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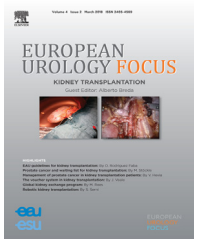

Review – Prostate Cancer

Prostate-specific Membrane Antigen Biology in Lethal Prostate Cancer and its Therapeutic Implications

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Abstract

Context: Prostate-specific membrane antigen (PSMA) is a promising, novel theranostic target in advanced prostate cancer (PCa). Multiple PSMA-targeted therapies are currently in clinical development, with some agents showing impressive antitumour activity, although optimal patient selection and therapeutic resistance remain ongoing challenges.

Objective: To review the biology of PSMA and recent advances in PSMA-targeted therapies in PCa, and to discuss potential strategies for patient selection and further therapeutic development.

Evidence acquisition: A comprehensive literature search was performed using PubMed and review of American Society of Clinical Oncology and European Society of Medical Oncology annual meeting abstracts up to April 2021.

Evidence synthesis: PSMA is a largely extracellular protein that is frequently, but heterogeneously, expressed by PCa cells. PSMA expression is associated with disease progression, worse clinical outcomes and the presence of tumour defects in DNA damage repair (DDR). PSMA is also expressed by other cancer cell types and is implicated in glutamate and folate metabolism. It may confer a tumour survival advantage in conditions of cellular stress. PSMA regulation is complex, and recent studies have shed light on interactions with androgen receptor, PI3K/Akt, and DDR signalling. A phase 2 clinical trial has shown that ^{177}Lu-PSMA-617 causes tumour shrinkage and delays disease progression in a significant subset of patients with metastatic castration-resistant PCa in comparison to second-line chemotherapy. Numerous novel PSMA-targeting immunotherapies, small molecules, and antibody therapies are currently in clinical development, including in earlier stages of PCa, with emerging evidence of antitumour activity. To date, the regulation and function of PSMA in PCa cells remain poorly understood.

Conclusions: There has been rapid recent progress in PSMA-targeted therapies for the management of advanced PCa. Dissection of PSMA biology will help to identify biomarkers for and resistance mechanisms to these therapies and facilitate further therapeutic development to improve PCa patient outcomes.

Patient summary: There have been major advances in the development of therapies targeting a molecule, PSMA, in PCa. Radioactive molecules targeting PSMA can cause tumour shrinkage and delay progression in some patients with lethal disease. Future

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studies are needed to determine which patients are most likely to respond, and how other treatments can be combined with therapies targeting PSMA so that more patients may benefit.

1. Introduction

Prostate-specific membrane antigen (PSMA) is a promising novel theranostic target in advanced prostate cancer (PCa), which remains a leading cause of male cancer mortality [1]. PSMA is overexpressed in PCa cells and is associated with worse clinical outcomes. Normal tissue expression of PSMA is restricted to the proximal renal tubules, glial cells, small intestine, and salivary and lacrimal glands [2–7]. PSMA has various aliases, including glutamate carboxypeptidase II, used primarily in a neurological context, and folate hydrolase 1 (*FOLH1*), used when describing the gene encoding PSMA. The physiological role of PSMA in the brain is to facilitate neuronal glutamate synthesis and its enzymatic role in the intestine is to facilitate folate absorption. Its physiological role in prostate cells remains poorly defined. Given the rapid development of PSMA-targeted therapies and imaging agents, it is now critical to elucidate the regulation and function of PSMA in PCa to improve the precision and maximise the benefits of PSMA-targeted therapies. This review first focuses on PSMA biology in PCa, then summarises key clinical studies of PSMA-targeted therapies in PCa, and finally provides insights into how an understanding of PSMA biology can inform future therapeutic strategies to improve patient selection and treatment outcomes.

2. Evidence acquisition

We performed a review of preclinical and clinical studies focusing on PSMA-targeted therapies in PCa following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The search was performed on PubMed using the search terms “Prostate Specific Membrane Antigen”, “Prostate-Specific Membrane Antigen”, “PSMA”, “FOLH1”, “Glutamate Carboxypeptidase II” or “Folate Hydrolase” in conjunction with “Prostate Cancer” or “Prostate” in the title or abstract, up to February 2021. Only English language publications were included. Editorials, guidelines, letters, commentaries, and review articles were excluded. Conference abstracts of the American Society of Clinical Oncology and European Society of Medical Oncology up to February 28, 2021 were also reviewed and included. When there were multiple reports for the same patient cohort, the most recent and comprehensive publication was selected. Studies on refining PSMA imaging protocols, not directly relevant to PCa treatment or PSMA targeting therapies, or on agents only being evaluated in the preclinical setting were excluded.

Authors B.S. and C.G. performed article selection and review independently. Articles were included in the review after agreement between the authors. Keywords searched in study titles and abstracts were used to refine studies for initial consideration. The authors then reviewed the full texts of studies fitting the inclusion/exclusion requirements outlined above. In addition, select articles that provided background for PSMA regulation and physiological function were included (Fig. 1).

3. Evidence synthesis

3.1. *The PSMA gene (*FOLH1*) and protein*

PSMA is encoded by the *FOLH1* gene located on chromosome 11p11.12 [8]. Consisting of 19 exons and 18 introns within a 60-kb region, the gene is under the control of an upstream promoter and an enhancer region present within the third intron [9]. It has been shown that SOX-7 (repressor), the TMPRSS2-ERG gene fusion (repressor), and NFATC-1 (activator) regulate *FOLH1* gene expression [10–12]. However, none of these transcription factors are entirely responsible for PSMA expression, suggesting that additional factors contribute to the regulation of PSMA in PCa.

PSMA is a glycosylated, transmembrane carboxypeptidase subdivided into three major regions: a short cytoplasmic tail, a transmembrane segment, and a large extracellular portion [13]. The role of PSMA depends on the site of expression. In glial cells, PSMA catalyses the synthesis of glutamate from the neuropeptide *N*-acetyl-aspartyl-glutamate (NAAG), thereby promoting excitatory neural transmission [13]. In the duodenum, PSMA cleaves glutamate moieties from dietary polyglutamated folates to produce monoglutamated folates that are more readily absorbed [14].

3.2. *Regulation of PSMA expression in PCa*

3.2.1. *Regulation by the androgen receptor*

The dichotomous relationship between PSMA and androgen receptor (AR) signalling has been described in the preclinical and clinical settings. Studies using hormone-sensitive prostate cancer (HSPC) cell lines and xenografts showed that treatment with testosterone, dihydrotestosterone, or the synthetic analogue R1881 reduces PSMA expression, while androgen deprivation therapy (ADT) increased PSMA expression [15,16].

By contrast, a clinical imaging study using ^{68}Ga-PSMA positron emission tomography (PET) showed that ADT acutely downregulated PSMA expression (maximum [SUVmax] and mean [SUVmean] standardised uptake values) in the majority of patients with HSPC who also experienced a marked decrease in prostate-specific antigen (PSA)

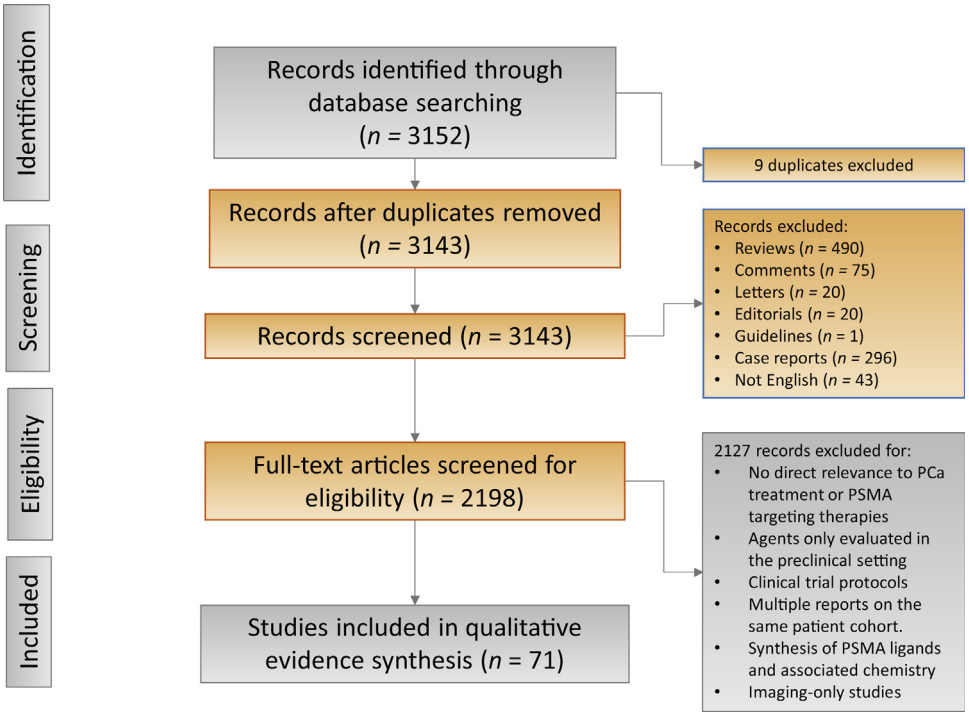

Fig. 1 – PRISMA flow diagram PCa = prostate cancer; PSMA = prostate-specific membrane antigen.

[17]. Since PSMA expression on PSMA-PET is related to both the number of cells expressing PSMA and target level expression, an initial reduction in PSMA expression on PET imaging in the castration-sensitive setting is likely to be in part attributable to tumour shrinkage in response to ADT as opposed to reduced PSMA expression per cell.

In the castration-resistant setting, enzalutamide or abiraterone led to a marginal increase in PSMA expression on PSMA PET. Notably, this group did not have a significant decrease in PSA [17]. Moreover, a separate study showed that PSMA expression on immunohistochemistry (IHC) was elevated in biopsy tissue from metastatic castration-resistant prostate cancer (mCRPC), which has higher AR signalling [18,19]. The heterogeneity in PSMA expression in advanced mCRPC may also be explained by the fact that AR-negative PCa cell lines and human PCa cells that have transitioned to an AR-negative neuroendocrine/basal phenotype either have significantly reduced or no PSMA expression [19,20].

3.2.2. *Regulation by PI3K/Akt/mTOR*

PI3K/Akt/mTOR pathway activation occurs in approximately half of advanced PCa. There is significant crosstalk between PI3K/Akt/mTOR and AR signalling [21]. The enzymatic activity of PSMA is probably critical for the crosstalk between PI3K/Akt/mTOR signalling and PSMA [7]. Glutamate, cleaved by PSMA from folates, can drive the PI3K/Akt/mTOR axis by activating G-coupled protein receptors (GPCRs) upstream of the β-isoform of PI3K to perpetuate its signalling (Fig. 2). PSMA expression is also correlated with increased phosphorylation of 4EBP-1, which is modulated by the drug rapamycin, in PCa tumour samples. Inhibition of downstream targets of the PI3K/Akt/mTOR signalling pathway, such as mTOR1, by rapamycin increases PSMA expression, perhaps as a compensatory mechanism [22].

Rapamycin-sensitive genes significantly associate with “PSMA high” patient samples. This was reiterated by gene set enrichment analysis of PSMA-positive cell lines (LNCaP-Ctrl and PC3-PSMA) and their PSMA-negative counterparts (LNCaP-KD and PC3-Ctrl); genes regulated by Akt and mTOR were significantly linked to PSMA expression. Given the reciprocal feedback between AR and PI3K/Akt signalling, it is likely that modulation of PSMA expression is dependent on the point at which the signalling cascades are targeted and the *PTEN* status of the cells. Overall, these studies indicate that modulation of the PI3K/Akt pathway should be explored as a strategy to upregulate PSMA expression.

3.2.3. *Regulation by DNA damage*

PSMA may regulate glutamate and folate availability to cells. These molecules are fundamental to nucleotide synthesis, a process upregulated in cells requiring DNA damage repair (DDR). Both the PI3K/Akt/mTOR and AR signalling axes can regulate DDR pathways, and it has been reported that their blockade sensitises to DNA-damaging agents [23,24]. Mechanistically, PI3K inhibitors reduce nucleoside pools, which can induce replication stress, and AR inhibition reduces receptor mediation of DDR with co-regulators [24,25]. This hypothesis is further underscored by the observation that DDR-defective mCRPCs have higher PSMA expression than those without DDR defects [19]. Another study showed that *BRCA2* knockout in PCa cell lines results in an increase in PSMA expression [26]. Interestingly, PCa

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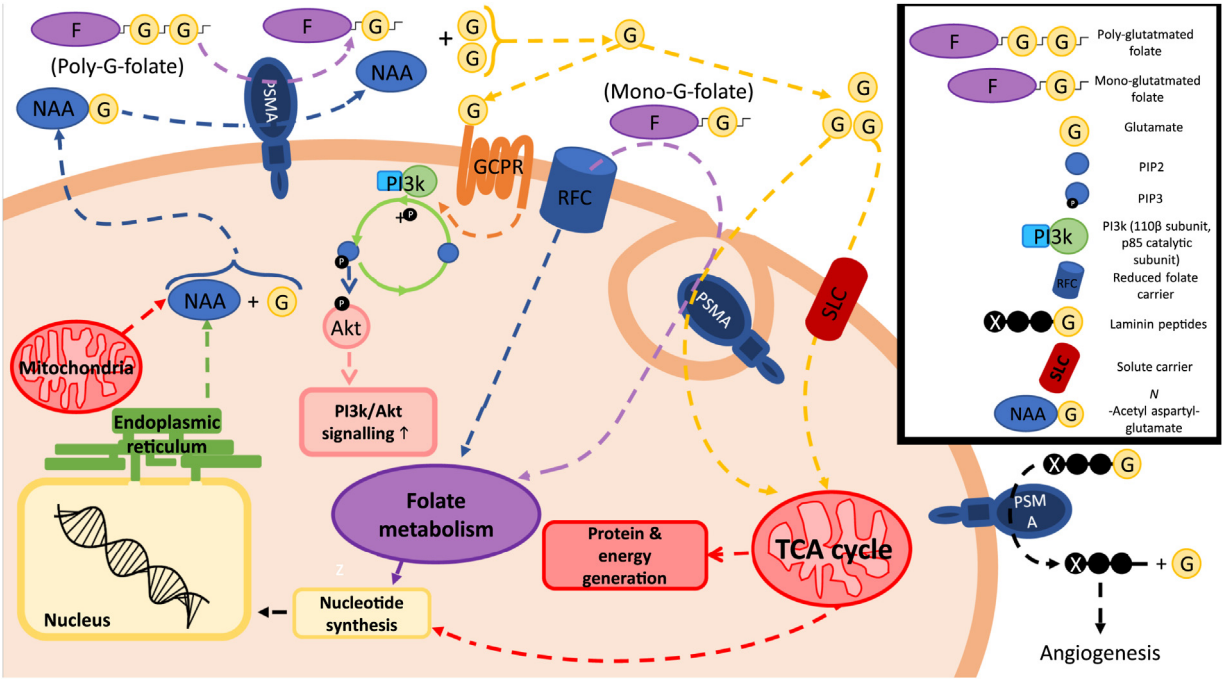

Fig. 2 – Schematic of cellular function of PSMA in PCa cells based on established in vitro studies. PSMA enzymatic function cleaves glutamate from poly-G-folates, NAAG, laminin peptides, and other unknown glutamated substrates. The liberation of glutamate and its subsequent binding to glutamate receptors (GCPRs) results in upregulation of the oncogenic PI3K(110β subunit)/Akt/mTOR signalling pathway. Synthesis of NAAG by recurrent ovarian and pancreatic tumour cells generates a local glutamate reservoir only accessed by PSMA expression. Mono-G-folate generation from PSMA enzymatic action on poly-G-folate increases locally available folate and therefore uptake by the RFC and other means in PCa cells. This is particularly relevant in low folate conditions, in which pathways to increase folate uptake are essential for normal folate homeostasis. Concurrent liberation of glutamate from various substrates by PSMA contributes to energy generation by cancer cells, feeding into the TCA cycle. Enzymatic action on previously MMP-degraded laminin peptides generates both glutamate and angiogenic peptides. This glutamate conceivably activates GCPRs to activate PI3K(110β) or is utilised metabolically within the cell.
PSMA = prostate-specific membrane antigen; PIP2 = phosphatidylinositol-(4,5)-bisphosphate; PIP3 = phosphatidylinositol-(3,4,5)-trisphosphate; PI3K = phosphoinositide 3-kinase; RFC = reduced folate carrier; Akt = protein kinase B; TCA = tricarboxylic acid cycle; GCPR = G-coupled protein receptor; MMP = matrix metallopeptidase.

with *TP53* loss exhibited resistance to PSMA-targeted β-particle therapy *in vivo* [27] and to α-particle therapy in patients with CRPC [28]. This is somewhat counterintuitive, as a defective DDR response would presumably sensitise a cell to a DNA-damaging agent, as it cannot recover its genomic integrity correctly. This may be down to which genes in the various DDR cascades are defective. Genomic defects coding for proteins that inhibit cell cycle progression, such as *CDC25A*, can lead to radioresistant DNA synthesis [29]. Therefore, the cell can still repair its DNA but it cannot stop cell cycle progression, even when there is irreparable DNA damage. Mutations in central DDR mediators, such as *BRCA2*, often sensitise to radiation [30] as the DDR mechanism is comparatively limited. In either situation, cells are likely to be in a state of stress due to uncontrolled proliferation or continually increasing DNA damage, with the significant metabolic requirements of these scenarios. Therefore, PSMA expression is likely to be expressed because of cell stress; however, high PSMA expression may not necessarily indicate resistance to DNA-damaging agents. In order to substantiate these relationships, changes in PSMA expression as a direct consequence of cell stress, including the specific DDR defects noted in PSMA-positive CRPC, should be investigated.

3.3. *Function of PSMA in PCa*

PSMA has been implicated in folate and glutamate mobilisation, uptake, and signalling (Fig. 2). Both glutamate and folate are involved in wide-ranging cellular processes, including DDR, bioenergetics, protein synthesis, and cellular signalling. Interestingly, it has been reported that PSMA is involved in folate transport in PCa cells and converts locally synthesised NAAG to *N*-acetyl asparate and glutamate [31].

PSMA overexpression in PCa cells confers a survival advantage over non–PSMA-expressing cells in folate-depleted conditions [32]. PCa cells are likely to be highly sensitive to folate deprivation because of their greater demand for folate for polyamine synthesis [33]. PSMA generates monoglutamated folates, which can pass across the cell membrane, from polyglutamated folates [31,32]. The uptake of folic acid, a synthetic form of dietary folate, is higher in PSMA-positive cells [33]. Furthermore, metabolic scores comprising genes associated with folate metabolism, the one-carbon cycle, and polyamine synthesis are considerably higher in a cohort of localised prostate cancers (TCGA-PRAD), compared to other tumour types, suggesting that PCa cells have higher demand for the products of folate metabolism [34]. PSMA is probably critical in this process.

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It has also been reported that glutamine is an alternative energy source in PCa cells through glutaminolysis. During glutaminolysis, glutamine is broken down into glutamate and ammonia as products of the first step; the former is used as a substrate for the tricarboxylic cycle [35,36]. PSMA is implicated in the generation of glutamate via its enzymatic action on glutamate moieties of NAAG, polyglutamated folates, and laminin peptides in the extracellular matrix [32,37,38]. PSMA is required for liberation of glutamate from tumour-derived NAAG, although this relationship has not yet been investigated in the context of PCa [37]. It has been shown that PSMA generates a localised reservoir of glutamate from NAAG and fuels tumour growth in high-grade ovarian serous adenocarcinoma cells [37]. Matrix metallopeptidases, which are also upregulated in PCa [39,40], break down laminin peptides to generate peptide components with glutamate moieties. PSMA can then act on these to generate proangiogenic peptides and glutamate [38,41]. Other enzymes involved in glutaminolysis, a process that converts glutamate into substrate for the tricarboxylic acid cycle, are upregulated in PCa [42]. Furthermore, it has been shown that patients with high-risk PCa have high serum glutamate levels [43]. While the role of PSMA in glutaminolysis in PCa is not fully understood, it is possible that increasing cellular stress and metabolic demand for glutamate could increase PCa cell vulnerability to PSMA targeting.

3.4. *PSMA as a therapeutic target and biomarker in PCa*

Given the high expression of PSMA by PCa cells and its biological functions, targeting of PSMA has been the focus of intense clinical research in PCa. Numerous PSMA-targeting agents, including radionuclide therapy (RLT; with an antibody or small molecule), PSMA-targeting immunotherapies (bi- and tri-specific T-cell engagers), and antibody-drug conjugates (ADCs) are currently in clinical development. Many of these agents have demonstrated promising antitumour activity, with a 177-Lutetium (^{177}Lu) conjugated small-molecule peptide (^{177}Lu-PSMA-617) the furthest in clinical development (NCT03511664; Table 1) [44–46,51].

3.4.1. *PSMA-directed radiopharmaceuticals*

PSMA-targeting radiopharmaceuticals can be labelled with different radionuclides for diagnostic (eg, positron emitter gallium-68) or therapeutic (eg, the β-particle emitter ^{177}Lu and the α-particle emitters Actinium-225 [^{225}Ac] and Thorium-227 [^{227}Th) purposes. Changes to the radionuclide linker, chelator, and PSMA binding domains can alter the pharmacokinetic and pharmacodynamic properties, and consequently impact the antitumour activity and toxicity profile. α-particles have higher linear energy transfer but a shorter range than β-particles; the result is more DNA damage to nearby cells but less penetration into surrounding tissue. Thus, α and β emitters are likely have different advantages depending on the disease pattern [47,48].

3.4.2. *β-emitting RLTs*

PSMA ligands such as PSMA-617, MIP-1095, and PSMA-I&T ("imaging and therapy") can be labelled with β-emitters such as ^{177}Lu or Iodine-131 (^{131}I) for RLT. A nonrandomised phase 2 study of ^{177}Lu-PSMA-617 (up to 6 cycles, 6 wk apart) in 50 patients who had experienced progression after taxane chemotherapy and second-generation novel antiandrogens, selected on the basis of high PSMA avidity and the absence of discordant PSMA-negative metastases on fluorodeoxyglucose (FDG) PET imaging, reported that 64% of patients achieved the primary endpoint of a PSA decline of ≥50%. This was subsequently shown to be associated with longer overall survival. Of the 27 patients who had measurable soft-tissue disease, 15 (56%) had a partial radiological response [45,49]. Notably, 11/15 (73%) patients who had previously responded to ^{177}Lu-PSMA-617 and were retreated with ^{177}Lu-PSMA-617 achieved a PSA decline of ≥50% with retreatment. The most common treatment-emergent adverse effects were self-limiting xerostomia (all grade 1–2; 66%), transient nausea (all grade 1–2; 48%), thrombocytopenia (grade 3–4; 10%), and anaemia (grade 3; 10%) [45,49]. This treatment was subsequently evaluated in a randomised phase 2 study comparing ^{177}Lu-PSMA-617 with cabazitaxel in patients selected using the same imaging criteria. Eighty of the 291 participants registered were excluded on the basis of imaging criteria. Patients receiving ^{177}Lu-PSMA-617 had significantly higher rates of PSA response (decrease by ≥50%: 66% vs 33%) and radiological response (49% vs 24%), and longer progression-free survival. The most common treatment-emergent adverse effects were fatigue and cytopenias, although treatment was well tolerated when compared with cabazitaxel [50]. The phase 3 VISION trial randomised mCRPC patients (2:1) who had progressed after at least one line of novel androgen axis-targeted therapy and at least one taxane regimen with PSMA-positive metastatic disease and no moderately-sized PSMA-negative metastatic disease to ^{177}Lu-PSMA-617 or best supportive care. This trial had a high screen positive rate of 87%. The trial initially suffered from a high dropout rate partly because radium-223 and chemotherapy were not permitted in the control arm. Dropout improved with mitigation measures including site education. The study met its primary and secondary endpoints with the ^{177}Lu-PSMA-617 arm demonstrating a significant improvement in overall survival, radiologic progression-free survival, PSA and RECIST response. These results will likely see ^{177}Lu-PSMA-617 become a part of the prostate cancer treatment armamentarium to be sequenced after AR-targeted agents and chemotherapy [51].

A ^{177}Lu-labelled diagnostic or therapeutic PSMA ligand (DOTAGA-[I-y]fk[Sub-KuE], also called PSMA-I&T) is being prospectively evaluated. In a series of 56 patients with progressive mCRPC for whom PSMA uptake was determined via ^{68}Ga-PSMA, 59% achieved a PSA decline of >50% after receiving ^{177}Lu-PSMA-I&T. Objective, partial radiological response was observed in 20% of the 25 patients with measurable disease. There was no clinically significant haematological toxicity, nephrotoxicity, or xerostomia. Similar to studies of ^{177}Lu-PSMA-617, the most common adverse

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Table 1 – Key clinical studies of PSMA-directed therapies in clinical development

Class	Agent	Setting	Phase	Clinical trial registration	Publication
β-Emitting small molecule	**Monotherapy**				
	^{177}Lu-PSMA-617	High-risk localised or locoregional APC (neoadjuvant)	Phase 1/2	NCT04430192	
	^{177}Lu-PSMA-617	mCRPC	Phase 2	ACTRN12615000912583 [a]	Published [45,49]
	^{177}Lu-PSMA-617 vs cabazitaxel	mCRPC	Phase 2	NCT03392428	Published [50]
	^{177}Lu-PSMA-617 vs best supportive/ standard of care	mCRPC	Phase 3	NCT03511664	Published [99] [b]
	^{177}Lu-PSMA-617 (fractionated dosing)	mCRPC	Phase 1	NCT03042468	
	^{177}Lu-PSMA-I&T	Oligometastatic HSPC	Phase 2	NCT04443062	
	^{177}Lu-PSMA-617 vs AR-targeted therapy	mCRPC	Phase 3	NCT04689828	
	^{177}Lu-PSMA-I&T	Neoadjuvant for localised APC	N/A	NCT04297410	
	^{177}Lu-PSMA-I&T vs abiraterone or enzalutamide	mCRPC	Phase 3	NCT04647526	
	Combinations				
	^{177}Lu-J591 and ^{177}Lu-PSMA-617	mCRPC	Phase 1/2	NCT03545165	
	^{177}Lu-PSMA-617 and pembrolizumab	mCRPC	Phase 1 Phase 1/2	NCT03805594 NCT03658447	
	^{177}Lu-PSMA-617 followed by docetaxel vs docetaxel	Metastatic HNPC	Phase 2	NCT04343885	
	^{177}Lu-PSMA-617 plus olaparib	mCRPC	Phase 1	NCT03874884	
	^{177}Lu-PSMA-617 plus enzalutamide	mCRPC	Phase 2	NCT04419402	
β-Emitting antibodies	**Monotherapy**				
	^{90}Y- or ^{177}Lu-J591 mAbs	CRPC	Phase 1	N/A	Published [58,59]
	^{90}Y-J591	CRPC	Phase 1	N/A	Published [54]
	^{177}Lu-J591	CRPC	Phase 1	N/A	Published [55]
	^{177}Lu-J591	mCRPC	Phase 2	NCT00195039	Published [56]
	^{177}Lu-J591 (fractionated dosing schedule)	mCRPC	Phase 1	NCT00538668	Published [57,97]
	Combinations				
	Docetaxel/prednisone plus ^{177}Lu-J591 Ab (fractionated)	mCRPC	Phase 1	NCT00916123	Published [88]
	^{177}Lu-J591 plus KCZ and HC vs 111In-J591 (Ab without radioactive particle) plus KCZ and HC	Micrometastatic CRPC	Phase 2	NCT00859781	
α-Emitting antibody	^{225}Ac-J591	mCRPC	Phase 1	NCT03276572	Published [61]
	Thorium-227 conjugate PSMA (BAY 2315497)	mCRPC	Phase 1	NCT03724747	
PSMA/CD3BiTE/TriTAC	AMG160 monotherapy and combination with pembrolizumab	mCRPC	Phase 1	NCT03792841	Published [71]
	Pasotuximab (BAY2010112)	mCRPC	Phase 1	NCT01723475	Published [68,72]
	HPN424	mCRPC	Phase 1/2	NCT03577028	Published [69,98] [b]
PSMA ADC	PSMA ADC (IgG1 Ab with monomethyl auristatin E)	mCRPC	Phase 1	NCT01414283	Published [73]
	PSMA ADC (IgG1 Ab with monomethyl auristatin E)	mCRPC (post taxane)	Phase 2	NCT01695044	Published [75] [b]
	PSMA ADC (IgG1 Ab with monomethyl auristatin E)	mCRPC (post abiraterone/ and/or enzalutamide)	Phase 2	NCT02020135	Published [74] [b]

Ab = antibody; ADC = antibody-drug conjugate; APC = advanced prostate cancer; AR = androgen receptor; BiTE = bispecific T-cell engager; CRPC = castration-resistant prostate cancer; HNPC = hormone-naïve prostate cancer; HSPC = hormone-sensitive prostate cancer; mAb = monoclonal Ab; mCRPC = metastatic CRPC; HC = hydrocortisone; KCZ = ketoconazole; N/A = not applicable; PSMA = prostate-specific membrane antigen; TriTAC = trispecific T-cell engager.
[a] Australian New Zealand Clinical Trials Registry.
[b] Publication in abstract form at the literature review cutoff date.

events were grade 1–2 anaemia, leukopenia, and transient xerostomia, although thrombocytopenia was not reported [52]. Several studies are also evaluating whether ^{177}Lu-PSMA-617 and ^{177}Lu-PSMA-I&T would be beneficial in earlier stages of disease (NCT04343885, NCT04443062, NCT04297410, NCT03828838, and NCT04430192) (Table 1).

3.4.3. *PSMA-targeting antibodies*

Apart from conjugated small molecules, PSMA-targeting antibodies conjugated with a β-emitter are also in clinical development. The anti-PSMA monoclonal antibody J591 has been conjugated with Yttrium-90 (^{90}Y) and ^{177}Lu using dodecane tetraacetic acid as the chelate. The unarmed

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antibody had minimal antitumour activity [53]. Antitumour activity was first demonstrated in a phase 1 trial of ^{90}Y-J591 in which partial radiological responses and a PSA decline of >50% occurred in two patients [54]. Subsequently, ^{177}Lu-J591 has been evaluated in five published phase 1/2 clinical trials in patients with mCRPC without imaging selection. These studies demonstrated dose-dependent antitumour activity. The phase 1 study established a recommended phase 2 dose (RP2D) of 70 mCi/m^2 [55,56]. The most common side effects were cytopenias, which were mostly reversible [55–59]. Given the larger size of the antibody compared to small-molecule inhibitors of PSMA, ^{177}Lu-J591 has a longer circulating time. ^{177}Lu-J591 also has less exposure at the renal tubules and small intestinal brush border than small-molecule RLTs, so has a different side-effect profile; it causes more haematological toxicities, but potentially has less impact on the kidneys, salivary glands, and small intestine [55,56].

In light of dose-limiting myelotoxicity in the phase 1 study, a phase 1/2 study evaluated the effect of fractionation (20–45 mCi/m^2, 2 doses, 2 wk apart). It showed that this approach improved the therapeutic window by decreasing the radioactivity per dose to the bone marrow and increasing the total tumour dose [57]. At the highest RP2D (45 mCi/m2, 2 doses), 29% of patients had a PSA decline of >50%; 35% had reversible grade 4 neutropenia and 59% had thrombocytopenia [57]. A subsequent pilot study evaluating hyperfractionation (25 mCi/m^2 every 2 wk until grade 2 toxicity), with the intention that this may allow the delivery of even higher cumulative doses, did not demonstrate an additional benefit. Further studies using the two-dose fractionation schedule are planned [60]. An important question remains as to how PSMA-targeting small molecules compare to antibodies in terms of antitumour activity and overall safety.

3.4.4. *α-emitting RLTs*

Several α-emitting PSMA-targeting RLTs are in clinical development, including the antibody-based RLT ^{225}Ac-J591 (NCT03276572), a PSMA-targeted ^{227}Th conjugate (PSMA-TTC; BAY 2315497; NCT03724747), and the small-molecule conjugates ^{225}Ac-PSMA-I&T and ^{225}Ac-PSMA-617 (NCT04597411) [61–63]. The antitumour activity of ^{225}Ac-PSMA-617 and ^{225}Ac-PSMA-I&T in mCRPC patients has been reported in retrospective case series, including in some patients who had previously experienced progression on a β-emitting PSMA-targeting RLT [63–65]. However, xerostomia led to weight loss and treatment discontinuation in some cases [64]. Efforts to mitigate α-emitter–induced glandular damage include conjugation with an antibody to reduce salivary gland distribution, fractionation, dose titration, and salivary gland protective measures [65], although their effectiveness remains unclear. To date, there has been no head-to-head comparison of α- and β-emitting PSMA-targeting RLTs.

3.4.5. *PSMA-targeting immunotherapies*

PSMA-directed bispecific T-cell engagers (BiTEs) consisting of an antibody targeting both PSMA and the CD3 T-cell receptor to induce T-cell activation, PCa-directed cell lysis, and growth inhibition have shown antitumour activity both in vitro and in vivo [66,67]. Several agents (AMG160, pasotuxizumab/AMG212/BAY2010112) are in clinical development as monotherapy (NCT03792841) or in combination with an anti–PD-1 antibody (NCT01723475). Pasotuxizumab demonstrated early evidence of clinical activity with partial response seen in a patient who had previously failed to respond to ^{177}Lu-PSMA-617, although administration is via continuous intravenous infusion [68]. Another PSMA-directed BiTE, AMG160, is currently being evaluated in a phase 1 dose-escalation study in patients with heavily pretreated mCRPC. According to a preliminary report, 6/10 patients had a PSA (>50%) response and one had a confirmed partial response among patients treated at the two highest dose levels. This included patients who had previously received PSMA-targeted RLT. Predictable and generally low-grade cytokine release syndrome was easily mitigated by dexamethasone premedication, prehydration, and a lower run-in dose. A trispecific T-cell–activating construct consisting of a PSMA-targeting domain, a CD3-targeting domain, and a third domain that binds noncovalently to serum albumin to extend the half-life (HPN424) is also in phase 1 clinical development (NCT03577028) [69]. While *in vitro* studies of AMG160 indicated that PSMA expression is necessary for antitumour activity [70], the clinical trial did not select patients on the basis of PSMA expression [71,72]. Since PSMA-targeting T-cell–activating therapies rely on indirect tumour lysis by T cells, which can potentially impact adjacent non–PSMA-positive tumour cells, it is plausible that heterogeneous or lower levels of expression are sufficient to confer antitumour immunity [67,70]. Biomarker studies from these clinical trials will further elucidate the biology of PSMA-directed T-cell–activating therapies.

3.4.6. *PSMA-directed therapies with nonspecific cytotoxic agents*

The high expression of PSMA in a significant subset of PCa makes it an ideal target for delivery of a nonspecific cytotoxic payload using an ADC. A fully humanised antibody to PSMA linked to the microtubule-disrupting agent monomethyl auristatin E (MMAE) was evaluated in a phase 1 dose-escalation study in which 52 patients with mCRPC who had experienced progression on taxanes were treated at doses ranging from 0.4 to 2.8 mg/kg. Neutropenia and peripheral neuropathy were early and late dose-limiting toxicities, respectively, which established the maximum tolerated dose of 2.5 mg/kg. A PSA decline of ≥50% was observed in 8/40 patients (20%) who received doses of ≥1.8 mg/kg [73]. Preliminary results from two phase 2 studies of this PSMA-targeting ADC, which included both taxane-refractory and chemotherapy-naïve mCRPC cases, demonstrated antitumour activity at both the 2.5 mg/kg and 2.3 mg/kg doses. Dosing was initiated at 2.5 mg/kg and adjusted to 2.3 mg/kg because of neutropenia [74,75]. Previous efforts to develop PSMA-targeting ADCs have been less successful. The development of MLN2704, a PSMA-targeting monoclonal antibody linked to the anti-microtubule chemotherapy agent maytansinoid, was terminated

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because of linker lability leading to payload deconjugation and peripheral neuropathy [76,77]. While promising, these approaches are also limited by the heterogeneity of PSMA expression, highlighting the need to elucidate the biology of PSMA regulation and expression. Therefore, combinatory treatments that enhance PSMA expression on PCa cells will arguably benefit this subset of therapies the most.

3.5. Biomarker development for PSMA-targeting

Given the rapid advances in and success of PSMA-targeting RLT, there is now an urgent need to optimise patient selection for these treatments. Prospective studies of ^{177}Lu-PSMA-617 in patients often select according to the presence of tumour PSMA expression, defined as SUVmax for tumour involvement of at least 1.5 times the SUVmean for liver, and the lack of major discordant FDG-positive and PSMA-negative disease [49,78], although it remains unclear what the lower threshold of expression for benefit is. Studies of other α- and β-emitting PSMA-targeted RLTs published to date have not selected patients on the basis of PSMA expression and are underpowered for biomarker analyses.

Intrapatient heterogeneity and the dynamic nature of PSMA expression present additional challenges for biomarker development [19,79]. Various methods to enhance tumour visualisation by reducing physiological or background uptake are under investigation. These include preimaging supplementation with monosodium glutamate or the "cold" radioconjugate; however, monosodium glutamate did not improve tumour visualisation [80,81]. PET imaging offers advantages over IHC in characterising heterogeneous PSMA expression across different metastatic sites and mapping longitudinal changes in PSMA expression, while IHC assays elucidate heterogeneity in PSMA expression at a cellular level. A study of primary PCa biopsies that were PSMA-negative on IHC predicted for the lack of avidity on PSMA-PET. It is unclear, however, whether patients with PSMA-PET–negative disease, for whom low-level or heterogeneous expression on IHC is observed, may still benefit from PSMA-targeted therapy through bystander and/or crossfire effect [82]. Nevertheless, PSMA IHC expression in diagnostic tumour biopsy samples is unlikely to be representative of expression at metastatic sites and in advanced later-stage disease [15,83–85]. As discussed earlier, standard-of-care PCa treatments alter PSMA expression, and expression generally increases with disease progression. Overall, fresh tumour biopsies for IHC analyses and PET imaging are likely to be complementary. Prospective studies incorporating serial and orthogonal measures of PSMA expression, as well other biomarkers measuring vulnerability to radiotherapy or payload chemotherapy, are needed to identify potential responders.

3.6. Overcoming resistance

It has now been shown that PSMA-targeted RLT benefits a significant subset of patients with mCRPC, with additional studies evaluating its efficacy in earlier stages of disease. Further studies are now needed to broaden the benefit of these treatments and to develop strategies to overcome secondary resistance. Measures to improve the therapeutic window through dose fractionation, enhanced drug delivery, and retention are being pursued. Other strategies to overcome primary and secondary resistance to RLT include combining existing agents with drugs that upregulate PSMA expression, synergise with the cytotoxic agents or radiation, or target pathways, such as PI3K/Akt/mTOR, that have crosstalk with PSMA [86,87]. In addition, PSMA-independent pathways may play a role in resistance to PSMA-targeted therapies, so unbiased analyses of pre- and post-treatment samples are critical in these studies. Strategies that increase the dependence of PCa cells on PSMA for survival (e.g. by altering glutaminolysis) also merit further study.

Given that PSMA is involved in generating folate and glutamate, with expression associated with defective DDR, PSMA-targeting radionuclides may also synergise with treatments that cause further DNA damage or inhibit the DDR response [19]. Radiation and chemotherapy both cause DNA damage. When combined with inhibition of PSMA function, this approach may be synergistic. Moreover, DNA-damaging agents and DDR inhibitors increase replication stress, which in turn could upregulate PSMA [19]. This may be of particular relevance to patients with tumours harbouring defective DNA repair genes. Chemotherapies such as taxanes can also reduce tumour bulk and radiosensitise cells. The feasibility of this approach was studied in a phase 1 trial of the combination of dose-fractionated ^{177}Lu-J591 (2 doses, 2 wk apart, up to a planned dose of 2.96 GBq/m^2) and docetaxel in patients with mCRPC. As expected, haematological toxicities were common but reversible. Antitumour activity, as shown by a >50% PSA decline in 11/15 patients (73%) and a partial radiological response in 3/5 patients (60%) with measurable disease, was observed [88]. Given the aforementioned interaction between AR and PSMA [85,89,90], combining AR blockade and PSMA targeting may also improve their efficacy.

Radiotherapy may synergise with immunotherapy through abscopal effects, which occurs, in part, because of induction of systemic antitumour immunity [91,92]. Radiation induces genomic instability, neoantigen formation, and activation of both innate and adaptive immune responses that promote immune surveillance. Simultaneous upregulation of immune checkpoints, however, may limit this [93–95]. Thus, immunotherapy could enhance systemic antitumour immunity while specifically targeting compensatory immune evasive adaptations. A preclinical study indicated that the combination of ^{225}Ac-PSMA-617 and anti–PD-1 delayed tumour progression in immunocompetent syngeneic mouse tumour models [96]. Early-phase clinical trials combining PSMA-targeting radionuclides or PSMA-targeting radionuclides with AR blockade, PARP inhibitors, chemotherapy, and immunotherapy are ongoing (Table 1).

4. Conclusions

In conclusion, PSMA-targeting therapies have demonstrated impressive antitumour activity and clinical benefit

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in recent clinical studies. Orthogonal and serial characterisation of PSMA expression during these studies is now urgently needed to define the optimal biomarker selection strategies for PSMA-targeted therapies. Since PSMA expression is heterogeneous and dynamic, its regulation needs better elucidation to drive rational drug development efforts aimed at modulating PSMA expression to improve efficacy. Understanding the biological functions of PSMA will also help to identify cellular vulnerabilities to these therapies, leading to therapeutic combinations that overcome treatment resistance and maximise clinical benefit.

***Author contributions*:** Johann S. de Bono had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: de Bono, Sheehan, Guo.
Acquisition of data: Guo, Sheehan.
Analysis and interpretation of data: Sheehan, Guo.
Drafting of the manuscript: Guo, Sheehan.
Critical revision of the manuscript for important intellectual content: de Bono, Guo, Sheehan, Sandhu, Paschalis, Neeb.
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