Prostate-Specific Membrane Antigen–Targeted Theranostics: Past, Present, and Future Approaches

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Abstract: Although prostate cancer is the type of cancer most commonly survived by men in the United States, it remains the second most common cause of death from cancer, largely owing to metastatic disease. Patients with metastatic castration-resistant prostate cancer (mCRPC) whose disease has progressed on standard-of-care therapies have few options and a poor prognosis. Prostate-specific membrane antigen (PSMA) is a type II integral membrane protein that is commonly expressed in prostate cancer. Expression is limited on extra-prostatic tissues other than the salivary glands, lacrimal glands, duodenal epithelium, Kupffer cells, and renal tubules. PSMA-directed theranostics has emerged to exploit the specificity of PSMA for prostate cancer cells and has demonstrated promising results in the clinic. Radionuclides linked to PSMA inhibitors/binders have resulted in US Food and Drug Administration (FDA) approval of 2 radiodiagnostics for PSMA-directed positron emission tomography/computed tomography. In addition, these radionuclides have led to the development of lutetium Lu 177PSMA-617 therapy, which is currently under priority FDA review. Multiple novel PSMA-targeted modalities have been developed and are currently under clinical investigation, including ligand-drug and cellular immune therapies. In this review, we discuss the development of PSMA-directed theranostics, along with its clinical implications, limitations, and future directions.

Keywords
Antibody-drug conjugate, ligand-drug conjugate, metastatic castration-resistant prostate cancer, prostate-specific membrane antigen (PSMA), precision medicine, radiopharmaceuticals

Overview

Prostate cancer will develop in approximately 1 in 9 men in the

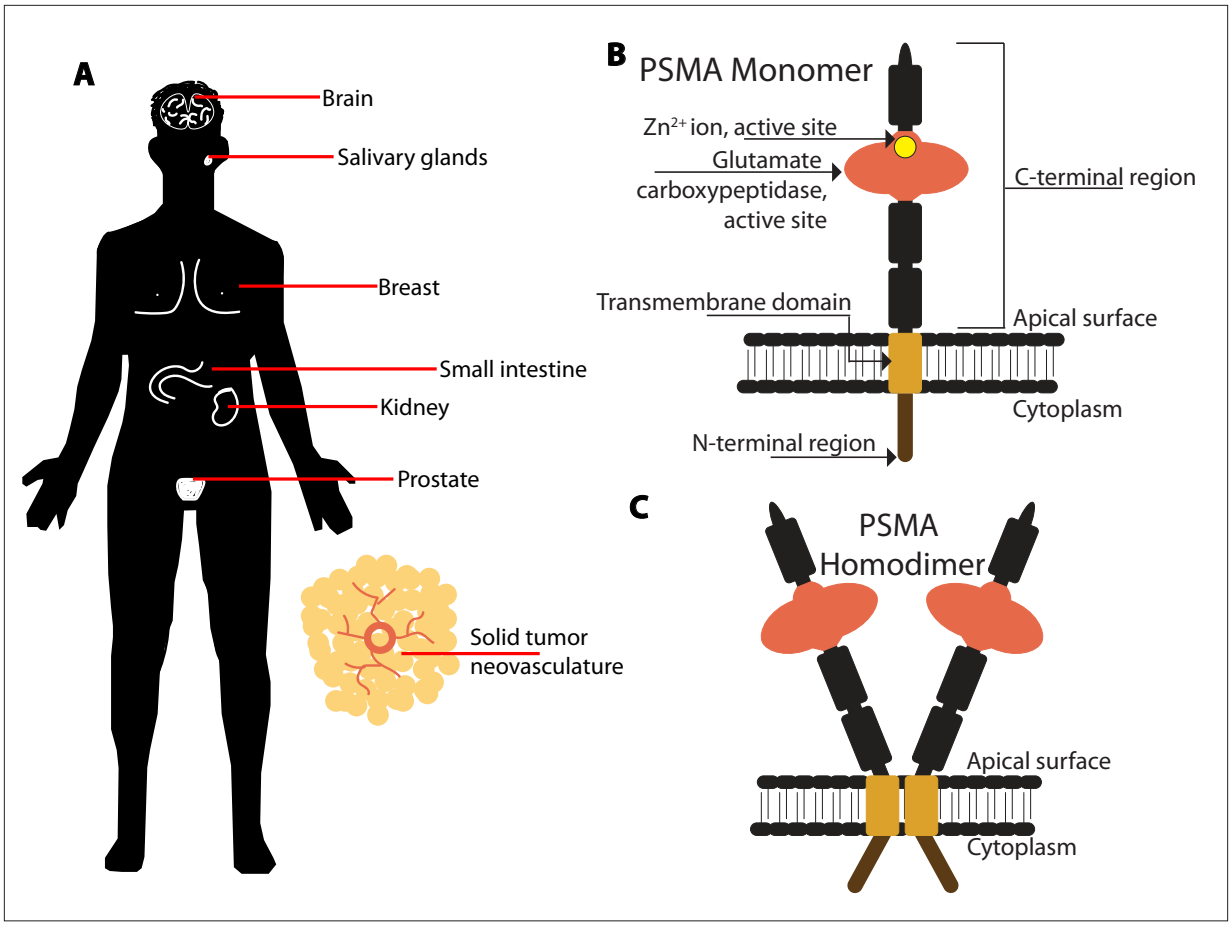

Figure 1. A, Tissues in the body that express prostate-specific membrane antigen (PSMA). Solid tumor neovasculature expresses PSMA, but normal endothelium does not express PSMA. **B,** Simplified representation of the PSMA type 2 transmembrane protein as a monomer. **C,** PSMA exists on cell membranes as a homodimer.

United States during their lifetime. Although most of these men will survive the disease for a time owing to early detection and local therapy, eventually approximately 20% of them will experience relapse after surgery and 40% after radiation, with the development of potentially life-threatening disease.[1-3] Metastatic prostate cancer is the second-leading cause of cancer-related death among American men, with a 5-year survival rate of approximately 30%.[4] The second-generation anti-androgen therapy and chemohormonal therapy introduced over the past decade have significantly improved outcomes in men with metastatic hormone-sensitive prostate cancer (mHSPC) and have become the standard of care (SOC).[5-8] Despite these improvements, castration resistance develops in nearly all patients with metastatic disease, and they will require further therapy over time. Despite the use of further taxane chemotherapy, radium Ra 223 dichloride (Xofigo, Bayer) or sipuleucel-T (Provenge, Dendreon) therapy, and selected precision medicine therapies such as pembrolizumab (Keytruda, Merck) and olaparib (Lynparza, AstraZeneca), remissions remain short and durable remissions remain rare in this setting. The paucity of therapeutic options after the failure of standard therapies underscores an urgent and unmet need. The recent emergence of PSMA-directed theranostics promises to address this unmet need.

In this review, we provide an overview of the discovery and biology of PSMA and how its physical and biological properties have been and are being exploited to develop novel theranostics, and of the potential effect these discoveries will have in the clinic and beyond.

The Discovery of PSMA and Its Biology

After establishing the LNCaP cell line in 1983, Horoszewicz and colleagues 4 years later isolated a monoclonal antibody (mAb), 7E11, from murine hybridomas immunized with LNCaP cells. These mAbs were highly

specific for the membranes of normal and malignant prostate epithelium. The mAbs did not appear to react with normal tissues of other organs that were tested; thus, the membrane protein was named prostate-specific membrane antigen (PSMA).[9] Outside the prostate gland, very low levels of PSMA expression are seen in the brain, the proximal tubules of the kidneys, the salivary and lacrimal glands, the peripheral ganglia, breast tissue, and duodenal brush border epithelium (Figure 1A).[10-12] The expression of PSMA is approximately 1000 times greater in prostate cancer than in normal prostate tissue and highest among poorly differentiated and castration-resistant tumor cells.[10,13] This finding led to the observation that PSMA expression decreased by 3- to 10-fold in the presence of androgen.[10] Subsequently, in one of the first clinical studies of PSMA, Ross and colleagues showed that PSMA overexpression in primary prostate tumors increases with the tumor grade and the presence of metastatic disease, and higher levels independently predict poor clinical outcomes.[14,15]

PSMA is a type II integral membrane glycoprotein located on the apical surface of cells as a monomer or homodimer (Figure 1B).[16-18] The protein structure includes a short cytoplasmic N-terminal region, a 24–amino acid transmembrane domain, and a 707–amino acid extracellular C-terminal region, which is strongly homologous with the human transferrin receptor.[19-23] The earliest functional studies of what is now known as PSMA, conducted in brain tissue, demonstrated that PSMA has N-acetylated-alpha-linked acidic dipeptidase (NAALDase) activity.[17] PSMA, also known as folate hydrolase 1 (FOLH1) or glutamate carboxypeptidase II (GCPII), plays a tissue-specific role in folate and glutamate metabolism by removing gamma-linked glutamate from folate and catalyzing the hydrolysis of N-acetyl-aspartyl-glutamate (NAAG) to form glutamate and N-acetylaspartate. Prostate cancer cells expressing PSMA take up a greater amount of folate and proliferate at significantly higher rates in response.[24-26] Glutamate released by the hydrolase activity of PSMA leads to activation of the phospholipase C signaling pathway and tumor growth (Figure 2).[27,28] Furthermore, the hydrolysis of NAAG by GPCII provides an important source of glutamate in high-grade tumors, and GPCII inhibition in vivo reduces glutamate concentration and tumor growth.[29]

Shortly after its discovery, PSMA was identified on the neovascular endothelium of several tumor types, including colon adenocarcinoma, renal cell carcinoma, lung cancer, and melanoma, but not on normal endothelial cells.[30] In-human imaging studies confirmed these findings.[31,32] In its functional role on tumor neovascular endothelium, PSMA is thought to contribute to interactions with integrin and endothelial activation.[33] These processes occur via PSMA-mediated laminin proteolysis, which generates a pro-angiogenic peptide.[33]

Since the initial discovery that PSMA is highly specific for prostate cancer, researchers have been eager to find ways to exploit its properties and develop prostate-targeted diagnostics and therapeutics. Over the past 3-plus decades, several approaches have been taken in the development of therapy—namely, antibodies, antibody-drug conjugates, cellular immune therapy, small-molecule inhibitors, bispecific antibodies, and PSMA-binding peptide conjugates. In the following sections, we review these approaches, the discoveries to which they have led, and their clinical implications.

The Evolution of PSMA Ligands for Use in Diagnostics and Therapeutics

The first PSMA ligands to be studied in the clinic were mAbs. Anti-PSMA mAbs can be categorized according to whether their epitopes are located in an intracellular or extracellular domain (Figure 3A). To achieve cytotoxic anticancer effects, the mAbs are labeled with radionuclides or conjugated with drugs. The mAb 7E11, which led to the discovery of PSMA, was the earliest radionuclide-labeled antibody (indium In 111 capromab pendetide [ProstaScint, Cytogen]) to receive FDA approval for the imaging of prostate cancer.[34-36] Despite its approval, the clinical utility of ProstaScint has been limited because mAb 7E11 binds only to the intracellular domain of PSMA. Highly hydrophilic antibodies do not cross the lipid membranes of viable cells; therefore, mAbs with intracellular domain epitopes react primarily with necrotic or apoptotic cells in vivo.[37]

The limitations of the first anti-PSMA mAbs motivated researchers to focus on extracellular domain targets. In 1997, Liu and colleagues reported the first 4 immunoglobulin G (IgG) mAbs that bind to the external domain of PSMA (J591, J415, J533, and E99).[38,39] This group also demonstrated that extracellular domain PSMA antibodies are internalized through endocytosis of the PSMA-antibody complex (Figure 3A-C).[40] Their findings inspired an effort to utilize these antibodies to deliver cytotoxic payloads.

Studies of radionuclide-labeled mAbs demonstrated that their uptake in viable tumor cells was greater than that of 7E11.[39] The first humanized mAb to be developed, Hu-J591, was adopted as a backbone in the development of radioligand and antibody-drug conjugate therapies.[41-43]

The earliest in-human studies of PSMA-targeted therapy were 2 independent phase 1 trials conducted in patients whose progressive castration-resistant prostate cancer (CRPC) was treated with Hu-J591 labeled with either yttrium 90 (^{90}Y) or lutetium 177 (^{177}Lu) (Figure

3D).[44,45] Both trials demonstrated acceptable safety profiles; dose-limiting toxicities were characterized as grade 3 thrombocytopenia and neutropenia. Both radionuclide antibodies showed anticancer activity; in the ^{177}Lu and ^{90}Y trials, 4 of 35 and 2 of 29 patients, respectively, demonstrated a decline in prostate-specific antigen (PSA) of more than 50% for 8 months, and 16 of 35 and 6 of 29 patients, respectively, demonstrated PSA stability for 60 days.

Compared with ^{90}Y-Hu-J591, ^{177}Lu-Hu-J591 emits a lower-energy beta particle, has a more than 2-fold longer physical half-life (2.7 vs 6.7 days, respectively), and has a longer tumor-residence time. Therefore, ^{177}Lu provides a higher level of antitumor activity with less toxicity to normal tissue. Furthermore, the emission of gamma particles by ^{177}Lu makes it useful as an imaging agent at treatment doses.[46] These differences led investigators to concentrate on developing ^{177}Lu-Hu-J591 in preference to its ^{90}Y-labeled counterpart.

Subsequently, in a phase 2 trial, men with progressive metastatic CRPC (mCRPC) were treated with 1 dose (65 or 70 mCi/m^2) of ^{177}Lu-Hu-J591, and disease response was evaluated at 12 weeks. Of the 47 patients enrolled after disease progression on hormonal therapy, 55% had received prior chemotherapy. At the 12-week endpoint, 59.6% had a decline in PSA. A reduction in PSA by 50% or more was noted in just 10.6% of the patients. More patients in the 70-mCi/m^2 than in the 65-mCi/m^2 dose group had a PSA decline of 30% or greater (46.9% vs 13.3%; P=.048). Survival was longer in the 70-mCi/m^2 than in the 65-mCi/m^2 dose group (21.8 vs 11.9 months; P=.03), but grade 4 hematologic toxicity was increased. A partial response was noted in only 1 of 12 patients with radiographically measurable disease. The rates of reversible hematologic toxicity were 46.8% (grade 4 thrombocytopenia) and 25.5% (grade 4 neutropenia).[47]

Despite its high specificity for prostate cancer and excellent safety profile, phase 1 and 2 studies of ^{177}Lu-Hu-J591 have highlighted some important limitations of using mAb ligands as a backbone of radioligand therapy. mAbs have a long circulation time, so that non-prostate tissues are exposed to extensive radiation, and they do not permeate solid tumors as well as small molecules. Therefore, PSMA-targeted low-molecular-weight biomolecules were developed. One approach utilized antibody variable regions while reducing molecular weight with antibody fragments. Anti-PSMA minibodies and single-chain fragments (scFvs) are 2 examples of antibody fragments that are under investigation for use in radioligand theranostics. To construct scFvs, portions of the mAb variable heavy and light chains are linked together. Minibodies contain scFv domains linked to an IgG1 hinge and a CH3 domain.[48]

A first-in-human phase 1 trial in which a minibody, zirconium Zn 89 desferrioxamine-IAB2M (^{89}Zr-DF-IAB2M), was used to treat men with metastatic prostate cancer demonstrated safe and effective targeting of metastatic skeletal and nodal lesions.[49] Imaging was performed 48 hours after injection, making widespread clinical utility a challenge. In a subsequent, phase 2 trial, when ^{89}Zr-DF-IAB2M was used for positron emission tomography/computed tomography (PET/CT) before prostatectomy, it performed similarly to gallium Ga 68 PSMA-11 (^{68}Ga-PSMA-11). Smaller scFv radioligands in preclinical in vivo studies have shown more favorable results. Specifically, in a comparison with larger antibody fragments, an scFv developed from D2B antibody and labeled with a radioisotope (iodine I 124 scFvD2B) showed a high uptake fraction and specificity in PSMA-positive cells at a better time point after infusion.[50] In-human trials of ^{124}I-scFvD2B are pending.

The desire to minimize molecular size and retain specificity for PSMA has led to the development of small-molecule PSMA inhibitors. In 1996, a potent inhibitor of neuropeptidase NAALDase was developed to study and treat neurologic disease.[51] The compound, 2-phosphonomethyl pentanedioic acid (2-PMPA), is a phosphonate derivative that acts as a substrate or transition-state analogue. Because the enzymatic function of PSMA is identical to that of NAALDase and FOLH1, interest shifted toward utilizing 2-PMPA as a small-molecule PSMA inhibitor. Fluorine F 18 2-PMPA (^{18}F-2-PMPA; BAY 1075553) was developed and studied in a phase 1 trial in men with metastatic prostate cancer, but it was shown to be inferior to fluorocholine in PET/CT because it had less specificity for lymph node and bone marrow disease.[52]

Subsequently, the race to optimize small-molecule PSMA inhibitors took hold with the introduction of phosphinic and phosphoramidate scaffolds (as NAALDase transition states).[53] In a phase 1 trial, a phosphoramidate compound, CTT1057, demonstrated promise as a radiopharmaceutical and radioligand therapeutic.[54] Thiol-based small-molecule PSMA inhibitors have been tested in humans as oral prodrugs.[55,56] However, further clinical development of thiol-based molecules has been hampered by their biological instability and unfavorable toxicity profiles.[56] Substantial improvements in small-molecule inhibitors came with the discovery of urea-based compounds. Although similar in structure to the phosphoramidate compounds, urea-based inhibitors were found to have a high degree of affinity for PSMA and were simpler to synthesize and modify, features making their use highly feasible (Figures 3B and 3D).[57]

Urea-based PSMA inhibitors consist of a 2–amino acid backbone joined to a urea group (glutamate-urea-X,

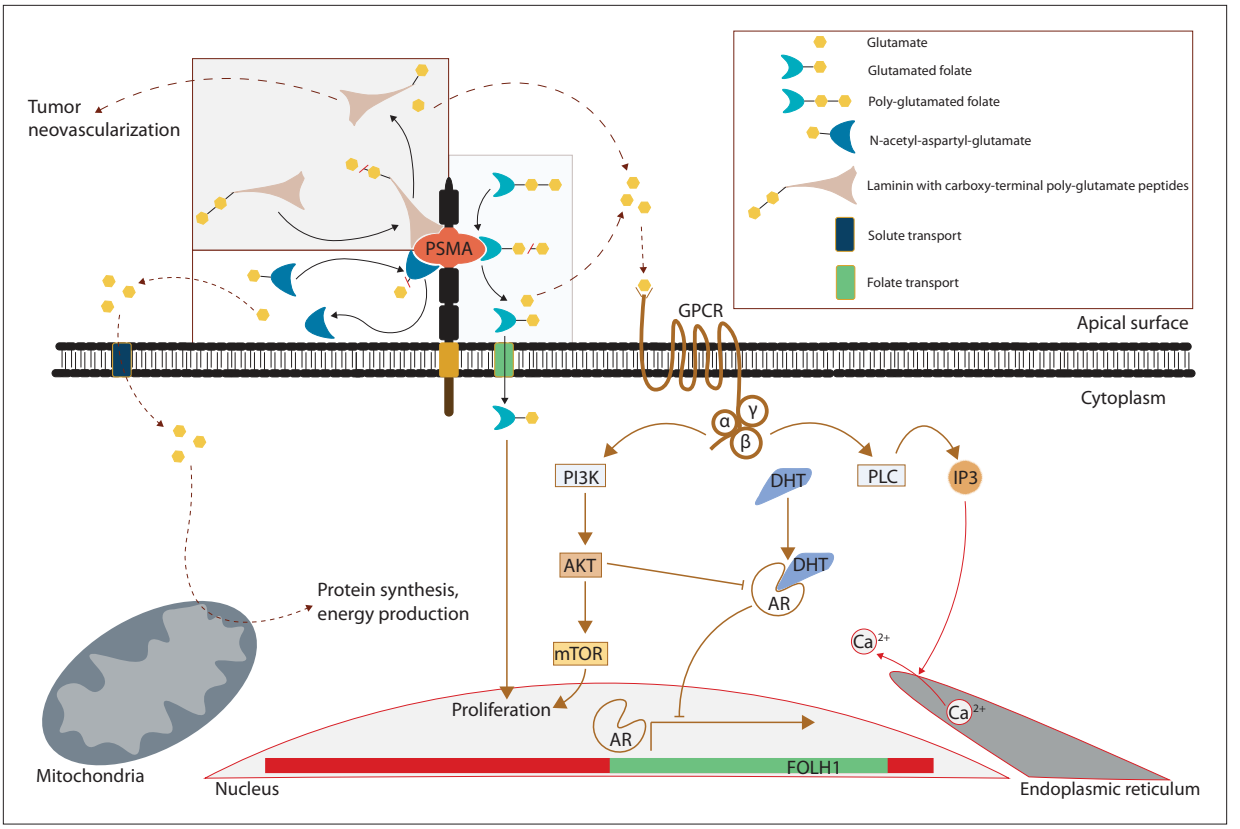

Figure 2. Graphic representation of the molecular functions of PSMA and its role in prostate cancer. Through its enzymatic activity, PSMA removes glutamate from folate, N-acetyl-aspartyl-glutamate, and laminin. After glutamate is removed from folate, it is taken up into surrounding cells and contributes to DNA synthesis. PSMA-mediated laminin proteolysis leads to a pro-angiogenesis environment and tumor neovascularization. The glutamate freed by PSMA enzymatic activity is available for uptake by cells for protein synthesis and increased energy production. Freed glutamate also binds to its G protein–coupled receptor, thereby activating PI3K/AKT/mTOR and PLC/IP3/Ca^{2+} pathways. AR, free of its ligand, promotes PSMA expression by promoting transcription of FOLH1.

AKT, protein kinase B; AR, androgen receptor; DHT, dihydrotestosterone; FOLH1, folate hydrolase 1; GPCR, G-protein coupled receptor; IP3, inositol triphosphate; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase; PLC, protein lipase C; PSMA, prostate-specific membrane antigen.

where X is glutamate, cysteine, or lysine).[58] These urea-based backbones can be labeled with radioactive isotopes for theranostics or joined to cytotoxic agents, as we discuss in subsequent sections. The glutamate-urea-lysine motif was the backbone of the first radioisotope-labeled compounds to be studied in humans, ^{123}I-MIP-1095 and ^{123}I-MIP-1072.[59] It has subsequently become the primary backbone of radiopharmaceuticals used in the clinic. In December 2020, ^{68}Ga-PSMA-11 became the first FDA-approved PSMA-targeted radiopharmaceutical for PET/CT in men with prostate cancer.[60,61] Using a similar strategy, Chen and colleagues linked [^{18}F]fluoropyridyl to a Glu-urea-Lys backbone, ^{18}F-DCFPyL. Recently published phase 2/3 clinical trials (OSPREY and CONDOR) resulted in its approval by the FDA in May 2021.[62-64]

Emergence of Radioligand Therapy

To take advantage of the excellent pharmacodynamic properties of small-molecule ligands, ^{177}Lu was linked to a Glu-urea-Lys backbone to form ^{177}Lu-PSMA-617. In a phase 1 trial of 56 men with progressive mCRPC, the patients experienced no serious adverse effects with up to 5 doses of ^{177}Lu-PSMA-617 (median dose per cycle, 5.76 GBq; range, 3.6-8.7 GBq), which was excellent tolerability.[65] A subsequent, single-arm phase 2 trial administered a median of 4 cycles of ^{177}Lu-PSMA-617 at a median dose of 7.5 GBq (range, 4-8.9 GBq) to 50 men with progressive mCRPC and positive findings on PSMA PET/CT.[66,67] A single decline in PSA of 50% or greater from baseline was seen in 64% of the patients,

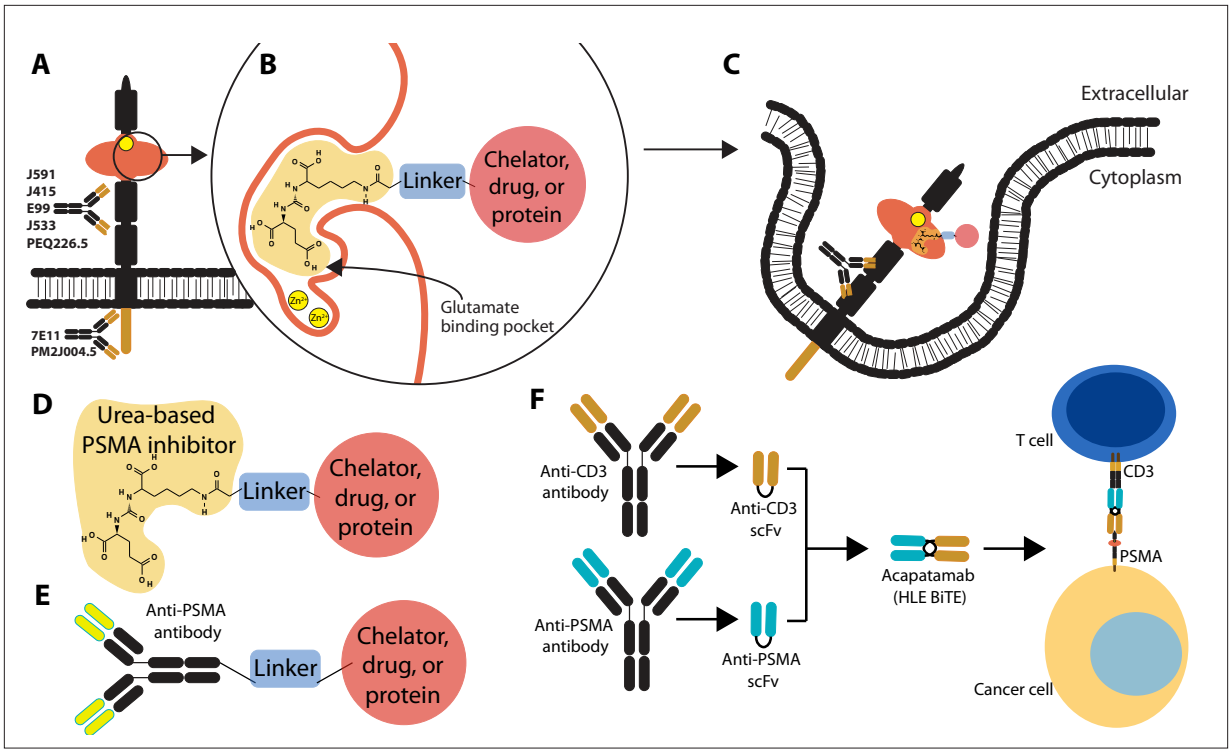

Figure 3. A, Anti-PSMA antibodies and their known binding location (N-terminal region or extracellular region). **B,** Representation of a urea-based small-molecule inhibitor linked to a therapeutic agent (chelator, drug, or protein). **C,** After PSMA is bound by antibody or a small-molecule inhibitor, PSMA undergoes endocytosis. **D,** Basic schematic of a small-molecule PSMA inhibitor linked to a therapeutic compound. **E,** Basic schematic of an antibody-drug conjugate. **F,** The half-life extended bispecific T-cell engager is formed from the scFvs of anti-CD3 and anti-PSMA antibodies. The scFvs are linked together and bind to PSMA and CD3 on T cells, leading to T-cell activation against the cancer cells.

HLE BiTE, half-life extended bispecific T-cell engager; PSMA, prostate-specific membrane antigen; scFv, single-chain variable fragment.

and among those with nodal and visceral disease, an objective response (complete/partial by RECIST 1.1) was seen in 56% at 3 months. Thrombocytopenia, the only grade 3 or 4 toxicity, occurred in 13% of patients. A subset of patients with an initial response were re-treated with ^{177}Lu-PSMA-617 at the time of progression, and 73% had an unconfirmed PSA decline of 50% or greater. To compare ^{177}Lu-PSMA-617 with standard therapies, an unblinded randomized phase 2 trial (TheraP) was conducted in men with mCRPC who were eligible for treatment with cabazitaxel (Jevtana, Sanofi-Aventis).[68] In this study, patients were treated with ^{177}Lu-PSMA-617 or cabazitaxel. Treatment with ^{177}Lu-PSMA-617 vs cabazitaxel resulted in a PSA decline of 50% or greater in 66% vs 37% of patients, respectively, and a 12-month progression-free survival (PFS) rate of 19% vs 3%, respectively. Overall, the rates of grade 3/4 toxicities in the men treated with ^{177}Lu-PSMA-617 vs cabazitaxel were 33% vs 53%, respectively. Grade 3/4 neutropenia was less common with ^{177}Lu-PSMA-617 than with cabazitaxel (4% vs 13%, respectively), but thrombocytopenia was more common with ^{177}Lu-PSMA-617 than with cabazitaxel (11% vs 0%). In these studies, the patients treated with ^{177}Lu-PSMA-617 experienced a significant reduction in cancer-related pain.

In September 2021, results from the landmark prospective, randomized, international phase 3 VISION study were published.[69] This trial enrolled 831 men with mCRPC and positive results on ^{68}Ga-PSMA-11 PET/CT (defined as the presence of at least one PSMA-positive metastatic lesion and no PSMA-negative lesions) whose disease had progressed on at least one androgen receptor pathway inhibitor (ARPI) and one or more taxane regimens (39% and 43% of the ^{177}Lu-PSMA-617 and control groups, respectively, had received 2 taxane regimens). The subjects were randomly assigned to receive 4 cycles of ^{177}Lu-177-PSMA-617 at a dose of 7.4 GBq plus SOC or SOC alone. Permitted SOC therapies included approved ARPIs (eg, enzalutamide [Xtandi, Astellas] and abiraterone), radiation therapy, denosumab (Xgeva, Amgen),

bisphosphonates, or glucocorticoids. Patients had to maintain castrate-level testosterone throughout the study. SOC did not include chemotherapy, radioactive isotopes, immunotherapy, or experimental therapies owing to a lack of safety data for these in combination with experimental treatment. Up to 6 doses of ^{177}Lu-PSMA-617 were permitted. PFS and overall survival (OS) were better in the patients who received ^{177}Lu-PSMA-617 than in those who received SOC alone (median PFS, 8.7 vs 3.4 months; median OS, 15.3 vs 11.3 months). All subgroups benefited regardless of visceral pattern of spread, functional status, concurrent ARPI use, or age. In patients with liver metastases (n=48), the hazard ratio (HR) for OS was 0.87 (95% CI, 0.53-1.43). The proportion of men with a confirmed PSA response (PSA decline ≥50% from baseline) was 46% with ^{177}Lu-PSMA-617 vs 7% with SOC. Toxicities with ^{177}Lu-PSMA-617 included grade 1 or 2 xerostomia, dry eyes, thrombocytopenia, leukopenia, and nausea and vomiting. The rates of grade 3 or 4 toxicities were 23% for bone-marrow suppression, 1.5% for nausea and vomiting, and 3.4% for renal impairment, vs 7%, 0.5%, and 2.9%, respectively, with SOC alone. These results led the FDA to grant Breakthrough Therapy Designation to ^{177}Lu-PSMA-617, which is under priority review for use in men with progressive PSMA-positive mCRPC after ARPI therapy and chemotherapy.[70]

Further trials are in progress to evaluate the benefit of ^{177}Lu-PSMA-617 in various clinical scenarios and in combination with other therapies. Of active interest is the potential synergistic combination of ARPI and PSMA radioligand therapy. PSMA expression has been found to increase in response to androgen deprivation therapy (ADT) and ARPI treatment. An early study of metastatic and primary prostate tissues in the biopsy specimens of men at baseline and after ADT found that PSMA expression increased over baseline in 55% of primary prostate samples and in 100% of metastatic samples after ADT.[71] A more recent prospective study showed an increase in PSMA expression after men with mCRPC were started on an ARPI (such as enzalutamide). There were 7 men with mCRPC who underwent ^{68}Ga-PSMA-11 PET/CT at baseline (before ARPI) and on days 9, 18, and 28 (after ARPI). After androgen inhibition had been started, the men with mCRPC demonstrated a median 45% increase in PSMA PET/CT maximum standardized uptake value (SUV_{max}) by day 9, which plateaued by day 28, and a median PSA decline of 15%.[72] Given the increase in PSMA expression seen with androgen inhibition, interest is being shown in combining PSMA-targeted radioligand therapy and ARPI therapy, with the anticipation that increased PSMA expression will result in a synergistic therapeutic effect. This will be studied in men with mCRPC in Enza-p (NCT04419402), a phase 2 trial investigating the activity and safety of the combination of ^{177}Lu-PSMA-617 plus enzalutamide vs enzalutamide alone.[73]

Additional trials include giving ^{177}Lu-PSMA-617 before docetaxel chemotherapy, before prostatectomy, in low-volume mCRPC, in combination with stereotactic body radiation, in comparison with androgen-directed therapy in chemo-naive mCRPC, in mHSPC, and in combination with poly(ADP-ribose) polymerase (PARP) inhibition and programmed death 1 (PD-1)–based immunotherapy.

The PSMA-617 ligand continues to provide a foundation for multiple PSMA-targeted therapies under clinical investigation. By replacing ^{177}Lu with the alpha emitter actinium 225 (^{225}Ac), researchers hope to improve upon the antitumor efficacy and toxicity profile of ^{177}Lu-PSMA-617; the alpha particles of ^{225}Ac have a higher energy level and shorter path length.[74] A recent prospective cohort study evaluated the efficacy and safety of ^{225}Ac-PSMA-617 in patients with mCRPC that was refractory or naive to ^{177}Lu-PSMA-617.[75] A PSA decline of 50% or greater was seen in 25% and 39% of patients with disease that was refractory or naive, respectively, to ^{177}Lu-PSMA-617. Importantly, no grade 3 or 4 events occurred. Phase 1 studies are ongoing to evaluate ^{225}Ac-PSMA-617 (NCT04597411) and ^{225}Ac-HuJ591 (NCT04946370).

Antibody-Drug Conjugates

Like PSMA radioligands, antibody- or ligand-drug conjugates aim to deliver a payload of cytotoxic therapy to maximize tumor drug concentrations and minimize toxicity to normal tissues (Figure 3E). Approaches to the development of PSMA-targeted drug therapies have been analogous to the initial approach to radioligand therapy. In 2 antibody-drug conjugates studied in men with mCRPC after progression on ARPI therapy or taxane chemotherapy, the anti-PSMA antibody HuJ591 was linked to maytansinoid-1 (MLN2704) and pyrrolobenzodiazepine (MEDI3726).[76,77] Only 8% and 3% of the treated patients showed a PSA decline of 50% or greater with MLN2704 and MEDI3726, respectively. Doses of MLN2704 were limited by neurotoxicity, with 71% of patients overall experiencing neuropathy. Grade 3 or 4 events with MEDI3726 occurred in 55% of patients and included elevated gamma-glutamyltransferase (21.2%), thrombocytopenia (9%), and capillary leak syndrome (9%). A third antibody-drug conjugate, fully humanized IgG1 anti-PSMA antibody conjugated to monomethylauristatin E (MMAE), has also been tested in men with mCRPC after progression on ARPI therapy.[78,79] The phase 1 dose-limiting toxicity was neutropenia, which occurred in 33% of patients; other toxicities were nausea (29%),

vomiting (19%), and transaminitis (25%). An overall PSA decline of 50% or greater was seen in 15% of patients. In all 3 cases, the PSA response and doses were limited by toxicity. This may have been a result of decreased tumor penetration and premature separation of the drug from the antibodies.

PSMA Ligand-Drug Conjugates

As discussed, the limitations of antibody-based therapy can be overcome with high-affinity, small-molecule PSMA ligands. The investigational drug EC1169 is a small-molecule ligand-drug conjugate that has undergone initial clinical testing.[80,81] The ligand contains the Glu-urea-Lys motif, which is analogous to PSMA-617, and is linked to tubulysin B. Preliminary phase 1 results in men with progressive mCRPC showed promising efficacy; disease was stable or improved in 6 of 6 evaluable patients, with rare grade 3 and no grade 4 toxicity. In a preclinical study, Rogers and colleagues linked a Glu-urea-Lys–based PSMA inhibitor, MU2, to human protease granzyme B (GZMB) plus a *Pseudomonas* exotoxin fragment, PE35.[82]

Small-molecule PSMA inhibitors have also been explored as ligands for the delivery of taxane chemotherapy. BIND-014 is a nanoparticle encapsulating docetaxel linked to small-molecule PSMA ligands.[83,84] In a phase 2 study of BIND-014 in men with chemotherapy-naive mCRPC, the agent was well tolerated for up to 21 cycles. No grade 3 or 4 neuropathy occurred, and grade 1 or 2 neuropathy was seen in 33% of patients. Overall, 30% of the patients had a reduction in PSA of more than 50%. Median radiographic PFS was 9.9 (95% CI, 7.1-12.6) months. PSMA expression before enrollment was not evaluated. No current clinical trials are using this agent.

Mipsagargin is a prodrug-ligand compound in which PSMA hydrolysis of the ligand activates thapsigargin, an anticancer drug whose use is limited by toxicity.[85] In a first-in-human phase 1 study, mipsagargin showed tolerability; however, response was not monitored. Results of a phase 2 study of mipsagargin before radical prostatectomy are pending (NCT02381236).

PSMA-Specific Immunotherapy

Despite encouraging results preclinically, immune checkpoint inhibitors (ICIs) in patients with prostate cancer have yet to find widespread clinical success. Dostarlimab (Jemperli, GlaxoSmithKline) has been approved for patients with DNA mismatch repair deficiency (dMMR), and pembrolizumab has been approved for patients with microsatellite instability high (MSI-H) tumors, a high tumor mutational burden (TMB-H), and dMMR. However, these scenarios account for just 3% of cases.[86] Therefore, efforts are underway to harness PSMA to expand ICI therapy to more patients.

Preclinical studies demonstrated a synergistic effect of programmed death ligand 1 (PD-L1) blockade plus PSMA-targeted radiotherapeutics.[87] Phase 1 trials are underway evaluating the addition of pembrolizumab to ^{177}Lu-PSMA-617 (NCT03805594) and ^{225}Ac-J591 (NCT04946370). The phase 1b trial of ^{177}Lu-PSMA-617 was presented at the 2021 European Society for Medical Oncology (ESMO) annual meeting, and preliminary results showed that this combination was well tolerated, with promising efficacy. An unconfirmed PSA decline of greater than 50% was seen in 27 of 37 patients, and 7 of 9 patients showed a radiographic partial response.[88] A major challenge is determining the relative benefits of adding PD-1 inhibition to an active agent without a control; therefore, controlled studies are needed. Novel anti-PSMA therapies are being developed to enhance the efficacy of ICI therapy. A bispecific antibody, REGN5678, was designed to target PSMA and CD28.[89] A phase 1/2 clinical trial is underway in patients with mCRPC treated with REGN5678 plus anti–PD-1 therapy (NCT03972657).

Similarly, acapatamab (formerly AMG 160), a half-life extended bispecific T cell engager (HLE BiTE), is designed to bind PSMA and CD3 (Figure 3F).[90] Phase 1 dose expansion results from 33 patients have been reported (NCT0379284). The most common toxicity was grade 3 or lower cytokine release syndrome in 84% of patients. A PSA reduction of 50% or greater was demonstrated in 60% of patients at cycle 5 or 6.[91] Current studies of patients with mCRPC given acapatamab alone or in combination with pembrolizumab (NCT03792841) are ongoing.

Additional PSMA-targeted BiTEs are under clinical investigation. Results from a phase 1 study of a PSMA-targeted BiTE, pasotuxizumab (AMG 212/BAY 2010112), have been reported.[92] This study demonstrated the safety and efficacy of the PSMA-targeted BiTE; a PSA decline greater than 50% occurred in 9 of 31 patients who received a subcutaneous formulation. Recently, CCW702, an anti-CD3 antibody linked to a urea-based small-molecule binder, showed excellent preclinical activity.[93] A first-in-human clinical trial of CCW702 is currently underway (NCT04077021).

Cellular immunotherapy has been used in prostate cancer for over a decade. Sipuleucel-T, a cellular therapy that stimulates T-cell activity against prostatic acid phosphatase protein, was approved in 2010 for men with minimal symptoms or asymptomatic mCRPC.[94] Analogously, investigators have developed cellular T-cell therapies against PSMA. Chimeric immunoglobulin T-cell receptors specific for PSMA showed promising results in preclinical studies.[95] Initial results from a

phase 1 trial of 5 patients with advanced prostate cancer showed a PSA reduction of greater than 50% in 2 of 5 patients.[96] Recently, PSMA-directed/TGFβ-insensitive chimeric antigen receptor (CAR) T-cell therapy has been developed and has undergone phase 1 testing in men with mCRPC.[97] Preliminary results demonstrated a good safety profile and a PSA decline in 6 of 10 patients. Final analyses are pending (NCT03089203).

Clinical Implications, Limitations, and Future Directions

Almost 40 years after the discovery of PSMA, the relentless work of scientists and clinicians has led to the first FDA-approved PSMA-targeted PET imaging drugs to facilitate treatment decisions regarding local vs systemic therapies in early disease, and has opened the door to promising novel PSMA-directed therapies in more advanced disease. The radiopharmaceutical drug ^{177}Lu-PSMA-617 has completed phase 3 testing and is now awaiting FDA approval for men with mCRPC after progression on SOC. This appears to be the first of many future agents that will help to fill a major clinical need in the treatment of patients with mCRPC, and PSMA-targeted approaches are expected to move into earlier mCRPC and mHSPC, given the frequent expression of PSMA in early disease and the tolerability of targeted approaches.

PSMA-based imaging has multiple important clinical implications. Improvements in the early detection and localization of recurrent prostate cancer may make it possible for patients to receive local therapies. These tandem strategies are under active investigation. Furthermore, PSMA-based PET/CT will become an important tool for biomarker detection. As explained in this review, the response to PSMA-targeted therapies depends on tumor expression of PSMA. The heterogeneity of PSMA expression is a major limitation, and downregulation of PSMA expression is common during mCRPC progression, particularly because of lineage plasticity.[98,99] A higher level of PSMA expression may be seen in men with DNA repair defects, whereas a lower level of expression is observed in patients with neuroendocrine transformation and visceral metastases. Approximately 15% of patients in the VISION study screen failed treatment because of the lack of a PSMA-dominant lesion and/or the presence of PSMA-negative metastases. Therefore, it will likely be necessary to evaluate all patients for PSMA expression status to determine their candidacy for PSMA-directed therapy. Because PSMA PET/CT is becoming widely available since the recent FDA approval, the PET determination of PSMA status in patients with mCRPC will likely become routine practice as a companion diagnostic at the time of progression. Some investigators have used PSMA expression in circulating tumor cells (CTCs) as a surrogate for response, and liquid biopsy assays for PSMA heterogeneity are an area of active investigation.

The most obvious limitation of PSMA-directed theranostics is their dependence on tumor PSMA expression. Patients who have no PSMA detected clinically by PSMA PET/CT or no CTC PSMA expression will not be candidates for PSMA-directed therapy. It has also been demonstrated that PSMA expression can show substantial intra-patient and inter-patient heterogeneity. In one study of PSMA-positive prostate biopsy samples, foci with no detectable PSMA were found in 100% of HSPCs and in 84% of mCRPCs.[99] Furthermore, patients with neuroendocrine or small-cell differentiation are deficient in PSMA expression. As PSMA-directed therapies evolve and become integrated into the SOC, more biomarkers and clinical predictors may be needed to identify patients who are unlikely to respond, and to anticipate resistance due to a loss of PSMA expression and the emergence of transformed neuroendocrine prostate cancer or androgen receptor indifference caused by lineage plasticity.

After the anticipated approval of ^{177}Lu-PSMA-617 by the FDA, this agent will become part of the SOC for patients with mCRPC and positive PSMA PET/CT results after progression on ARPI treatment and taxane chemotherapy. Although this is a very important development, it is important to note existing limitations. Some men are not candidates for taxane chemotherapy, and the use of ^{177}Lu-PSMA-617 seems reasonable in such patients. The PSMAfore (NCT04689828) and SPLASH (NCT04647526) trials, which will evaluate the efficacy of ^{177}Lu-PSMA-617 and ^{177}Lu-PSMA I&T, respectively, after progression on ARPI therapy without the requirement of prior taxane chemotherapy, will help address this limitation. Additionally, in comparison with the beta emission of higher-energy radionuclide isotopes (^{90}Y), the beta emission of ^{177}Lu has demonstrated decreased efficacy in bulky disease.[46] ^{177}Lu-PSMA-617, although generally very well tolerated, is of limited use in patients with bone marrow metastases. This limitation may be addressed with the investigational agent ^{225}Ac-PSMA-617, which is less toxic to marrow. However, ^{225}Ac-PSMA-617 has caused higher levels of xerostomia, which can be dose-limiting.[100]

The excellent tolerability of PSMA-directed therapy provides an opportunity to use it in combination with alternate modalities. Preclinical studies have demonstrated synergy between PSMA radionuclide therapy and immunotherapy. Current clinical trials are investigating these findings. Because PSMA expression is associated with aberrations in DNA damage repair, interest has also been shown in combining PARP inhibitors with PSMA-targeted therapies. Combinations with radiopharmaceuticals or immunotherapies targeting neuroendocrine prostate

cancer antigens seem reasonable, given the emergence of PSMA-negative tumors over time.

Of course, as PSMA-targeted therapies become prevalent in the clinic, the inevitability of disease resistance will become a secondary challenge. PSMA-negative foci within or across sites of disease may remain unresponsive to therapy and become a dominant clone. Evolutionary pressures from PSMA-targeted therapies may lead to the development of neuroendocrine and small-cell differentiation. Modifications to PSMA protein may also introduce mechanisms of resistance. For example, the PSMA splice variant PSMA′ is a cytosolic protein without a transmembrane domain. Normal PSMA, once bound by its ligand, becomes internalized through endocytosis (Figure 3C). In preclinical models, PSMA's lack of a cytoplasmic tail precludes internalization.[101] The identification of resistance mechanisms and the ability to detect patients at risk will be critical to the ongoing development of PSMA-directed therapy.

Conclusion

Prostate cancer–related death is caused by metastatic disease. Patients with mCRPC that has progressed on SOC have few options and a poor prognosis. PSMA is commonly expressed in prostate cancer, with higher degrees of expression in CRPC cells and little expression in extra-prostatic tissues. Exploiting these properties, PSMA-directed theranostics has emerged and demonstrated promising results in the clinic. Radionuclides linked to PSMA inhibitors have resulted in FDA approval of 2 radiodiagnostics for PSMA-directed PET/CT, and ^{177}Lu-PSMA-617 therapy is currently under priority FDA review. Several novel modalities have been developed and are currently under clinical investigation, including ligand-drug and cellular immune therapies.

Disclosures

Dr Sartor is a paid consultant with Advanced Accelerator Applications (AAA), Astellas Pharma, AstraZeneca, Bayer, Blue Earth Diagnostics, Bavarian Nordic, Bristol Myers Squibb, Clarity Pharmaceuticals, Clovis, Constellation Pharmaceuticals, Dendreon, EMD Serono, Fusion Pharmaceuticals, Isotopen Technologien München, Janssen, Myovant Sciences, Myriad Genetics, Noria Therapeutics, Novartis, Noxopharm, Progenics Pharmaceuticals, POINT Biopharma, Pfizer, Sanofi, Teneobio, Telix Pharmaceuticals, and Theragnostics and receives research funding (to his institution) from Advanced Accelerator Applications, Amgen, AstraZeneca, Bayer, Constellation Pharmaceuticals, Endocyte, Invitae, Janssen, Lantheus, Merck, Progenics Pharmaceuticals, and Teneobio. Dr Morris has a consulting or advisory role with Astellas Pharma, Bayer HealthCare Pharmaceuticals, Endocyte, and Advanced Accelerator Applications and receives research funding (to his institution) from Bayer HealthCare Pharmaceuticals, Sanofi, Endocyte, and Progenics Pharmaceuticals. Dr Armstrong is a paid consultant with Pfizer, Astellas Pharma, Janssen, Bayer HealthCare Pharmaceuticals, AstraZeneca, Bristol Myers Squibb, Forma Therapeutics, Novartis, Dendreon, and Merck, and receives research funding (to his institution) from Pfizer, Astellas Pharma, Janssen, Bayer HealthCare Pharmaceuticals, Dendreon, Novartis, Genentech/Roche, Merck, Bristol Myers Squibb, AstraZeneca, Constellation Pharmaceuticals, BeiGene, Forma Therapeutics, and Amgen.

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