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Novel approaches for detection and targeted therapy of prostate cancer using antibodies, aptamers, and nanobodies

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Prostate cancer is the most frequently diagnosed malignancy in men and the second most common cancer globally, with significant annual incidence and mortality rates. Although benign in its early stages, it can progress to advanced and metastatic forms if not detected and treated early. Thus, early detection significantly improves treatment outcomes, relying primarily on screening for specific biomarkers such as prostate-specific antigen (PSA) and prostate-specific membrane antigen (PSMA) using antibodies, aptamers, and nanobodies. These small biomolecules offer notable advantages over conventional diagnostic and therapeutic approaches and are highly effective in targeting PSA and PSMA in prostate cancer. Each biomolecule possesses unique strengths and weaknesses, making them valuable tools for biomedical applications. To date, numerous anti-PSA and anti-PSMA diagnostic and therapeutic strategies, using antibodies, aptamers, and nanobodies, either in free form or conjugated with toxins or radionuclides, have been investigated in both preclinical and clinical studies. This review explores the fundamentals, diagnostic and therapeutic applications in prostate cancer, and the challenges and potential solutions associated with antibodies, aptamers, and nanobodies. Subsequent sections comparatively analyze these biomolecules in terms of stability, cost, and clinical application, highlighting both complementary advantages and critical limitations. Finally, we explore the integration of computational biology, artificial intelligence (AI), machine learning (ML), and deep learning (DL) into prostate cancer diagnosis and therapy to enhance early detection and improve the performance of anti-PSA and anti-PSMA molecules for future advancements.

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1. Introduction

Prostate cancer originates in the prostate gland and is one of the most prevalent malignancies in men, particularly those over 50 years of age.[1,2] In many cases, it initially exhibits a benign nature and remains asymptomatic, progressing slowly during the early stages without causing significant clinical complaints. Such cases may be managed with minimal or even no medical intervention. However, in some instances, prostate cancer can become aggressive and metastatic, rapidly spreading throughout the body.[3] Clinical manifestations range from urinary difficulties such as: pain, increased frequency, and nocturia in the early stages, to urinary retention and back pain in advanced and metastatic stages. These symptoms primarily arise from prostatic hypertrophy.[2,4] According to the global cancer observatory (2022), prostate cancer is the most frequently diagnosed malignancy in men and the second most common cancer worldwide, with approximately 1.4 million new cases and more than 375 000 deaths annually.[5] The highest incidence rates are reported in developed Western countries, including North America and Northern and Western Europe. In contrast, mortality rates are disproportionately higher in low- and middle-income countries due to limited access to advanced screening, diagnostic techniques, and comprehensive medical care.[6,7] The latest statistics reveal that prostate cancer accounts for 29% of all cancers among men.[8]

Given the growing global burden of prostate cancer, effective diagnostic strategies have become essential for enabling early intervention and improving patient outcomes.[9] Current diagnostic tools include the prostate-specific antigen (PSA) test, digital rectal examination (DRE), prostate biopsy, multiparametric

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magnetic resonance imaging (mpMRI), transrectal ultrasound (TRUS), and advanced imaging modalities such as positron emission tomography (PET) and single-photon emission computed tomography (SPECT).[10] PSA testing and DRE remain the most common and widely applied methods for early detection. PET and SPECT, which utilize radiolabeled tracers, are more accurate for detecting metastatic disease; however, they have limited sensitivity in differentiating recurrent or metastatic lesions, representing a major limitation.[11] Despite advances in diagnostics, the choice of treatment remains heavily dependent on disease stage and aggressiveness. Available treatment options include active surveillance, radical prostatectomy, radiation therapy, hormone therapy, and chemotherapy.[12–15] While these approaches are generally effective for early, localized, and less aggressive disease, advanced and metastatic forms often display resistance.[16,17] Additionally, current detection and treatment methods are frequently invasive, costly, and incapable of reliably distinguishing benign from aggressive tumors or cancerous from non-cancerous tissues. Such limitations not only increase the risk of infection but also reduce quality of life through sexual dysfunction, urinary complications, and pain.[9,17] Therefore, identifying new detection and treatment approaches that target specific markers of prostate cancer could help overcome the limitations and side effects of existing methods.

Classical antibodies have the capability to detect cancer cell markers, but these large molecules are unstable, their production processes are costly and laborious, and they may have issues with random immobilization.[9,18,19] As a result, the design of human-specific antibodies against cancerous antigens has attracted scientists' attention as a precise choice for cancer therapy.[19] For example, the prostate-specific membrane antigen (PSMA) is highly expressed by prostate cells and is one of the most commonly used markers for the detection of prostate cancer.[20] Recent advances in molecular engineering have led to the development of alternative targeting agents such as aptamers and nanobodies, which offer improved stability, lower production costs, and high binding specificity. For example, engineered camelid single-domain antibodies (VHHs, or nanobodies) demonstrate high stability, ease of production, and strong diagnostic sensitivity.[21] In recent years, several PSMA-based approaches have been designed and developed using small molecule inhibitors such as antibodies, aptamers, and nanobodies for the detection of prostate cancer.[22]

In this review, we first discuss the structure, function, and mechanisms of action of antibodies, aptamers, and nanobodies. We then highlight their applications as novel approaches for the detection and targeted therapy of prostate cancer. Finally, we examine the limitations and outline future directions for overcoming current challenges associated with these molecules.

2. Antibodies in prostate cancer detection and therapy

Since PSA is highly expressed by prostate cells, monitoring its levels is considered one of the most effective approaches for the detection and treatment of prostate cancer, particularly in the early stages of the disease. In this section, we first provide an overview of the basic principles of antibodies, followed by their applications in detection and therapy (Fig. 1). Finally, the biomedical limitations of these molecules are discussed in the subsequent sections.

2.1. Overview of antibodies

The antibodies, also called immunoglobulins (Igs), are among the most well-studied Y-shaped glycoproteins.[23] These proteins are key components of the immune system, produced by B lymphocytes in response to the presence of foreign antigens.[24] The basic structure of all human antibodies is similar, consisting of four polypeptide chains: two heavy chains and two light chains.[25,26] These two heavy chains are held together by a disulfide bond, which is formed between two sulfide atoms in the amino acids of each chain. The heavy and light chains of antibodies have two main regions: the variable (V) region are located in N-terminal domains while constant (C) regions are

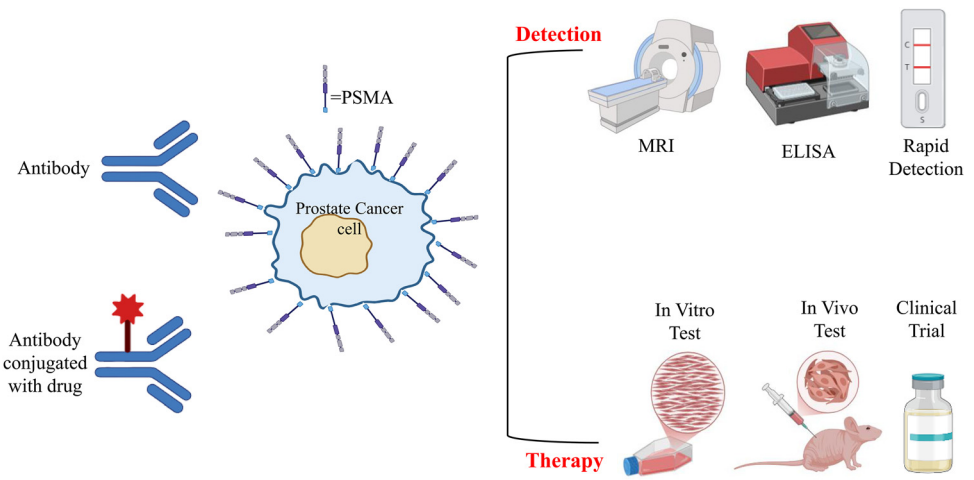

Fig. 1 The role of antibodies in the detection and therapy of prostate cancer.

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placed at the C-terminal domains of antibody.[27] The V regions, which have highly diverse amino acid sequences, are found at the tips of the Y arms and are responsible for the specific recognition of antigenic epitopes, whereas the C region determines the antibody class.[25,28] Structurally, the light chain has only one variable domain (VL) and constant domain (CL), while the heavy chain has one variable domain (VH) which followed by three constant domains named CH1, CH2, and CH3.[25,27] Sequence analysis of variable domains has revealed hypervariable segments, also known as complementarity-determining regions (CDRs), in which certain amino acid residues vary greatly at specific positions.[25,29] Each variable region of both heavy and light chains contains three CDRs (CDR1–CDR3), which vary significantly in sequence and/or length among different antibodies and determine the specificity of antigen–antibody interactions. These hypervariable regions are interspersed with four framework regions (FR1–FR4), which are conserved and show minimal variability compared to CDRs.[28]

Antibodies exist in five main classes (isotypes)—IgG, IgM, IgD, IgA, and IgE—based on differences in the amino acid sequence of the heavy chain constant regions: γ, μ, δ, α, and ε, respectively. In contrast, there are only two light chain isotypes, κ and λ, which have no functional differences. IgG, IgD, and IgE are monomeric and secreted, IgA exists as a monomer or dimer, and IgM forms a pentamer.[25] Upon antigen exposure, B cells differentiate into plasma cells and memory B cells, producing antibodies.[30,31] The antigen-binding sites are located in the variable regions of both heavy and light chains. Structurally, the Y-shaped antibody consists of: (i) one fragment crystallizable (Fc) region, located in the stem, which mediates immune-related functions, and (ii) two fragment antigen-binding (Fab) regions, located in the arms, which are responsible for antigen binding.[25,31,32] IgM is the primary antibody produced during initial immune responses, providing strong binding to pathogens.[33,34] IgG is the most abundant antibody in serum and is produced during secondary immune responses. It plays key roles in placental transfer, complement activation, and pathogen elimination *via* Fc receptor-mediated phagocytosis.[25] IgA provides mucosal protection and is present in secretions such as tears, breast milk, and saliva. IgE mediates allergic responses by activating basophils and mast cells, whereas the function of IgD remains unclear.[31] Most antigens possess multiple epitopes recognized by antibodies from different B cell clones, producing polyclonal antibodies (pAbs)—mixtures of immunoglobulins capable of binding different epitopes on the same antigen. In contrast, monoclonal antibodies (mAbs) are derived from a single B cell clone and recognize only one specific epitope.[35] Both pAbs and mAbs are widely used in the targeted detection and treatment of various cancers and other diseases, including prostate cancer.

2.2. Applications in prostate cancer detection

Building on the structural and functional properties of antibodies described in Section 2.1, numerous PSMA-targeting strategies have emerged over the past two decades as key diagnostic and therapeutic tools in prostate cancer. Various radiolabeled tracers against PSMA have been developed and optimized for imaging and detection in both preclinical and clinical settings.[11] Indium-111 capromab pendetide (^{111}In-labeled, ProstaScint®) was the first and remains the only FDA-approved mAb for prostate cancer detection, selectively binding to an intracellular epitope of PSMA. Its diagnostic performance was evaluated by injecting the labeled antibody into patients, showing 60% sensitivity, 70% negative predictive value, and 60% positive predictive value for soft-tissue metastases. However, its use is limited by low sensitivity and poor detection of bone metastases, primarily because it targets an intracellular epitope, thus binding only to necrotic or apoptotic tumor cells.[22] To overcome these limitations, Pandit-Taskar *et al.* developed ^{111}In-labeled J591, a second-generation mAb targeting extracellular PSMA segments. This approach showed ~93.7% detection of skeletal lesions identified by standard imaging, and in 13 of 18 cases (72.2%), bone metastases were confirmed exclusively with J591.[36] Subsequent developments included 89Zr- and 64Cu-labeled mAbs for PET imaging of nodal, skeletal, and soft-tissue metastases in animal models.[37,38] A phase I clinical trial evaluated 177Lu-labeled J591 (177Lu-J591) in 35 patients, determining biological activity, dose optimization, and toxicity. Sixteen patients received up to three doses, with 70 mCi m^{-2} (single dose) and 45–60 mCi m^{-2} (repeat dose) as maximum tolerated levels; 30 mCi m^{-2} was deemed safe for multiple administrations. Notably, 177Lu-J591 successfully targeted all metastatic sites detected by MRI and topography, with no anti-J591 antibody formation regardless of dosing schedule.[39]

More recently, a dose-escalation study assessed 225Ac-labeled J591 (225Ac-J591), an alpha-emitting radiotherapeutic, in patients with metastatic castration-resistant prostate cancer (mCRPC) who had failed conventional treatments. Among 32 patients, no maximum tolerated dose (MTD) was reached, and 93.3 kBq kg^{-1} was recommended as the phase II dose (RP2D). Safety analysis showed mostly low-grade nonhematologic adverse events, with high-grade effects limited to hematologic toxicity. A single injection demonstrated acceptable safety and preliminary efficacy in imaging mCRPC.[40] In parallel, Jens Cardinale *et al.* developed 18F-PSMA-1007, a PET tracer structurally related to PSMA-617. Following synthesis *via* solid-phase chemistry and prosthetic group coupling, binding assays confirmed high affinity (6.7 ± 1.7 nM) for PSMA-positive LNCaP cells, with 8.0 ± 2.4% ID per g tumor uptake *in vivo*. Small-animal PET clearly visualized LNCaP tumors in mice, suggesting that 18F-PSMA-1007 is a promising, noninvasive PET/CT and PET/MRI tracer, pending large-scale clinical validation. Another innovation involved gold nanoparticle (GNP)-conjugated anti-PSMA antibodies for tumor monitoring *via* X-ray fluorescence CT (XFCT). In mice with subcutaneous prostate tumors, intravenous GNP-antibody injection produced a 4-fold increase in PSMA targeting efficiency and maximum tumor uptake at 24 h post-administration. Combining GNP-antibody conjugates with XFCT markedly enhanced sensitivity, speed, and performance *in vivo*.[41] Beyond these examples, numerous clinical and preclinical investigations have assessed

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Table 1 The summarized details of various studies conducted for detection of prostate cancer *via* anti-PSMA labelled with radiometals and radioligands

Type of study	Subjects (*n*)	Name of radiolabel antibody	Treatment schedule	Main results	Ref.
Clinical phase 1	Patients (7)	^{123}I-MIP-1072 and ^{123}I-MIP-1095	370 MBq (10 mCi) of ^{123}I-MIP-1072 and ^{123}I-MIP-1095 for 2 weeks	Both mAbs detect lesions in soft tissue, bone, and the prostate gland at as early as 1–4 h.	42
Preclinical	Xenograft mice cmodels of prostate cancer (n = 10)	uSPIO-5D3-DM1-AF488	Two dose of 10 mg kg^{-1} at days 1 and 14	MRI images showed that 1 and 24 h postinjection uSPIO-5D3-DM1-CF750 accumulated in the target sites of PSMA(+) tumors and a 42% reduction was seen in PSMA(+) tumors *vs.* 16.7% reduction in PSMA(−) tumors.	43
Preclinical	Murine bearing cancer models	GRFLT-ECG-ROX radiolabelled with Tc-99m	IV injection of 55.5 MBq (200 nM in 150 μl) of radiolabelled mAb *via* the tail vein.	Tc-99m GRFLT-ECG-ROX had binding affinity value (K_d): 9.5 ± 1.3 nM for LNCaP cells and the removal of visible nodules was performed during optical imaging.	44
Phase I/IIa	Patients (17)	^{89}Zr-Df-IAB2M	74 MBq (2 mCi) of target antibody at total mass doses of 10 mg	^{9}Zr-Df-IAB2M was well-tolerated, there were administration related reactions and adverse effects. 0.68 ± 0.08 mSv MBq^{-1} of ^{89}Zr-Df-IAB2M was defined as an effective dose.	45
Preclinical	BALB/c nu/nu mice bearing prostate cancer	125I/^{111}In-capromab	Either 10 MBq of ^{124}I-capromab or 25 MBq of ^{111}In- capromab in 100 μl PBS at 10 μg of protein/animal.	125I/^{111}In-capromab had more rapid clearance of iodine radioactivity from liver, spleen, kidneys, bones, colon tissue, as well as tumors. The highest tumor uptake was reprted at 13 ± 8% ID per g for iodine and 29 ± 9% ID per g for indium. High tumor accumulation and low uptake of radioactivity in normal organs werer detected *via* microPET/CT 5 days post-treatment.	46
Preclinical	BALB/c nude mice bearing prostate cancer	^{111}In-DTPA-D2B-IRDye800CW	IV injection 2 μg, 0.55 MBq per mouse	Micro-SPECT/CT and NIRF imaging showed that ^{111}In-DTPA-D2B-IRDye800CW successfully accumulated in tumor sites of subcutaneous PSMA-positive LNCaP tumors at 168 h after administration and the target antibody caused specific and sensitive tumor detection.	47
Preclinical	Immunodeficient NOG (NOD/SCID/IL-2Rγ^{null}) mice (6- to 10-weeks-old)	X770-scFvD2B	The tail vein injection of 80 μg of X770-scFvD2B was monitored by FRI and FMT at 1, 3, 4 and 10 days	X770-scFvD2B was specifically bound to PSMA and internalized in LNCaP cells. Also, caused a high affinity contrast agent for *in vivo* detection of prostate cancer	48
Clinical	Patients (3)	^{89}Zr-Df-IAB2M	IV injection of 4 MBq (2 mCi) of ^{89}Zr-IAB2M and imaging upon 24 and 48 h using a PET/CT scanner	PET imaging clinical samples showed that there was a positive correlation between the degree of ^{89}Zr-Df-IAB2M uptake and PSMA expression in tumor samples	49

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the diagnostic value of radiometal- and radioligand-labeled anti-PSMA mAbs for imaging various prostate cancer stages. A detailed summary of these studies and their main findings is provided in Table 1.

2.3. Applications in prostate cancer therapy

The number of studies focusing on antibody-based cancer therapies has significantly increased in recent years. These therapeutics, often small molecules, are utilized as monoclonal antibodies (mAbs), either alone or as antibody–drug conjugates (ADCs) conjugated with payloads such as toxins or radionuclides.[50] Monoclonal antibodies exert their anticancer effects by inducing antibody-dependent cellular cytotoxicity (ADCC), leading to the direct destruction of cancer cells by immune cells, such as macrophages and neutrophils. Their efficacy is enhanced when used in conjugated forms, which improve targeted delivery to cancer cells and enhance cell death induction.[50] Prostate-specific membrane antigen (PSMA) is an ideal target for prostate cancer therapy, and numerous PSMA-targeted therapeutic approaches have emerged in recent years.[51] Three main types of radionuclides are used for labeling anti-PSMA antibodies: α-particle emitters (211At, 212Bi, 213Bi, 225Ac), β-particle emitters (67Cu, 90Y, 131I), and Auger electron emitters (111In, 125I). Among these, lower-energy radionuclides like 177Lu are suitable for treating smaller tumors, while higher-energy radionuclides like 90Y are more effective for larger tumors.[52] The radiolabeled antibody capromab (CYT-356) was the first therapeutic anti-PSMA antibody introduced, and its clinical application was investigated in men with metastatic prostate cancer.[52] However, capromab's clinical use was limited due to previously discussed drawbacks.[53] Consequently, next-generation anti-PSMA antibodies were developed, with significant preclinical and clinical focus on J591, a highly effective humanized mAb targeting PSMA. Advances in radiolabeling have enabled successful conjugation of J591 with 90Y and 177Lu for therapeutic purposes.[54,55]

In vitro and *in vivo* studies using 177Lu-J591 in prostate cancer cell lines and NOD mice bearing prostate xenografts demonstrated that both 90Y- and 177Lu-labeled J591 induced antitumor responses, with a clear dose–response relationship. The maximum tolerated dose (MTD) of 177Lu-J591 was significantly higher than that of 90Y-DOTA-J591.[56] Based on these findings, 177Lu-J591 emerged as a promising radiopharmaceutical for prostate cancer treatment. Multiple phase I and II clinical trials were conducted in patients with metastatic castration-resistant prostate cancer (mCRPC) administered 177Lu-J591 starting in October 2000.[56]

Two medical centers conducted a phase II trial of 177Lu-J591 in mCRPC patients, administering 65 mCi m^{-2} and 70 mCi m^{-2} to an initial and second cohort, respectively. The results were promising, with imaging demonstrating accurate targeting of prostate cancer in 94% of patients. The higher-dose cohort showed a greater reduction in prostate-specific antigen (PSA) levels (71% *vs.* 46%). However, thrombocytopenia was the most common toxicity associated with 177Lu-J591, with no reported non-hematologic toxicitie.[57] A subsequent phase I trial evaluated the safety, adverse effects, dose-limiting toxicity (DLT), and MTD of 177Lu-J591 combined with docetaxel in mCRPC patients. Patients received 75 mg m^{-2} of docetaxel every three weeks, alongside escalating fractionated doses of 177Lu-J591 (1.48 GBq m^{-2} up to a maximum of 2.96 GBq m^{-2}) in cycle 3. Treatment-related toxicities, including prolonged myelosuppression, platelet transfusions, febrile neutropenia, and grade ≥ 3 non-hematologic toxicities, were monitored. PSA levels were assessed before each treatment cycle, and serial CT and bone scans were performed. Fifteen mCRPC patients received dose-escalated 177Lu-J591 across four cohorts up to the highest planned dose. Notably, no grade ≥ 3 non-hematologic toxicities or DLTs were observed at any dose. Grade 4 neutropenia without fever occurred in 8/15 (53.3%) patients, and thrombocytopenia in 2/15 (13.3%), with two requiring prophylactic platelet transfusions. PSA levels declined by $>50\%$ in 11/15 (73.3%) patients. The combination of 177Lu-J591 and docetaxel was feasible, with CT and bone scans confirming accurate targeting of all known prostate cancer sites and a strong preliminary efficacy signa.[58]

A dual-center phase II study further evaluated the safety and therapeutic efficacy of 177Lu-J591 in 57 mCRPC patients.[59] Fifteen patients received 65 mCi m^{-2}, while 17 received 70 mCi m^{-2}. Administration of the phase I MTD (70 mCi m^{-2}) resulted in a $>30\%$ PSA decline in a significant proportion of patients and improved median overall survival. However, this dose was associated with increased rates of grade 4 hematologic toxicity and a higher need for platelet transfusions. Following a single dose of 177Lu-J591, 10.6%, 36.2%, and 59.6% of patients experienced $\geq 50\%$, $\geq 30\%$, and any PSA decline, respectively. Importantly, 177Lu-J591 accurately targeted metastatic sites in 93.6% (44/47) of patients and was well-tolerated with reversible myelosuppression and no serious non-hematologic toxicities.[59]

Additional studies aimed to enhance treatment efficacy and reduce adverse effects of radiolabeled antibodies by focusing on PSMA-targeted ADCs, immunotoxins, or nanoparticles. Daniel Peter Petrylak and colleagues investigated the clinical antitumor activity of an anti-PSMA antibody conjugated with monomethyl auristatin E (MMAE) in taxane-refractory mCRPC patients previously treated with taxanes in two separate phase I and II dose-escalation studies.[60,61] Phase I results showed that the conjugated anti-PSMA antibody was well-tolerated at doses up to 2.8 mg kg^{-1}, with 2.5 mg kg^{-1} selected as the MTD for taxane-treated patients. Antitumor effects were observed at the highest tested doses.[60] The phase II study involved 34 patients treated with 2.5 mg kg^{-1} or 2.3 mg kg^{-1} of the conjugated anti-PSMA antibody every three weeks. The 2.3 mg kg^{-1} dose showed a longer treatment duration and fewer grade ≥ 3 drug-related adverse effects (37% *vs.* 59% for 2.5 mg kg^{-1}). This dose was well-tolerated and demonstrated activity in treated patients.[61]

Recently, an engineered ADC, MEDI3726, was developed, comprising J591 mAb conjugated with pyrrolobenzodiazepine dimer (PBD) tesirine, which targets extracellular PSMA, internalizes, and induces cancer cell death by releasing PBD to bind

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DNA. Preclinical studies in cell cultures and xenograft mice bearing LNCaP and castration-resistant CWR22Rv1 prostate tumors demonstrated significant antitumor activity, with increased phosphorylated histone H2AX in tumor models.[62] A phase I clinical study administered MEDI3726 (0.015–0.3 mg kg^{-1} every three weeks) to mCRPC patients resistant to chemotherapeutics like abiraterone or enzalutamide. Results were moderately acceptable, with no MTD identified. Favorable clinical responses were observed at higher doses, with antidrug antibodies detected in only 9.4% (3/32) of patients. However, skin toxicities and effusions were reported in 90.9% (30/33) of patients, with 33.3% (11/33) discontinuing due to these adverse effects.[63]

In a subsequent preclinical study, a tubulin inhibitor-conjugated anti-PSMA antibody, ARX517, was evaluated. This humanized antibody selectively internalized, catabolized, and delivered a cytotoxic payload, inducing apoptosis. *In vivo* studies demonstrated significant tumor growth inhibition in mCRPC mouse models and a tolerable safety profile in monkeys.[64]

Beyond these approaches, antibody-conjugated toxins, or immunotoxins, have emerged as novel therapeutic options targeting prostate cancer biomarkers.[65] For example, P. Wolf and colleagues developed a recombinant immunotoxin, A5-PE40, targeting PSMA-positive prostate cancer cells. A5-PE40 comprises a single-chain antibody fragment (scFv) of an anti-PSMA mAb and a Pseudomonas exotoxin A (PE40) lacking its natural binding domain. The scFv was generated using phage display technology and direct selection on antigen-expressing cells, with the toxin domain expressed and purified in a bacterial system. A5-PE40 selectively bound PSMA-positive cells with potent anticancer activity (IC50 of 20 pM) and no effect on PSMA-negative cells.[66] Similarly, Fayun Zhang and colleagues developed a bivalent immunotoxin comprising a truncated diphtheria toxin (DT) and Fv fragments of an anti-PSMA mAb for imaging and therapeutic purposes. The immunotoxin, A-dmDT390-scfbDb (PSMA), demonstrated selective toxicity and cellular uptake in PSMA-positive cells but not in PSMA-negative cells. It markedly induced apoptosis in LNCaP cells, with uptake correlating with treatment time and dose. MRI and optical imaging in nude mice bearing PSMA-positive tumors confirmed its specific targeting and therapeutic potential.[67]

More recently, an anti-PSMA recombinant immunotoxin, JVM-PE24X7, was developed, consisting of a single-domain antibody (sdAb) and a Pseudomonas exotoxin A (PE24X7) toxin. Produced in a soluble form in *E. coli* to avoid complex renaturation, JVM-PE24X7 exhibited high stability and strong binding affinity to PSMA receptors. *In vitro* studies showed potent cytotoxicity against PSMA-positive cells (EC50 of 15.3 pM) with >300-fold selectivity over PSMA-negative cells. Preclinical data demonstrated complete suppression of prostate cancer cells with an MTD >15 mg kg^{-1}, indicating robust antitumor activity and reduced off-target effects due to the PE24X7 toxin.[68]

Immunotoxin are not only used in monotherapy, but also in combination with other agents.[69] For instance, a preclinical study evaluated an anti-PSMA recombinant immunotoxin combined with docetaxel, the first-line chemotherapeutic for CRPC. The immunotoxin, comprising an anti-PSMA scFv and a truncated *Pseudomonas aeruginosa* exotoxin A (PE40), induced apoptosis and reduced viability in androgen-dependent (LNCaP) and androgen-independent (C4-2) prostate cancer cells. A synergistic anticancer effect was observed with docetaxel, with IC50 values reduced in both LNCaP cultures and SCID mice bearing tumor models.[70]

Another preclinical study combined the immunotoxin J591PE with the pan-PI3K inhibitor ZSTK474 for prostate cancer treatment. J591PE consisted of an anti-PSMA scFv and a truncated *Pseudomonas aeruginosa* exotoxin A (PE38QQR). *In vitro* combination treatment increased apoptosis in PSMA-positive cells (LNCaP, C4-2, C4-2Luc) within 6 hours, with no significant effect on PSMA-negative cells (PC3, BT549). In mice bearing C4-2Luc tumors, a single dose of the combination significantly reduced luminescence within 6 days, suggesting ZSTK474 and J591PE as a promising approach for advanced prostate cancer.[71] Besides the mentioned studies that focused on evaluation of preclinical and clinical-anti-prostate action of antibodies, ADC, and immunotoxins, rest of them are represented in Table 2.

2.4. Challenges and limitations

Since the introduction of indium-111-labeled capromab as the first FDA-approved monoclonal antibody (mAb) for prostate cancer, numerous radiolabeled anti-PSMA mAbs have been developed for diagnostic and therapeutic purposes in preclinical and clinical studies. Although ^{111}In-capromab could detect soft tissue metastases, its application was significantly limited due to low sensitivity and poor capability for detecting bone metastases. These limitations stem from its recognition of intracellular PSMA epitopes, which target only tumor cells undergoing necrotic or apoptotic processes.[22] To address these issues, next-generation radiolabeled anti-PSMA mAbs were developed to target extracellular PSMA domains for improved diagnostic and therapeutic outcomes. Despite advances in monitoring and treating prostate cancer with these mAbs, several challenges persist.[22] The first major limitation is the heterogeneous expression of PSMA among prostate cancer patients and within different tumor sites. PSMA expression levels depend on disease progression, increasing from primary prostate cancer to metastatic castration-resistant prostate cancer (mCRPC). This heterogeneity significantly reduces the sensitivity of radiolabeled anti-PSMA monoclonal antibodies (mAbs) in cancer imaging, leading to suboptimal therapeutic outcomes.[83,84] For example, clinical investigations have estimated that approximately of mCRPC patients exhibit PSMA-negative metastases, significantly limiting the applicability of PSMA-targeted therapies in these cohorts.[85] This heterogeneity necessitates patient stratification strategies, such as pre-treatment PSMA PET imaging, to identify suitable candidates, though such approaches increase diagnostic complexity and costs.[86]

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Table 2 The summarized details of the studies have done for treatment of prostate cancer by anti-PSMA labelled with radiometals and radioligands

Type of study	Subjects (n)	Name of radiolabel antibody	Treatment schedule	Main results	Ref.
Clinical phase II	Patients (55)	J591 labelled with 177Lu or 111In	A single dose of J591 with 2 : 1 randomization to 177Lu (70 mCi m^{-2}) or 111In (5 mCi) in double-blinded fashion for 4 weeks	>50% PSA decline in 82% with 177Lu and 71% with 111In and combination of 177Lu with secondary hormonal therapy improved 18-mo MFS *vs.* to 111In	72
Preclinical	mice bearing LNCaP tumor	AMG 160	6–42 pmol L^{-1}	MG 160 showed potential antitumor activity *in vitro* and *in vivo*	73
Clinical phase II	Patients (55)	J591 labelled with 177Lu or 111In	A single dose of J591 with 2 : 1 randomization to 177Lu (70 mCi m^{-2}) or 111In (5 mCi) in double-blinded fashion for 4 weeks	More than 50% PSA decline in 82% with 177Lu and 71% with 111In and J591 in combination with keto/HC when radiolabeled with 177Lu improved 18-month met-free survival *vs.* 111In	74
Clinical	Patients (3)	PSMAxCD3 antibody in Fab- and IgG-based formats	A daily dose escalation, ranged from 20–40 μg up to 2.6 mg	Upon treatment of three patients with metastasized prostate carcinoma with the IgGsc administration led to significantly T cells activation and rapid reduction of PSA levels	75
Preclinical	NOD/SCID mice bearing prostate tumor (n = 4 in each group)	Anti-PSMA- CAR NK-92	IV injection of 5 × 10^6 CAR NK-92 cells on days 8, 10, 12, 14 and 16, 1 day after PD-L1/PD-1 antibody therapy	Combination of CAR NK-92 and anti-PD-L1 mAb led to improvements in the antitumor activities	76
Clinical phase II	Patients (n : 42)	ADC conjugated with MMAE	IV injection of 0.4 to 2.8 mg kg^{-1} of target antibody over approximately 90 min at 3-week intervals for four cycles	There was neutropenia and peripheral neuropathy and also MTD was 2.5 mg kg^{-1}. Reductions in PSA level and CTCs in patients treated was seen at doses of ≥1.8 mg kg^{-1} of conjugated ADC durable and often concurrent.	77
Preclinical	SCID mouse xenograft bearing prostate cancer	^{177}Lu-DOTA-3/F11	A single dose of 1 MBq of ^{177}Lu-DOTA-3/F11	A single dose of 1 MBq of target antibody inhibited tumor growth and prolonged survival rate. Also, an increasing tumor uptake of ^{177}Lu DOTA-3/F11 was seen over time with maximum tumor-to-muscle and tumor-to-blood ratios at three days post treatment.	78
Clinical	Patients (3)	^{89}Zr-Df-IAB2M	IV injection of 4 MBq (2 mCi) of ^{89}Zr-IAB2M and imaging upon 24 and 48 h using a PET/CT scanner	PET imaging clinical samples showed that there was a positive correlation between the degree of ^{89}Zr-Df-IAB2M uptake and PSMA expression in tumor samples	49
Preclinical	Xenograft model	JVM-PE24X7	EC_{50} value of 15.3 pM against PSMA-positive LNCaP cells	5 × 0.5 mg kg^{-1} once every three days completely suppressed the growth of prostate cancer	68
Preclinical	LNCaP, C4-2, and PC-3 cell lines and male SCID CB17/lcr-Prkdc scid/Crl mice	Immunotoxin hD7-1(VL-VH)-PE40	Combination regime of the BAD-like mimetic ABT-737 and immunotoxin hD7-1(VL-VH)-PE40	The combination regime inhibited Bcl-2, Bcl-xl, and Bcl-w, which led to apoptosis induction in PSMA-positive cells. Also, a significantly prolonged survival rate and inhibition of tumor growth were seen mice bearing cancer models.	79
Preclinical	C4-2 cells and mice xenografts bearing cancer model	Immunotoxin A5-PE40	*In vitro*: 50 ml per well of the immunotoxin A5-PE40. *In vivo*: IV injection of single doses between 1 μg and 20 μg of A5-PE40	A5-PE40 specifically target and bound to C4-2 cells and showed a dose dependent-cytotoxicity at IC_{50} of 220 pM. The treatment of mice models led to a significant suppression of tumor growth was seen in C4-2 xenografts *versus* DU145 xenografts.	80
Preclinical	C4-2 cells and mice xenografts bearing cancer model	Immunotoxin D7-PE40	*In vitro*: 10 to 25 nM per well of D7-PE40. *In vivo*: IV injection of various single doses between 0 and 40 μg in 500-mL PBS of A5-PE40	D7-PE40 selectively targeted C4-2 cells at IC_{50} of 140 pM. The treatment of mice bearing C4-2 tumor models led to a significant inhibition of tumor growth, while there was no anticancer effect in mice with PSMA-negative DU 145 tumor models.	81
Preclinical	LNCaP, CWR22Rv1, and PC-3 cell lines and athymic male nude mice	hJ591–SAZAP conjugate	*In vitro*: 10 μg ml^{-1} of hJ591 alone, 100 nM of unconjugated saporin, and 25 nM of hJ591–SAZAP for 72 h *In vivo*: 200 ml PBS solution containing 37.5 μg of hJ591 plus 7.5 μg of saporin per mouse	hJ591–SAZAP internalization into PSMA-positive at IC_{50} of 0.14 nM, 1.99 nM, and 100 nM in LNCaP, CWR22Rv1, and PC-3 cells, respectively. hJ591–SAZAP showed anticancer action in LNCaP mice models	82

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Another critical challenge is the toxicity profile of radiolabeled anti-PSMA mAbs. Clinical and pre-clinical studies have reported significant side effects, including prolonged myelosuppression, febrile neutropenia and grade ≥ 3 non-hematologic toxicities, associated with agents such as 225Ac-J591, 18F-PSMA-1007, 90Y-DOTA-J591, and 177Lu-J591.[39,40,56,57,87]

For instance, phase II trials of 177Lu-J591 demonstrated PSA decreases of $\geq 50\%$ in approximately 10.6–73.3% of patients, however, grade 4 thrombocytopenia was observed in up to 13.3% of cases, with 2/15 patients requiring platelet transfusions in one study.[59] Likewise, 225Ac-J591, while achieving higher PSA response rates (up to 80% in some cohorts), was associated with severe salivary gland toxicity in 30% of patients, limiting dose escalation.[88] These outcomes highlight a excahnged between efficacy and toxicity, where higher therapeutic doses often correlate with increased adverse events. The small sample sizes in these trials (*e.g.*, 15–57 patients) further limit generalizability, underscoring the need for larger, multicenter studies to establish robust safety profiles.[89,90]

Another drawback is the non-specific uptake of radiolabeled anti-PSMA mAbs by non-target organs and their slow clearance, which delays imaging in prostate cancer patients. This can result in false-positive imaging outcomes and unwanted treatment-related toxicities.[52] For instance, a phase I study of BAY 1075553 demonstrated its ability to detect prostate cancer cells, lymph node metastases, and bone metastases; however, its high uptake in degenerative bone lesions may limit its utility for evaluating bone disease.[91] Similarly, non-specific uptake in the liver and spleen reduces the signal-to-noise ratio in during of imaging, with some studies reporting up to 25% of administered 177Lu-PSMA-617 accumulating in non-target tissues, necessitating improved radiolabeling techniques or organ-protective agents.[92]

Furthermore, the large size of antibodies hinders their penetration into tumor tissue, particularly in solid tumors, significantly reducing their therapeutic potential. To overcome this, approaches using antibody fragments, such as minibodies or small bivalent antibody fragments, have been developed and tested in clinical studies.[93] Additionally, some anti-PSMA mAbs have been discontinued in clinical trials due to high immunogenicity and allergic reactions, despite promising preclinical results. Anti-drug antibodies (ADAs) may develop during intravenous administration in some patients, limiting therapeutic efficacy.[94] Moreover, the biomedical application of these mAbs is constrained by complex production processes and high costs.[94,95] The production of radiolabeled mAbs requires specialized facilities, stringent quality control, and compliance with good manufacturing practice (GMP) standards, with costs estimated to be 10–20 times greater than those of small-molecule drugs.[96] Moreover, the requirement for personalized dosing based on PSMA expression introduces logistical difficulties, especially in settings with limited resources.[97] Removing these limitations requires innovative approaches, such as bispecific antibodies to target heterogeneous tumors, organ-protective strategies to reduce toxicities, and streamlined production to lower costs, all of which are critical for advancing anti-PSMA mAb therapies.[98]

3. Aptamers in prostate cancer detection and therapy

Aptamers represent a promising class of molecules for the diagnosis and treatment of prostate cancer due to their unique properties and versatility. This section provides an overview of aptamers, focusing on their development as small oligonucleotides, followed by a detailed discussion of anti-prostate-specific antigen (PSA) and anti-prostate-specific membrane antigen (PSMA) aptamers for prostate cancer monitoring and therapy. Despite their significant therapeutic and diagnostic potential, clinical translation of aptamers faces several challenges, which are addressed at the end of this section.

3.1. Overview of aptamers

Aptamers are short, single-stranded DNA or RNA oligonucleotides (typically 20–100 nucleotides) that adopt specific three-dimensional conformations, enabling them to bind selectively and with high affinity to diverse targets, including small molecules, proteins, cell surface receptors, and whole cells.[99–101] The SELEX process involves three core steps: selection, partitioning, and amplification.[102,103] Today, SELEX remains the cornerstone of aptamer development, enabling the generation of highly specific binders for applications in cancer diagnostics and therapy, including prostate cancer (Fig. 2).[104,105]

The SELEX process involves three core steps: selection, partitioning, and amplification.[106,107] Briefly, a random oligonucleotide library, comprising approximately 10^{12} to 10^{14} unique sequences of 20–80 nucleotides, is incubated with a target molecule, such as prostate-specific antigen (PSA) or prostate-specific membrane antigen (PSMA). Non-binding sequences are removed through washing, while target-bound sequences are recovered and amplified *via* polymerase chain reaction (PCR) for subsequent rounds of selection. Typically,

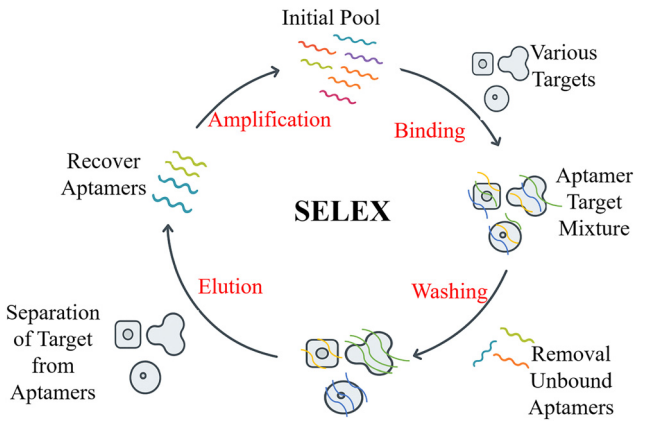

Fig. 2 SELEX procedure for isolation of specific aptamers. For detail, see Section 3.1.

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8–15 rounds are performed to enrich high-affinity Aptamers.[108] A negative selection step can be incorporated to enhance specificity by eliminating sequences that bind non-target molecules, such as serum proteins, ensuring minimal off-target interactions in complex biological environments.[109] Advanced SELEX variants, such as cell-SELEX and *in vivo* SELEX, have been developed to select aptamers directly against cancer cells or tissues, improving their relevance for clinical applications.[110]

Aptamers offer several distinct advantages in cancer diagnostics and therapy. Their high specificity and affinity enable precise recognition of cancer biomarkers like PSA and PSMA.[111] Structurally, aptamers form secondary elements, such as hairpins, internal loops, and bulges, which contribute to their flexibility and create specific binding pockets for target recognition. Tertiary structures, including pseudoknots, base triples, and stacking interactions, further stabilize these conformations, enhancing binding affinity (with dissociation constants, K_d, often in the picomolar to nanomolar range). For example, the A10 anti-PSMA RNA aptamer forms a stable hairpin structure that binds PSMA with a K_d of approximately 2 nM, making it highly effective for targeting prostate cancer cells.[112]

3.2. Applications in prostate cancer detection

Due to their favorable characteristics, aptamers have garnered attention in recent years for the detection and imaging of prostate cancer, particularly by targeting PSA and PSMA. For instance, Ye Zhu and colleagues developed an ultrasensitive electrochemical assay by combining rolling circle amplification (RCA) with poly(thymine)-templated copper nanoparticles (CuNPs). A gold nanoparticle–aptamer–primer bioconjugate was designed for PSA detection using a sandwich-type format. This approach achieved a linear detection range of 0.05–500 fg mL^{-1} with a limit of detection (LOD) of 0.020 ± 0.001 fg mL^{-1}. It also demonstrated promising sensitivity a nd specificity in human serum samples, suggesting strong potential for clinical applications. The obtained results showed that this method is easy, specific, and ultrasensitive and also has great potential for detection of prostate cancer in patients.[113] In an *in silico* study, several DNA aptamers (PSAG221, ΔPSap4#5, and truncated forms of TA87) were evaluated for PSA binding. The molecular dynamics (MD) simulations offered that PSAG221 enhanced PSA affinity over ΔPSap4#5. As well as, the affinity constant (KD) values for the PSA was recorded 0.35, 0.33, 0.35, 0.56, 0.45, and 0.51 μM^{-1} for PSAp45, PSAG221, TA87, TA87M24, and TA87M49, respectively. The experimental findings revealed that aptamer PSAG221 had good PSA affinity, while its affinity was slightly inferior to ΔPSap4#5. Comparison of the tested aptamers showed that lowest PSA affinity was belonged to the two mutations, TA87M24, TA87M49, the truncated aptamers, and TA87. PSAG221 reported as a new and alternative probe for generation of anti-PSA aptamer platforms.[114]

Newly, a preclinical study introduced a dual-modality imaging probe ([^{68}Ga]Ga-NOTA-PSMA-Cy5) for both PET and NIRF imaging. They used NOTA-PSMA-Cy5 as probe precursor for generation of PET/NIRF dual-modality probe [^{68}Ga]Ga-NOTA-PSMA-Cy5. In the next step, *in vitro* binding specificity and capability of the generated probe to PSMA checked through flow cytometry, fluorescence imaging, and cellular uptake experiments in positive and negative PSMA cells. The obtained preclinical PET/NIRF imaging data showed a great specific and sensitive [^{68}Ga]Ga-NOTA-PSMA-Cy5 to PSMA. The authors concluded that this probe could be a viable tool for fluorescence-guided prostate cancer diagnostics.[115] In another work, an anti-PSA aptamer-based electrochemical biosensor was developed for highly sensitive detection of prostate cancer using a composite of a two-dimensional (2D):2D:2D reduced graphene oxide (rGO), graphitic carbon nitride (g-C_3N_4), and AuNPs. The aptamer was immobilized on a glassy carbon electrode (GCE), and the biosensor exhibited high selectivity and sensitivity in complex media containing fetal bovine serum (FBS), glucose, and bovine serum albumin (BSA). It achieved an LOD of 0.44 fM and a limit of quantification (LOQ) of 2.5 fM within 30 minutes.[116]

Fei Gao and colleagues engineered two label-free aptamer-based biosensing approach including, a single-ended clamped microcantilever biosensor and a qual-ended clamped with serpentine cantilever for detection of PSA. They immobilized the desired aptamers on the gold surface of the cantilever biosensor to record the stress change generated by aptamer and PSA binding. Both sensors showed linear response ranges from 50–2000 ng mL^{-1}, with LODs of 9.88 ng mL^{-1} and 7.08 ng mL^{-1}, respectively.[117] Last year, an aggregation-induced emission (AIE)-based aptasensor with double sensing site (a specific binding site with PSA and a ligand for targeting of PSMA) was also developed for simultaneous detection of PSA and PSMA. Functionally, in the presence of PSA, the specific attachment of free antigen with PSA led to the creation of fluorescence signal and PSA sensing. As well as, the present aptasensor was able to specific detection of PSMA by binding PSMA-targeted ligand of biosensor. It produced fluorescence signals upon specific binding to either marker, achieving LODs of 6.18 pg mL^{-1} for PSA and 8.79 pg mL^{-1} for PSMA. This method was proposed for early screening of this cancer in men population due to its high specificity and sensitivity.[118]

Beyond direct biomarker detection, aptamers have also been employed in targeting extracellular vesicles (EVs), particularly exosomes, for prostate cancer diagnosis. One study reported a capacitance-based electrochemical aptasensor that targeted CD63 (a general exosome marker) and PSMA. Structurally, the aptasensor was fabricated using rGO/MoS_2-modified screen-printed carbon electrodes and detected biomarkers in less than 15 minutes using only 10 μL of EV samples. The aptasensor introduced as a highly specific and sensitive and reagent-free technique for point-of-care diagnosis of prostate cancer. It achieved LODs of 4.83×10^2 EVs per μL for PSMA and 1.47×10^3 EVs per μL for CD63, without signal amplification. Further validation using urinary EVs and comparison with a commercial PSMA ELISA kit showed strong agreement.[119] Due to the growing volume of literature on aptamer-based detection of

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Table 3 The list of the aptamer-based diagnostic approaches against PSMA and PSA biomarkers of prostate cancer

Type of study	Aptamer name	Nanoparticles	Target	Main results	Ref.
In vitro	Thiolated aptamers for PSA and VEGF	Gold electrodes	PSA and VEGF	The aptamer could detect PSA with great sensitivity in linear detection ranges 0.08–100 ng mL^{-1} for PSA	120
In vitro	Triggerable aptamer-based nanostructure based on ssDNA/gold nanoparticle	AuNPs	PSMA	aPSMA-AuNPs-aggregates detected 50 pM of PSMA	121
In vitro	Nuclease-stabilized RNA aptamers (xPSM-A9 and xPSM-A10)	—	PSMA	xPSM-A9 inhibited PSMA with an average K_i of 2.1 nm, but xPSM-A10 targeted with an average K_i of 11.9 nm.	122
Preclinical	A10-3.2	Lipid targeted nanobubble	PSMA	The targeted nanobubbles aggregated in C4-2 xenograft tumors. Also, A10-3.2 aptamer selectively targeted prostate cancer tissues and cells.	123
Computational	Truncated RNA aptamer (A9L; 41mer)	—	PSMA	According to the computational predictions, A9L; 41mer showed specific binding activity to PSMA.	124
Human sample and *in vitro*	Anti-PSMA-aptamer Apt3	AuNPs	PSMA	Anti-PSMA-aptamer Apt3 could detect with sensitivity—94%, specificity—76%, and accuracy—87%	125
In vitro	Chronoimpedimetric anti-PSMA aptasensor	Gold nanoparticle	PSMA	The aptamer detected PSAM in real serum samples with a linear range from 1 to 40 cells per mL with LOD of 0.62 cells per mL.	126
In vitro	High-throughput micro-sampling unit (HTMSU) anti-PSMA aptasensor	Gold nanoparticle	PSMA	The constructed microchip PSMA on the surface of LNCaP cells in linear flow rate of 2.5 mm s^{-1} during 29 min	127
In silico and *in vitro*	A9g RNA aptamer	Gold nanoparticles	PSMA	A9g RNA aptamer showed selective and competitive action for targeting of prostate cancer cells	128
Human sample and *in vitro*	QDs-Ab/PSA/AuNPs-PAMA/aptamer	AuNPs	PSA	Their aptamer detected PSA in linear range of 0.01 ng mL^{-1} to 100 ng mL^{-1} with LOD of 1 pg mL^{-1}	129

VEGF: vascular endothelial growth factor, quantum dots: QDs, gold nanoparticles: AuNP.

prostate cancer *via* PSA and PSMA, additional studies are summarized in Table 3.

3.3. Applications in prostate cancer therapy

Since the introduction of the SELEX process for generating aptamers, these small oligonucleotides have been extensively used in various biological fields, including the development of new therapeutics for prostate cancer, as summarized in Table 4. Farokhzad *et al.* designed the first nanoparticle-RNA-PSMA aptamer conjugates for specific targeting of prostate cancer cells. The studied nanoparticles (NPs) have three valuable features: (1) a negative surface charge, which reduces nonspecific interactions; (2) the presence of carboxylic acid groups on the particle surface, enabling potential modifications and covalent conjugations; and (3) the presence of PEG on the particle surface, which increases circulating half-life and reduces off-target uptake. The results showed that the generated anti-PSMA nanoparticle-RNA aptamer bioconjugates efficiently targeted and delivered to LNCaP cells, which are PSMA-positive, with a 77-fold increase in binding compared to the negative control. Conversely, cellular uptake of these NPs was not increased in PSMA-negative cells.[130] In subsequent years, Dhar *et al.* constructed aptamer-functionalized Pt(IV)-encapsulated PSMA-targeted NPs composed of poly(DL-lactic-*co*-glycolic acid) (PLGA)–poly(ethylene glycol) (PEG) to reduce dose toxicity and deliver cisplatin specifically to prostate cancer. They demonstrated that the aptamer-functionalized Pt(IV) prodrug-PLGA–PEG NPs specifically delivered cisplatin into PSMA-positive LNCaP cells, showing marked differences between NPs with or without aptamers targeting PSMA.[131] Another early report described anti-PSMA-RNA aptamers generated on gold nanoparticle surfaces for therapeutic and diagnostic purposes. Therapeutic evaluation of gold nanoparticles conjugated with PSMA aptamer was performed after loading with doxorubicin (DOX). The results showed significant cytotoxic effects against PSMA-positive LNCaP cells compared to PSMA-negative PC3 cells.[132]

Profiling of miRNAs in various cancers revealed that miR-15a and miR-16-1 can act as tumor suppressors or oncogenes; however, in prostate cancer, they function as tumor suppressor genes. Therefore, delivery of these miRNAs to prostate cancer cells can be considered a safe and effective therapeutic approach. Although RNA aptamer A10 has been extensively used as a ligand for PSMA targeting, second-generation aptamers such as A10-3.2 are more efficient. Accordingly, an anti-PSMA-A10-3.2 aptamer was designed using polyamidoamine (PAMAM) dendrimers to carry miR-15a and miR-16-1. PAMAM was conjugated with the aptamer (PAMAM-PEG-APT) *via* a polyethylene glycol (PEG) spacer to construct a miRNA-targeted delivery system. PSMA-positive LNCaP and PSMA-negative PC3 cells were used as model and control cell lines, respectively, to assess specificity. Luciferase assay data showed that transfection efficiency of the DNA/PAMAM-PEG-APT system was significantly higher than that of DNA/PAMAM-PEG. *In vitro* cell viability assays revealed that the IC_{50} value of the miRNA/PAMAM-PEG-APT system was approximately 4.7-fold

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Table 4 The detailed list of the aptamers to target PSMA and PSA biomarkers and prostate cancer treatment

Type of study	Treatment strategy	Nanoparticles	Target	Main results	Ref.
Preclinical	Anti-PSMA aptamer-decorated nanoparticles for DTX delivery (DTX-apt-NPs)	Polymeric nanoparticles	PSMA	DTX-apt-NPs significantly enhanced *in vitro* antitumor effect and cellular uptake. DTX-apt-NPs delivery system was effective to drug delivery to tumor site and enhance antitumor efficacy.	140
In vitro	Anti-PSMA-aptamer conjugated cationic-lipid coated mesoporous silica platform for targeted delivery of PTX	Mesoporous silica nanoparticles	PSMA	The target aptamer based delivery strategy promoted of cellular uptake of particles by LNCaP cells and led to around 80% of cell death compared with pure PTX.	141
In vitro	Aptamer-conjugated polyplexes (APs)	Polyethylene glycol (PEI–PEG)	PSMA	Co-delivery of an anti-cancer drug and shRNA may allow for the selective destruction of cancer cells	142
Preclinical	ΔPSap4#5	Poly(lactic-*co*-glycolic acid) (PLGA)	PSMA	pt-ABR-NP therapy minimized off-target cytotoxicity, reduced drug loss due to site-specific delivery	143
In vitro	RNA aptamer conjugate toxin (gelonin)	—	PSMA	The toxin conjugate RNA aptamer selectively lysed PSMA-positive cancer cells with an IC_{50} of 27 nmol L^{-1} and showed an increase in toxicity of 600-fold in comparison with PSMA-negative cells	137
In vitro	A RNA–DNA hybrid aptamer (A10-3-J1)	Super paramagnetic iron oxide nanoparticles	PSMA	SPIO-Apt-Dox specifically targeted the PSMA+ cells and inhibited nonspecific uptake of DOX to the non-target cells	144
Computational	Truncated RNA aptamer (A9L; 41mer)	—	PSMA	Computational modeling and dockings of A9L predicated that it could bind to PSMA and inhibit its enzymatic activity	124

PTX: paclitaxel, DTX: doxorubicin, apt: aptamer.

lower than that of miRNA/PAMAM-PEG.[133] In another study, an A9g RNA aptamer was developed and evaluated preclinically as a smart drug for prostate cancer metastases by inhibiting PSMA enzymatic activity. Treatment with this aptamer reduced cancer cell migration and invasion *in vitro* and metastasis in animal models. Remarkably, A9g was non-toxic and safe in murine models bearing prostate cancer and was non-immunogenic in human cells. Biodistribution studies showed that A9g specifically targeted cancer tissues without off-target effects, suggesting its potential as a smart drug for advanced prostate cancer.[134]

Kyoungin Min and colleagues designed a dual-aptamer system for specific targeting of both PSMA-positive cells *via* an A10 RNA aptamer and PSMA-negative cancer cells through a DUP-1 peptide aptamer. DOX was loaded onto the stem region of the A10 RNA aptamer to induce apoptosis in both cell types. They used a rapid and simple electrochemical technique to assess *in vitro* cell death and a transparent indium tin oxide (ITO) glass electrode for direct observation of treated cells. A10 and DUP-1 aptamers were immobilized for *in vivo* therapeutic applications. The dual-aptamer system effectively delivered DOX to cancer cells and enhanced cellular uptake.[135] A novel quantum dot (QD)-labeled anti-PSMA aptamer conjugated to pH-responsive niosomal particles was developed for docetaxel (DTX) delivery, aiming to reduce systemic toxicity. Aptamer A10 was conjugated to niosomes *via* disulfide bonds. CdSe/ZnS QDs functionalized with mercapto propanoic acid (MPA) ligands were bound to the 3′ terminal of the aptamer sequence to complete the fabrication of the conjugated aptamer. Characterization confirmed a homogeneous population of round niosomes approximately 200 nm in size and successful bioconjugation. The anti-PSMA aptamer specifically delivered DOX to PSMA-positive cells, increasing cellular uptake and cytotoxicity. The inclusion of QDs also enabled treatment tracking.[136]

Aptamers have also been conjugated with toxins for prostate cancer targeting.[137] For instance, the E3 aptamer conjugated with Pseudomonas exotoxin (PE-LR-8) selectively targeted PSMA-positive cells. The conjugate was specifically internalized into prostate cancer cells and exhibited cytotoxicity with IC_{50} values ranging from 0.4 to 1.1 nM, without toxicity to normal cells. *In vivo*, the aptamer-toxin conjugates targeted prostate tumors and patient-derived colon cancer tumors, as confirmed by near-infrared imaging.[138] Furthermore, an *in vitro* study was conducted on anti-PSMA aptamer-toxin conjugates to improve the challenges that are seen in *in vivo* administration of immunotoxins. The chemically synthesized minimized variants of the A9 aptamer conjugated with Pseudomonas exotoxin showed specificity toward PSMA-positive LNCaP cells with an IC_{50} of about 60 nM and *in vivo* serum half-life of approximately 4 hours.[139]

3.4. Challenges and limitations

Despite the hopeful therapeutic and diagnostic potential of aptamer for prostate cancer, clinical application of these small oligonucleotides have faced challenges.[145] A key limitation

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especially in the RNA aptamers stability, are highly susceptible to nuclease-mediated degradation, thereby shortening their circulatory half-life and therapeutic window. Although various chemical modifications, such as 2′-*O*-methyl and phosphorothioate substitutions, have been employed to enhance nuclease resistance, these alterations can inadvertently compromise binding affinity or disrupt the structural conformation essential for target recognition.[146] *In vivo* delivery efficiency represents another major problem. Due to their small molecular size, aptamers exhibit rapid renal filtration and systemic clearance, often resulting in suboptimal tumor accumulation and limited therapeutic efficacy. Moreover, the dense stromal architecture and poor vascularization characteristic of solid tumors such as prostate cancer exacerbate these delivery challenges, restricting deep tissue penetration and reducing overall bioavailability at the tumor site.[147] From a production perspective, the SELEX process lacks standardized protocols for selection, optimization, and validation, leading to inconsistencies in aptamer performance across studies. This methodological variability complicates clinical translation, as reproducibility and scalability are critical for regulatory approval.[102,148] In addition to the mentioned issues, heterogeneity of prostate tumor cells and the variability in expression level of PSA and PSMA, immunogenicity of aptamer due to chemical modifications, and off-target effects are other main concerns. Together, these elements underscore the necessity for enhanced preclinical validation, systematic refinement of SELEX processes, and standardized pharmacokinetic assessments to confirm aptamer-based therapies as dependable clinical instruments.[102,149] More preclinical and clinical investigations and studies are required to get rid of these concerns and develop more effective aptamer based approaches.

4. Nanobodies in prostate cancer detection and therapy

Besides the anti-PSMA-antibodies-and aptamer based approaches for detection and treatment using, nanobodies (Nbs) because of unique benefits including, small size, great stability, and cheap and easy production process have drowed the attractions for utilization in prostate cancer detection and treatment. Hence, we firstly discussed basics and structure of Nbs in this section, then the paper was continued by focusing on the published various preclinical and clinical investigations of anti-PSMA nanobodies for monitoring and treatment of prostate cancer. Drawbacks of nanobodies is the main heading at the end of this section.

4.1. Overview of nanobodies

Nbs, the naturally single-domain antibodies (sdAbs), discovered in the early 1990s when scientists isolated them from the serum of camelids, such as llamas, camels, and alpacas. Next, functional VHH domains are isolated and expressed independently.[150] Structurally, Nbs (size: ~12–14 kDa) are smaller than conventional antibodies and consist of only variable domain of the heavy chain (VHH) and lock light chains and first constant segment (CH1).[151] VHHs could detect cryptic epitopes of target antigen because of having larger size CDR3 domain, which is one the main limitations of classic Abs.[152] Currently, these small natural molecules are the existing antigen-binding fragments (ABFs) with complete binding capability with antigens. HVVs have various unique features, which introduce them as an attractive option for diagnosis and treatment of diseases especially cancers.[153,154] They share common advantages including, natural source, low toxicity, small size, and good sensitivity, safety, and stability.[150] Biological production of Nbs with a great affinity and yields on microorganisms including, *S. cerevisiae*, *E. coli*, and even plant cells is very easy and cost effective because there is no need to light chain.[155] Furthermore, Nbs have strong binding potential and high affinity for target antigens and could tolerate 3.0–9.0 pH range, 60–80 °C of temperature, and 500–750 MPa of pressure. Low immunogenicity and efficient penetration into tumor tissues are derived from their small size.[150] All of these suggest Nbs as the effective options for clinical use.

4.2. Applications in prostate cancer detection

In recent years, various nanobody-based approaches have been designed and developed targeting PSA and PSMA for prostate cancer detection and monitoring in both preclinical and clinical settings. For example, a ^{111}Inradiolabeled nanobody (Nb) was developed for imaging and therapeutic purposes by targeting PSMA. The Nb library was generated through llama immunization with four human prostate antigens, followed by biopanning to isolate anti-PSMA Nbs. JVZ-007 was selected as a probe for cancer imaging.[156] The C-terminal His-tag of JVZ-007, initially used for purification and detection, was replaced by a single cysteine at the C-terminus to enable site-specific chelate binding for radiolabeling. Radiolabeling was performed with p-SCN-DTPA for JVZ-007-His and maleimide-DTPA for JVZ-007-Cys. Various *in vitro* assays, including flow cytometry, autoradiography, and internalization studies, were used to assess the binding capability of the radiolabeled Nbs on primary patient-derived xenografts and prostate cancer cell lines.[156] Data showed that both ^{111}In-JVZ-007-His and ^{111}In-JVZ-007-Cys internalized into LNCaP cells (PSMA-positive), while there was no internalization in PSMA-negative cells. Preclinical tumor targeting of both VHHs was evaluated in mice bearing PSMA-positive PC-310 and PSMA-negative PC-3 tumors using SPECT/microCT imaging and biodistribution analyses. Both radiolabeled VHHs demonstrated selective tumor targeting and rapid blood clearance. However, the use of ^{111}In-JVZ-007-His was somewhat limited by high renal uptake, which was markedly reduced by administration of lysine and gelofusine. Replacing the His-tag with cysteine effectively lowered renal uptake without affecting Nb function.[156]

Another study developed an anti-PSMA Nb labeled with ^{99m}Tc for preclinical imaging. ELISA and flow cytometry confirmed the binding affinity of the Nbs before labeling the hexahistidine tail with ^{99m}Tc. *In vitro* internalization assays were conducted on LNCaP (PSMA-positive) and PC3 (PSMA-negative) cells. *In vivo* tumor targeting was then evaluated in

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xenograft-bearing mice *via* SPECT/microCT imaging. Among the candidates, ^{99m}Tc-PSMA6 and ^{99m}Tc-PSMA30 were selected, with ^{99m}Tc-PSMA30 showing higher binding and internalization in LNCaP cells. Imaging results aligned with *in vitro* data, indicating superior tumor uptake of ^{99m}Tc-PSMA30 in LNCaP-bearing mice.[157]

Besides their use in radiolabeling for imaging, anti-PSMA and anti-PSA nanobodies are also employed in biosensor-based prostate cancer detection due to their small size, high stability, and ease of production. These nanoscale molecules can be immobilized on sensor surfaces *via* covalent bonds, metal chelation, or streptavidin–biotin interactions.[19] For example, a surface plasmon resonance (SPR) biosensor using the anti-PSA Nb cAbPSA-N7 was developed for specific prostate cancer detection. The sensor used a BAD-tag for Nb immobilization on streptavidin chips, extra lysine residues to stabilize binding on self-assembled monolayer (SAM) chips, and a His6-tag for binding on Ni–NTA surfaces. The sensor detected PSA concentrations below 1 ng mL^{-1} within 15 minutes.[21] In another study, a high-affinity nanobody was developed *via* phage display using M13 phage to recognize recombinant PSMA (rPSMA) on LNCaP cells. Recombinant PSMA epitopes were expressed in *E. coli* BL21 and used to immunize a camel, leading to the construction of a VHH-phage library. These Nbs bound effectively to rPSMA on LNCaP cells with an affinity of 3.5×10^{-7}.[18] In 2018, Xin Liu *et al.* designed a novel sandwich-format electrochemical immunosensor using two nanobodies (Nb2 and Nb40) for direct PSA detection in serum with high sensitivity and specificity. Nb40 was immobilized on reduced graphene oxide-gold nanoparticles (rGO@AuNPs), while Nb2 was fused with a streptavidin-binding peptide and coupled with horseradish peroxidase-streptavidin for detection. The immunosensor measured PSA concentrations from 0.1 to 100 ng mL^{-1}, with a clear correlation between differential pulse voltammetry (DPV) peak current and PSA concentration. The sensor showed excellent sensitivity, stability, specificity, and reproducibility, attributed to the nanobodies.[158]

More recently, Mahmoud Esraa Baghdadi *et al.* developed the first sensitive homogeneous immunoassay using an anti-PSA nanobody coupled with a tri-part nanoluciferase (NLu) system. Two small components of split-NLuc (β9 and β10) were fused with N7 and N23, respectively, and combined with Δ11S, the third component. These proteins were produced *via* bacterial expression. The assay detected PSA in a linear range of 1.0 to 20.0 ng mL^{-1} with a limit of detection (LOD) of 0.4 ng mL^{-1}, showing comparable results to ELISA. This platform offers a specific, sensitive, and rapid method for prostate cancer detection.[159]

4.3. Applications in prostate cancer therapy

Published results demonstrate the effectiveness of anti-PSMA VHHs in prostate cancer therapy. Lior Rosenfeld *et al.* developed and characterized four nanobodies (NB7, NB8, NB13, NB37) with high affinity and specific binding to PSMA-positive cells and tumor sites. Treatment involved intravenous injections of these Nbs or doxorubicin (DOX) for three weeks. NB7 conjugated with DOX (NB7cysDOX) showed specific internalization into PSMA-positive cells and triggered cytotoxic effects. Tumor growth suppression by NB7cysDOX was comparable to commercial DOX treatment, but using 42-fold less drug, highlighting nanobodies as promising agents for targeted chemotherapeutic delivery.[160]

PSMA targeting has been further explored by Nonnekens *et al.*, who investigated two α-emitting radionuclide-labeled molecules: ^{213}Bi-PSMA I&T (a chemical compound) and ^{213}Bi-JVZ-008 (a nanobody). Both demonstrated specific targeting and induced DNA double-strand breaks in LNCaP cells *in vitro* and in xenograft models *in vivo*. The preclinical data showed that two target molecules were capable to specific targeting of prostate cancer cells and increasing double-strand breaks DNA (DSBs) at 1 hour and 24 hours upon administration, supporting their use in imaging and therapy.[161] To overcome drug resistance, Tieu *et al.* employed nanobody-displaying porous silicon nanoparticles (pSiNPs) to co-deliver DOX and siRNA targeting MRP1, a gene linked to multidrug resistance, in prostate cancer cells. The pSiNPs were conjugated to anti-PSMA nanobodies *via* a PEG linker for targeted delivery. Results showed over 74% inhibition of MRP1 expression and enhanced sensitivity to DOX.[162] Recently, researchers have engineered chimeric antigen receptor T cells (CAR-T) expressing anti-PSMA nanobodies for prostate cancer immunotherapy. Mahmoud Hassani *et al.* developed NBPII-CAR T cells expressing anti-PSMA VHHs. *In vitro* co-culture with PSMA-positive LNCaP cells induced specific T cell activation, cytokine production (IL-2), CD69 expression, and proliferation, whereas no significant response was observed with PSMA-negative DU-145 cells. These data demonstrate that nanobody-based CAR-T cells are effective and specific for prostate cancer therapy.[163]

4.4. Challenges and limitations

Single-domain antibody fragments (VHHs) have emerged as promising alternatives to full-length antibodies for prostate cancer detection and therapy due to their compact size, high binding affinity, exceptional thermal, chemical stability and enhanced tumor tissue penetration.[19] However, despite these favorable attributes, several translational and pharmacological challenges continue to impede their broad clinical implementation. The main limitation of their pharmacokinetics is rapid renal clearance and preferential renal accumulation, which result in a markedly reduced systemic half-life. Although this rapid removal may be beneficial for imaging applications by improving background contrast, it is a major obstacle for therapeutic goals, where stable interaction with the target is essential.[151,164] Strategies such as fusing nanobodies to Fc regions, polyethylene glycol (PEG) conjugation, or multimerization have been proposed to improve pharmacokinetics, but these may adversely affect binding affinity or immunoreactivity.[19,164] Immunogenicity represents another concern, particularly with non-humanized nanobodies derived from camelid sources. Although their overall immunogenic risk is lower compared to conventional monoclonal antibodies, repeated administration

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has been shown to induce anti-drug antibody (ADA) formation, potentially leading to reduced efficacy or faster clearance upon multiple dosing cycles.[164] These findings underscore the need for systematic humanization strategies and comprehensive immunogenicity profiling during preclinical and early clinical development.

Furthermore, the large-scale manufacturing of nanobodies at clinical-grade purity remains technically demanding. Achieving consistent folding, yield, and post-translational quality in recombinant expression systems adds to the economic burden of production, particularly when modifications such as PEGylation or Fc-fusion are incorporated.[165] Establishing standardized purification workflows and scalable bioprocessing technologies is thus critical for ensuring reproducibility and regulatory compliance.

In summary, although nanobody-based therapeutic and diagnostic approaches show significant potential in the management of prostate cancer, optimization of their pharmacokinetics, immunogenicity, and manufacturability will be essential for successful clinical application. Future studies that integrate rational design, advanced drug delivery systems, and humanization techniques could help overcome these limitations and accelerate their adoption in precision oncology.

5. Comparative analysis of antibodies, aptamers, and nanobodies

Antibodies, aptamers, and nanobodies are small biomolecules capable of targeting PSA and PSMA for the therapeutic and diagnostic applications of prostate cancer. Each biomolecule has unique strengths and weaknesses, making them valuable tools for biomedical applications. Numerous anti-PSMA and anti-PSA-based strategies using antibodies, nanobodies, and aptamers have been published with promising results.[40,53,121,157] Comparing these biomolecules in terms of stability, cost, and clinical application reveals both complementary advantages and critical limitations, offering a comparative perspective on their potential for prostate cancer detection and therapy.

Antibodies are the first class of biological molecules discussed for targeting prostate cancer biomarkers. These Y-shaped proteins, especially monoclonal antibodies (mAbs), are relatively large, and their biomedical applications are defined by various characteristics. They are employed either in their single mAb form or as antibody–drug conjugates (ADCs) by linking with substances such as toxins or radionuclides.[50] Antibodies exhibit high specificity and strong affinity for their targets. Moreover, their Fc region can induce immune effector functions such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), which are crucial for therapeutic uses.[50,166] Clinically, antibodies are well established in prostate cancer diagnostics and therapeutics. However, while they are effective in imaging studies, their therapeutic outcomes have been less encouraging.[167] Radiolabeled anti-PSA and anti-PSMA antibodies have shown excellent performance in imaging and detection due to their high specificity and sensitivity.[22,41] This specificity allows for highly sensitive diagnosis in various biological samples blood, urine, or tissues, making them suitable for early cancer monitoring.[40] Nevertheless, the therapeutic efficacy of radiolabeled antibodies is limited by factors such as poor penetration into solid tumors, heterogeneous expression of target biomarkers, restricted distribution, and immune-related side effects.[19] To address these challenges, antibodies have been conjugated with toxins to reduce adverse effects in their free form.[68] Though these immunotoxins show promising *in vitro* and *in vivo* anti-tumor activity, their clinical use is hindered by concerns like bulky structure, complex production, and potential immunogenicity upon intravenous injection issues even more pronounced than those of antibodies in single form.[68,69] Furthermore, antibodies are costly to produce, sensitive to environmental factors like pH, temperature, and organic solvents, and may lose function under harsh conditions. Their large size, immunogenicity, and limited penetration into prostate tumors remain clinical challenges.[52]

Aptamers, single-stranded DNA or RNA oligonucleotides (10–30 kDa), are the second class of biomolecules reviewed for prostate cancer diagnosis and therapy. While aptamers present certain limitations, they generally possess more advantages than antibodies.[102] They offer high specificity and affinity for target molecules (*e.g.*, PSA and PSMA), enabling precise biomarker detection with enhanced imaging quality and reduced background noise.[68] Due to their small size, aptamers can penetrate tumor tissues more effectively and clear from non-target areas faster than antibodies. They are also effective carriers for targeted delivery of therapeutic agents, are non-immunogenic, and exhibit high stability under various conditions, providing a significant advantage over protein-based molecules.[112] Aptamer–toxin conjugates are particularly promising in prostate cancer therapy and are preferred over immunotoxins for their improved safety profiles. Their synthesis is cost-effective, as they are chemically produced rather than biologically expressed.[68,139] However, clinical applications remain limited, with few FDA-approved aptamer-based drugs to date. A major drawback is their vulnerability to nuclease degradation *in vivo*, resulting in short half-lives though this can be addressed with chemical modifications. Additionally, their high affinity may cause non-specific interactions in complex biological environments.[102]

Nanobodies (Nbs), the third class of biomolecules, are single-domain antibodies derived from the heavy-chain-only antibodies (VHH) of camelids. These molecules are significantly smaller than conventional antibodies but retain similar high specificity and affinity (pM to nM range).[19] Their compact size enhances tumor penetration and allows access to hidden epitopes, making them ideal candidates for targeting solid tumors, including prostate cancer.[160] Other advantages of nanobodies include low toxicity, high sensitivity, cost-effective microbial production, low immunogenicity, and remarkable stability under harsh conditions, all of which address limitations of full-sized antibodies.[19] Despite these benefits,

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nanobodies lack an Fc region, limiting their ability to trigger immune effector functions; however, this issue can be addressed *via* genetic engineering.[168] Their short half-life in circulation remains a major challenge for clinical use.[164,165] Clinically, nanobodies are an emerging field, with some approved applications for other diseases. Although numerous nanobody-based strategies are under development, no FDA-approved nanobodies targeting PSA or PSMA for prostate cancer imaging or therapy currently exist.

6. Emerging trends and future directions

To date, various diagnostic and therapeutic strategies based on antibodies, aptamers, and nanobodies either in free form or conjugated with toxins or radionuclides—have been developed to target PSA and PSMA *in vitro*, *in vivo*, and in clinical settings.[50] Since the approval of ^{111}In-labeled ProstaScint®, the first FDA-approved antibody for prostate cancer, significant progress has been made in improving the efficacy and specificity of these biomolecules over time.[22] Despite strong performance in preclinical and *in vitro* models, clinical application still faces major challenges. Therefore, new strategies are required to achieve greater efficacy and successfully transition from research to clinical use.

Recent advances in nanotechnology, biotechnology, and computational science have opened new opportunities for designing improved antibody-, aptamer-, and nanobody-based systems against PSMA and PSA for prostate cancer diagnosis and therapy. Key trends likely to impact the field include the integration of nanotechnology with molecular recognition agents, enhancing diagnostic sensitivity and enabling precise delivery of therapeutics and imaging agents.[169–171] Functionalized nanoparticles, particularly metallic ones, conjugated with antibodies, aptamers, or nanobodies, have shown remarkable improvements in diagnostic and therapeutic performance.[41,116,133,162]

In parallel, computational biology, artificial intelligence (AI), machine learning (ML), and deep learning (DL) are gaining attention for their ability to generate highly accurate clinical predictions.[172] AI-based tools are rapidly growing in their applications for prostate cancer prognosis, diagnosis, and therapy.[173,174] These technologies assist in designing new drugs, small-molecule inhibitors, and drug conjugates, helping overcome drug resistance and potentially saving millions of lives.[175,176] AI algorithms can predict binding affinities between biomolecules and their targets (such as PSMA and PSA), optimize antibody–antigen interactions, identify aptamer–ligand binding sequences, and engineer nanobodies.[177] These computational algorithms can also be applied for designing anti-PSA and anti-PSMA small biomolecules in single form or conjugated with toxin or radiomolecules. Furthermore, AI-driven design of immunotoxins and aptatoxins constructs combining a targeting moiety (*e.g.*, antibody or aptamer) with a cytotoxic payload offers a promising avenue for prostate cancer therapy, particularly in castration-resistant prostate cancer (CRPC), where traditional treatments often fail.

Clinically, prostate cancer detection currently relies on datasets such as PSA and PSMA levels, genetic markers, Gleason grading, and radiological imaging (MRI, ultrasound), as well as histopathological data.[178] An AI-based algorithm called ^{18}F-1007-PSMA PET-based radiomics has recently shown superior predictive performance over traditional PSA models for prostate cancer detection.[179] AI also accelerates drug discovery, shortens development timelines, and reduces costs.[180] Integrating these advanced technologies into prostate cancer diagnosis and therapy allows for early and precise disease monitoring and improved performance of synthetic anti-PSA and anti-PSMA molecules. Although challenges remain in terms of scalability and regulatory approval, collaboration between academia, industry, and regulatory bodies will be critical to bridging the gap between research and clinical application.

7. Conclusion

Prostate cancer accounts for approximately 30% of all male cancers and is responsible for millions of deaths worldwide. However, early detection at non-metastatic stages is key to improving treatment outcomes and survival rates. Currently available diagnostic and therapeutic techniques are chosen based on cancer stage and risk category, but they are often invasive, expensive, and lack sensitivity in distinguishing advanced or recurrent disease. Antibodies, aptamers, and nanobodies targeting PSA and PSMA have emerged as promising theranostic tools, enabling accurate detection and targeted treatment with high specificity and selectivity. Following the FDA approval of ^{111}In-labeled ProstaScint®, optimism for early prostate cancer detection and treatment has grown. However, clinical limitations such as low sensitivity and inability to detect bone metastases have led to further development of next-generation radiolabeled anti-PSMA mAbs and their toxin-conjugated forms in preclinical and clinical studies.

Simultaneously, smaller biomolecules such as aptamers and nanobodies have been developed against PSA and PSMA. While each biomolecule class offers distinct advantages and faces unique challenges, aptamers and nanobodies appear to offer more favorable profiles for addressing limitations seen with antibodies. Antibodies are highly specific with long circulation times and strong functional effects but suffer from limited tumor penetration, high production costs, and immunogenicity. Aptamers, by contrast, are stable, low-cost, and non-immunogenic, though their *in vivo* stability remains a concern. Nanobodies effectively bridge the gaps between these two classes, offering small size, stability, and strong binding.

Combining these molecules could yield synergistic diagnostic and therapeutic effects. Hybrid constructs such as immunotoxins, aptamertoxins, or antibody–aptamer/nanobody–aptamer fusions represent promising strategies for advanced prostate cancer diagnosis and treatment. Integration of

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nanotechnology, biotechnology, AI, and computational methods into the design of these biomolecules may further improve specificity, stability, and performance. However, more preclinical and especially clinical research is needed to optimize these platforms, realize AI's full potential in drug design, and translate laboratory findings into clinical application.

8. Future perspectives

Although, recent advances in molecular biotechnology, particularly the development of biomolecule such as antibodies, aptamers, and nanobodies, have significantly enhanced the accuracy, specificity, and therapeutic precision of prostate cancer management. Future research will likely focus on integrating these molecular tools with emerging technologies to achieve more effective and personalized clinical outcomes.

The integration of advanced biomolecular technologies offers one of the most promising directions for prostate cancer treatment. Combining antibodies, aptamers, and nanobodies within multifunctional therapeutic or diagnostic agents can improve tumor targeting, biodistribution, and pharmacokinetics, ultimately reducing systemic toxicity. For example, coupling these biomolecules with nanomaterials enables controlled drug release and enhanced tissue penetration, creating powerful theranostic systems that simultaneously detect and treat tumors. Such hybrid approaches could bridge the gap between molecular diagnostics and precision therapeutics, transforming how prostate cancer is both identified and managed.

Personalized medicine also represents a pivotal area for future development. The heterogeneity of prostate cancer, both at the molecular and cellular levels, underscores the need for individualized therapeutic approaches. Biomolecular markers such as PSA and PSMA, when combined with genomic and proteomic profiling, can guide precision medicine strategies that tailor treatments to each patient's tumor characteristics. Integrating these biomarkers with artificial intelligence (AI) and machine learning (ML) will further refine patient selection and treatment prediction, optimizing outcomes while minimizing adverse effects. AI and ML are poised to revolutionize prostate cancer drug development. These computational approaches can accelerate the design and optimization of biomolecules with high binding affinity and low immunogenicity, as well as predict pharmacokinetic behavior and toxicity. AI-based models can simulate molecular interactions, forecast therapeutic efficacy, and optimize dosing regimens, thereby streamlining preclinical and clinical development. This convergence of biotechnology and computational science promises to reduce development timelines and improve the translation of laboratory discoveries into clinical applications.

Improvements in imaging and theranostic technologies will further enhance early detection and treatment monitoring. The integration of radiolabeled antibodies, aptamers, and nanobodies with advanced imaging techniques such as PET, SPECT, and MRI can enable real-time, high-resolution visualization of tumor distribution and therapeutic response. Emerging hybrid imaging probes are being developed to serve both diagnostic and therapeutic purposes, offering simultaneous visualization and targeted drug release. Such dual-function systems could redefine the paradigm of prostate cancer management by merging diagnosis and therapy into a single, continuous process.

Finally, nanotechnology will remain central to the evolution of biomolecule-based cancer therapy. The use of nanoparticle carriers can greatly enhance the stability, bioavailability, and tumor-specific accumulation of the antibodies, aptamers, and nanobodies. There have been good studies on the use of nanotechnology for the diagnosis, delivery and treatment of prostate cancer. For instance, RESV–198AuNP, a nanoradiopharmaceutical combining resveratrol's anticancer properties with radioactive gold nanoparticles (198AuNPs) for targeted prostate cancer therapy and diagnostics, demonstrated $>85\%$ injected dose retention in prostate tumors at 24 hours and a >10-fold tumor volume reduction by week 4 compared to controls, highlighting its potential for dual theranostic applications and real-time treatment monitoring.[181] Similarly, nanomaterials enable highly sensitive and specific prostate cancer detection through nanosensors and nanoprobes, leveraging their nanoscale size and unique properties, while also serving as carriers for contrast agents to enhance safe, accurate visualization of prostate cancer tissues *in vivo*.[182] Additionally, enzalutamide-loaded 8P4 nanoparticles (ENZ-8P4 NPs) enhance drug delivery efficiency, increase cytotoxicity, and reduce the IC_{50} for treating castration-resistant prostate cancer, with *in vivo* studies showing preferential tumor accumulation and significant tumor growth inhibition.[183] Multifunctional nanoplatforms capable of delivering imaging agents alongside therapeutic payloads will enable precise, image-guided treatments with reduced systemic toxicity. Continued optimization of nanocarrier design, surface chemistry, and biocompatibility will be critical for translating these technologies into clinical success.

In summary, the convergence of biomolecular engineering, computational intelligence, and nanotechnology is poised to transform the landscape of prostate cancer diagnosis and therapy. A multidisciplinary approach integrating these innovations will be essential for overcoming current limitations and advancing toward a new era of truly personalized and precise oncology.

Conflicts of interest

The authors declare no conflicts of interest regarding the research, authorship, or publication of this manuscript.

Data availability

This article is a review synthesizing published literature on antibodies, aptamers, and nanobodies for prostate cancer detection and therapy. No new experimental data were generated or analyzed in this study. All data discussed are derived

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