q3W, 82% of patients achieved a $PSA_{50}$ decline, 53% achieved a $PSA_{90}$ decline, and a 45% objective response rate was observed among 11 RECIST-evaluable patients, with all confirmed responses demonstrating emerging durability at the highest dose cohorts [5]. These early signals of encouraging anti-tumor activity in a heavily pretreated

population provide clinical validation of the PROXTEN masking strategy as an advance in the therapeutic index of PSMA-directed T-cell engager therapy for mCRPC. Building on this profile, VIR-5500 has since entered dose-expansion cohorts in late-line mCRPC and combination cohorts with androgen receptor pathway inhibitors, with pivotal Phase 3 trials anticipated in 2027 [38].

A related and complementary engineering strategy is represented by JANX007 (Janux Therapeutics), a PSMA-directed Tumor Activated T Cell Engager (TRACTr) that similarly employs protease-cleavable masking of both the PSMA-binding and CD3 domains to achieve tumor-selective T-cell activation [39]. In updated Phase 1 interim data from the ENGAGER-PSMA-01 trial [40] (NCT05519449), 109 patients were treated across Phase 1a dose-escalation and Phase 1b expansion cohorts as of the October 15, 2025 data cutoff. Patients enrolled in the Phase 1a dose-escalation cohort were heavily pretreated with a median of four prior lines of therapy, while Phase 1b enrolled taxane-naive mCRPC patients. Among 27 RECIST-evaluable patients, JANX007 demonstrated a 30% objective response rate, including confirmed and unconfirmed partial responses, alongside high PSA response rates and deep PSA declines across cohorts [39]. A radiographic progression-free survival of 7.9–8.9 months was observed in the Phase 1a population, representing a meaningful durability signal in this heavily pretreated setting [39]. The engager was generally well tolerated with a manageable CRS profile, and an every-two-week dosing schedule was found to maintain efficacy while improving patient convenience [39].

Nezastomig (REGN5678) takes a distinct approach to T-cell engagement, targeting PSMA×CD28 to amplify co-stimulatory signaling rather than directly activating CD3 [41]. It is administered in combination with the PD-1 inhibitor cemiplimab, representing the first clinical evaluation of a co-stimulatory bispecific strategy in mCRPC [41]. Updated Phase 1/2 data demonstrated that at active dose levels of ≥30 mg, 25% of patients achieved PSA50 responses and 16% achieved $PSA_{90}$ responses [41]. The Kaplan-Meier estimated median overall survival was 17.3 months at these dose levels compared to 10.3 months at sub-therapeutic doses [41]. These data provide early clinical evidence that co-stimulatory bispecific approaches can engage mCRPC in a setting characterized by resistance to checkpoint blockade monotherapy [41].

Collectively, the convergent clinical signals from VIR-5500, JANX007, and Nezastomig demonstrate that the challenges encountered by first-generation PSMA BiTE therapy were construct-driven, not target-driven. Precision engineering of T-cell engagement through tumor-conditional activation and optimized co-stimulatory design is now beginning to demonstrate the anti-tumor potential of PSMA-directed immunotherapy in mCRPC.

## Future Directions: Combination Strategies, Antigen Co-Targeting, and Patient Selection

The collective experience across radioligand therapy, ADCs, CAR-T, and BiTE programs has substantially advanced the field's understanding of PSMA as a therapeutic target while affirming that continued investment in PSMA-directed strategies is well justified. The central challenge is not target validity, but an incomplete understanding of the tumor-intrinsic biology that governs response and resistance. While clinical data consistently demonstrate the presence of both responders and non-responders across PSMA-targeted modalities, the molecular determinants underlying this heterogeneity remain an active area of investigation. In the VISION trial, for example, only approximately one-third of patients achieved a $PSA_{90}$ response, indicating that target expression alone is insufficient to predict therapeutic outcome [4]. One contributor to this variability may be the dynamic regulation of PSMA expression itself. Epigenetic modulation of FOLH1 suggests that heterogeneity in PSMA expression across metastatic sites and molecular subtypes reflects reversible regulatory states rather than genomic loss, raising the possibility that therapeutic susceptibility may be modifiable [42]. In parallel, the oncogenic transcription factor HOXB13, implicated in resistance to androgen receptor-targeted therapies, has been identified as a significant correlate of PSMA expression at both the mRNA and protein levels, with PSMA-PET SUV values associating with HOXB13 expression across independent cohorts [43]. Given that HOXB13 expression itself is epigenetically regulated in prostate cancer, the convergence of these findings may suggest a coherent upstream regulatory axis in which epigenetic state drives PSMA-relevant transcriptional programs, with direct implications for predicting and potentially modulating therapeutic susceptibility.

A deeper mechanistic understanding of PSMA expression states and the immune cell populations that characterize disease progression following PSMA-di-

rected therapies will be essential for identifying rational combination strategies. Of particular importance is the immune landscape at the time of progression, whether dominated by exhausted T cells, expanded regulatory populations, or enriched myeloid-derived suppressor cells, as this will determine which combinatorial immune interventions are most likely to enhance therapeutic sensitivity. In this context, the rationale for combining radioligand therapy with immunotherapy is compelling: radiation-induced immunogenic cell death may remodel the tumor microenvironment, enhance T-cell infiltration, and potentially engage immunologically cold mCRPC toward a more therapeutically responsive state [44,45]. The ENZA-p phase II trial provided the first randomized evidence supporting combinatorial PSMA-directed radioligand therapy, demonstrating that adding $^{177}$Lu-PSMA-617 to enzalutamide reduced the risk of death by 45% compared to enzalutamide alone in poor-risk mCRPC patients, with a median overall survival of 34 months in the combination arm versus 26 months with enzalutamide monotherapy [46] (HR 0.55; 95% CI 0.36–0.84; $p = 0.005$). Early studies combining $^{177}$Lu-PSMA-617 with pembrolizumab have also shown encouraging preliminary signals [44]. The EVOLUTION trial, which combined $^{177}$Lu-PSMA-617 with ipilimumab and nivolumab, observed treatment-related myocarditis in a subset of patients, highlighting that combination regimen design requires careful attention to immune-related toxicity and mechanistic rationale in patient selection [47].

Realizing the full potential of PSMA-directed combination therapy will require parallel investment in high-resolution molecular profiling approaches, including functional and spatial genomics, high-throughput transcriptomics, and correlative histopathology. These tools are critical for resolving the spatial architecture of PSMA expression heterogeneity and immune cell distribution at the level of individual metastatic lesions. Recent spatial transcriptomic studies in prostate cancer have begun to provide granular biological details that bulk genomic approaches cannot. A specific SPP1$^+$/TREM2$^+$ tumor-associated macrophage subpopulation has been identified as spatially enriched within metastatic prostate tumor regions and independently associated with clinical outcomes [48]. Blockade of SPP1 in a murine model improved anti-PD-1 efficacy and increased CD8$^+$ T-cell infiltration, illustrating the kind of spatially resolved mechanistic insight that will be valuable in designing rational immune combinations [48]. Applying these approaches to paired pre- and post-treatment biopsies from patients receiving PSMA-targeted therapies represents a key and underexplored area of investigation. Capturing tumor state before treatment, at response, and at progression could directly inform the design of next-generation combination trials [45,49].

## Conclusion

The trajectory of PSMA-targeted therapeutic development in mCRPC spans four decades, from the initial identification of PSMA as a prostate-restricted antigenic marker [1] to the clinical validation of $^{177}$Lu-PSMA-617 in the VISION trial [4]. The construct-level insights generated by first-generation ADC, CAR-T, and BiTE programs [23,30,31,35–37] and the early efficacy signals now emerging from next-generation platforms such as VIR-55005 collectively reflect a field that has advanced through iterative cycles of biological discovery and therapeutic refinement. What has limited therapeutic outcomes is not the target itself, but rather construct design, tumor microenvironment biology, and the heterogeneous regulation of PSMA expression [18,21,22]. The epigenetic and transcriptional mechanisms governing PSMA expression across metastatic sites, the upstream regulatory roles of drivers such as HOXB13 [42,43], and the immune landscape at progression [28] remain the field's most important open questions. Answering these questions through rigorous correlative science embedded within clinical trials will be essential for designing rational combination strategies [44,46] and patient selection frameworks. Together, these advances will be necessary to extend durable benefit across the broader mCRPC population and realize the full therapeutic potential that decades of investment in PSMA biology have established.

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## Conflict of interest

The authors declare no conflicts of interest.

## References


1. Horoszewicz JS, Kawinski E, Murphy GP. **Monoclonal antibodies to a new antigenic marker in epithelial prostatic cells and serum of prostatic cancer patients**. *Anticancer Res*. 1987;7(5B):927-935.
2. Dorff TB, Narayan V, Forman SJ, et al. **Novel Redirected T–Cell Immunotherapies for Advanced Prostate Cancer**. *Clin Cancer Res*. 2022;28(4):576-584. doi:10.1158/1078-0432.CCR-21-1483
3. Galsky MD, Eisenberger M, Moore-Cooper S, et al. **Phase I Trial of the Prostate-Specific Membrane Antigen–Directed Immunoconjugate MLN2704 in Patients With Progressive Metastatic Castration-Resistant Prostate Cancer**. *J Clin Oncol*. 2008;26(13):2147-2154. doi:10.1200/JCO.2007.15.0532
4. Sartor O, de Bono JS, Chi KN, et al. **Lutetium-177–PSMA-617 for Metastatic Castration-Resistant Prostate Cancer.** *N Engl J Med*. 2021;385(12):1091-1103. doi:10.1056/NEJMoa2107322
5. de Bono JS, Arum S, Silva DMD, et al. **Preliminary phase 1 dose escalation results of VIR-5500 (AMX-500), a dual-masked PRO-XTEN T-cell engager, in metastatic castration resistant prostate cancer (mCRPC)**. *J Clin Oncol*. 2026;44(7_suppl). doi:10.1200/JCO.2026.44.7_suppl.17
6. Mesters JR, Barinka C, Li W, et al. **Structure of glutamate carboxypeptidase II, a drug target in neuronal damage and prostate cancer**. *EMBO J*. 2006;25(6):1375-1384. doi:10.1038/sj.emboj.7600969
7. Pinto T, Suffoletto P, Berzin T, Qiao C, Lin S, Tong W. **Prostate-specific Membrane Antigen: A Novel Folate Hydrolase in Human Prostatic Carcinoma Cells**. *Clin Cancer Res*. 1996;2(9):1445-1451.
8. Carter RE, Feldman AR, Coyle JT. **Prostate-specific membrane antigen is a hydrolase with substrate and pharmacologic characteristics of a neuropeptidase**. *Proc Natl Acad Sci*. 1996;93(2):749-753. doi:10.1073/pnas.93.2.749
9. Barinka C, Rojas C, Slusher B, Pomper M. **Glutamate Carboxypeptidase II in Diagnosis and Treatment of Neurologic Disorders and Prostate Cancer.** *Curr Med Chem*. 2012;19(6):856-870. doi:10.2174/092986712799034888
10. Silver D, Pellicer I, Fair W, Heston W, Cordon-Cardo C. **Prostate-specific Membrane Antigen Expression in Normal and Malignant Human Tissues**. *Clin Cancer Res*. 1997;3(1):81-85.
11. Bostwick DG, Pacelli A, Blute M, Roche P, Murphy GP. **Prostate-specific membrane antigen expression in prostatic intraepithelial neoplasia and adenocarcinoma**. *Cancer*. 1998;82(11):2256-2261. doi:10.1002/(SICI)1097-0142(19980601)82:11<2256::AID-CNCR22>3.0.CO;2-S
12. Benešová M, Schäfer M, Bauder-Wüst U, et al. **Preclinical Evaluation of a Tailor-Made DOTA-Conjugated PSMA Inhibitor with Optimized Linker Moiety for Imaging and Endoradiotherapy of Prostate Cancer**. *J Nucl Med*. 2015;56(6):914-920. doi:10.2967/jnumed.114.147413
13. Hofman MS, Violet J, Hicks RJ, et al. **[177Lu]-PSMA-617 radionuclide treatment in patients with metastatic castration-resistant prostate cancer (LuPSMA trial): a single-centre, single-arm, phase 2 study**. *Lancet Oncol*. 2018;19(6):825-833. doi:10.1016/S1470-2045(18)30198-0
14. Kratochwil C, Bruchertseifer F, Giesel FL, et al. **225Ac-PSMA-617 for PSMA-Targeted α-Radiation Therapy of Metastatic Castration-Resistant Prostate Cancer**. *J Nucl Med*. 2016;57(12):1941-1944. doi:10.2967/jnumed.116.178673
15. Sathekge M, Bruchertseifer F, Knoesen O, et al. **225Ac-PSMA-617 in chemotherapy-naive patients with advanced prostate cancer: a pilot study.** *Eur J Nucl Med Mol Imaging*. 2019;46(1):129-138. doi:10.1007/s00259-018-4167-0
16. Novartis Pharmaceuticals. **A Phase III, Open-Label, Multi-Center, Randomized Study Comparing AAA817+ARPI Versus Standard of Care in Adult Participants With PSMA-Positive Metastatic Castration Resistant Prostate Cancer.** *clinicaltrials.gov;* 2026. Accessed June 9, 2026. https://clinicaltrials.gov/study/NCT06855277
17. Novartis Pharmaceuticals. **PSMAcTION: A Phase II/III, Open-Label, International, Multicenter, Randomized Study of AAA817 Versus Standard of Care in the Treatment of Adult Participants With PSMA Positive**

**Metastatic Castration-Resistant Prostate Cancer Who Progressed on or After [177Lu] Lu-PSMA Targeted Therapy**. *clinicaltrials.gov*; 2026. Accessed June 9, 2026. https://clinicaltrials.gov/study/NCT06780670

18. Paschalis A, Sheehan B, Riisnaes R, et al. **Prostate-specific Membrane Antigen Heterogeneity and DNA Repair Defects in Prostate Cancer**. *Eur Urol*. 2019;76(4):469-478. doi:10.1016/j.eururo.2019.06.030
19. Seifert R, Seitzer K, Herrmann K, et al. **Analysis of PSMA expression and outcome in patients with advanced Prostate Cancer receiving 177Lu-PSMA-617 Radioligand Therapy**. *Theranostics*. 2020;10(17):7812-7820. doi:10.7150/thno.47251
20. Buteau JP, Martin AJ, Emmett L, et al. **PSMA and FDG-PET as predictive and prognostic biomarkers in patients given [177Lu] Lu-PSMA-617 versus cabazitaxel for metastatic castration-resistant prostate cancer (TheraP): a biomarker analysis from a randomised, open-label, phase 2 trial.** *Lancet Oncol*. 2022;23(11):1389-1397. doi:10.1016/S1470-2045(22)00605-2
21. Bakht MK, Yamada Y, Ku SY, et al. **Landscape of prostate-specific membrane antigen heterogeneity and regulation in AR-positive and AR-negative metastatic prostate cancer**. *Nat Cancer*. 2023;4(5):699-715. doi:10.1038/s43018-023-00539-6
22. Bakht MK, Beltran H. **Biological determinants of PSMA expression, regulation and heterogeneity in prostate cancer**. *Nat Rev Urol.* 2025;22(1):26-45. doi:10.1038/s41585-024-00900-z
23. Petrylak DP, Kantoff P, Vogelzang NJ, et al. **Phase 1 study of PSMA ADC, an antibody-drug conjugate targeting prostate-specific membrane antigen, in chemotherapy-refractory prostate cancer.** *The Prostate*. 2019;79(6):604-613. doi:10.1002/pros.23765
24. Shen J, Pachynski R, Nordquist LT, et al. **1804P APEX-01: First-in-human phase I/II study of ARX517, an anti- prostate-specific membrane antigen (PSMA) antibody-drug conjugate (ADC) in patients (pts) with metastatic castration-resistant prostate cancer (mCRPC)**. *Ann Oncol*. 2023;34:S974-S975. doi:10.1016/j.annonc.2023.09.2752
25. Dorff TB, Peer A, Sharma MR, et al. **A phase 1, first-in-human (FIH) study evaluating the safety, pharmacokinetics, and efficacy of ABBV-969 in patients with metastatic castration-resistant prostate cancer (mCRPC).** *J Clin Oncol*. 2026;44(16_suppl):5014-5014. doi:10.1200/JCO.2026.44.16_suppl.5014
26. Stultz J, Fong L. **How to turn up the heat on the cold immune microenvironment of metastatic prostate cancer.** *Prostate Cancer Prostatic Dis.* 2021;24(3):697-717. doi:10.1038/s41391-021-00340-5
27. Venkatachalam S, McFarland TR, Agarwal N, Swami U. **Immune Checkpoint Inhibitors in Prostate Cancer.** *Cancers*. 2021;13(9):2187. doi:10.3390/cancers13092187
28. Li M, Kwantwi LB, Wang C, Xiao Q. **Tumor microenvironment-mediated immune evasion and resistance in prostate cancer: mechanisms, cross-talk, and therapeutic opportunities**. *Clin Exp Med*. 2025;26(1):60. doi:10.1007/s10238-025-01944-0
29. Lu X, Horner JW, Paul E, et al. **Effective combinatorial immunotherapy for castration-resistant prostate cancer.** *Nature*. 2017;543(7647):728-732. doi:10.1038/nature21676
30. Narayan V, Barber-Rotenberg JS, Jung IY, et al. **PSMA-targeting TGFβ-insensitive armored CAR T cells in metastatic castration-resistant prostate cancer: a phase 1 trial**. *Nat Med.* 2022;28(4):724-734. doi:10.1038/s41591-022-01726-1
31. Slovin SF, Gao X, Wei XX, et al. **Phase I Trial of P-PSMA-101 CAR T Cells in Patients with Metastatic Castration-Resistant Prostate Cancer.** *Clin Cancer Res.* Published online March 31, 2026:OF1-OF12. doi:10.1158/1078-0432.CCR-25-3052
32. Mohanty S, Yun H, Gomez A, et al. **256 Preclinical development of AB-3028, a logic-gated engineered CAR T cell product intended for the treatment of metastatic castration-resistant prostate cancer (mCRPC)**. *J Immunother Cancer.* 2025;13(Suppl 2). doi:10.1136/jitc-2025-SITC2025.0256
33. Arsenal Biosciences, Inc. **An Open-Label, Multicenter Phase 1/2 Study to Evaluate the Safety and Efficacy of AB-3028 in Patients With Castration Resistant Prostate Cancer (CRPC).** *clinicaltrials.gov*; 2026. Accessed June 24, 2026. https://clinicaltrials.gov/study/NCT07285694

34. Palecki J, Bhasin A, Bernstein A, et al. **T-Cell redirecting bispecific antibodies: a review of a novel class of immuno-oncology for advanced prostate cancer.** *Cancer Biol Ther.* 2024;25(1):2356820. doi:10.1080/15384047.2024.2356820
35. Hummel HD, Kufer P, Grüllich C, et al. **Pasotuxizumab, a Bite® Immune Therapy for Castration-Resistant Prostate Cancer: Phase I, Dose-Escalation Study Findings**. *Immunotherapy*. 2021;13(2):125-141. doi:10.2217/imt-2020-0256
36. Lim EA, Schweizer MT, Chi KN, et al. **Phase 1 Study of Safety and Preliminary Clinical Activity of JNJ-63898081, a PSMA and CD3 Bispecific Antibody, for Metastatic Castration-Resistant Prostate Cancer.** *Clin Genitourin Cancer.* 2023;21(3):366-375. doi:10.1016/j.clgc.2023.02.010
37. Dorff T, Horvath LG, Autio K, et al. **A Phase I Study of Acapatamab, a Half-life Extended, PSMA-Targeting Bispecific T-cell Engager for Metastatic Castration-Resistant Prostate Cancer.** *Clin Cancer Res.* 2024;30(8):1488-1500. doi:10.1158/1078-0432.CCR-23-2978
38. Vir Biotechnology, Inc. **Vir Biotechnology Reports Positive Updated Phase 1 Results for PSMA-targeting, PRO-XTEN® Dual-masked T-Cell Engager VIR-5500 in Patients with Metastatic Prostate Cancer.** *Vir Biotechnology Investor Relations.* February 23, 2026. Accessed June 9, 2026. https://investors.vir.bio/news/news-details/2026/Vir-Biotechnology-Reports-Positive-Updated-Phase-1-Results-for-PSMA-targeting-PRO-XTEN-Dual-masked-T-Cell-Engager-VIR-5500-in-Patients-with-Metastatic-Prostate-Cancer/default.aspx
39. Janux Therapeutics, Inc. **Janux Announces Encouraging Efficacy and Safety Profile from Ongoing Phase 1 Clinical Trial for JANX007 in mCRPC**. *Janux Therapeutics Investor Relations.* December 1, 2025. Accessed May 31, 2026. https://investors.januxrx.com/investor-media/news/news-details/2025/Janux-Announces-Encouraging-Efficacy-and-Safety-Profile-from-Ongoing-Phase-1-Clinical-Trial-for-JANX007-in-mCRPC/default.aspx
40. Janux Therapeutics. **A Phase 1, Open-Label, Multicenter Study of JANX007 in Subjects With Metastatic Castration-Resistant Prostate Cancer.** *clinicaltrials.gov*; 2026. Accessed June 9, 2026. https://clinicaltrials.gov/study/NCT05519449
41. Siddiqui BA, Zhang J, Carneiro BA, et al. **Abstract CT064: Updated safety and efficacy results from a phase 1/2 study of nezastomig, a first-in-class co-stimulatory PSMA×CD28 bispecific antibody (bsAb), plus cemiplimab (anti-PD-1) in patients (pts) with metastatic castration-resistant prostate cancer (mCRPC)**. *Cancer Res.* 2025;85(8_Supplement_2):CT064. doi:10.1158/1538-7445.AM2025-CT064
42. Sayar E, Patel RA, Coleman IM, et al. **Reversible epigenetic alterations mediate PSMA expression heterogeneity in advanced metastatic prostate cancer.** *JCI Insight.* 2023;8(7). doi:10.1172/jci.insight.162907
43. Angappulige DH, Barashi NS, Pickersgill N, et al. **Prostate-Specific Membrane Antigen–Targeted Imaging and Its Correlation with HOXB13 Expression**. *J Nucl Med*. Published online June 27, 2024. doi:10.2967/jnumed.123.267301
44. Aggarwal R, Starzinski S, de Kouchkovsky I, et al. **Single-dose 177Lu-PSMA-617 followed by maintenance pembrolizumab in patients with metastatic castration-resistant prostate cancer: an open-label, dose-expansion, phase 1 trial.** *Lancet Oncol.* 2023;24(11):1266-1276. doi:10.1016/S1470-2045(23)00451-5
45. Hong J, Bae S, Cavinato L, et al. **Deciphering the effects of radiopharmaceutical therapy in the tumor microenvironment of prostate cancer: an in silico exploration with spatial transcriptomics**. *Theranostics*. 2024;14(18):7122-7139. doi:10.7150/thno.99516
46. Emmett L, Subramaniam S, Crumbaker M, et al. **Overall survival and quality of life with [177Lu]Lu-PSMA-617 plus enzalutamide versus enzalutamide alone in metastatic castration-resistant prostate cancer (ENZA-p): secondary outcomes from a multicentre, open-label, randomised, phase 2 trial**. *Lancet Oncol.* 2025;26(3):291-299. doi:10.1016/S1470-2045(25)00009-9
47. Sandhu S, Subramaniam S, Thomas H, et al. **177Lu-PSMA-617 with ipilimumab (ipi) and nivolumab (nivo) in metastatic castration-resistant prostate cancer (mCRPC):**

**An investigator-initiated phase 2 trial (EVOLUTION; ANZUP2001).** *J Clin Oncol.* 2025;43(16_suppl):5016-5016. doi:10.1200/JCO.2025.43.16_suppl.5016

48. Mei S, Zhang H, Hirz T, et al. **Single-Cell and Spatial Transcriptomics Reveal a Tumor-Associated Macrophage Subpopulation that Mediates Prostate Cancer Progression and Metastasis.** *Mol Cancer Res.* 2025;23(7):653-665. doi:10.1158/1541-7786.MCR-24-0791
49. Kiviaho A, Eerola SK, Kallio HML, et al. **Single cell and spatial transcriptomics highlight the interaction of club-like cells with immunosuppressive myeloid cells in prostate cancer**. *Nat Commun*. 2024;15(1):9949. doi:10.1038/s41467-024-54364-1