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Radionuclide Therapy in Prostate Cancer: from standalone to combination PSMA theranostics

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ABSTRACT

Despite significant advances in prostate cancer therapeutic development over the last two decades, metastatic prostate cancer remains a lethal disease. Prostate-specific membrane antigen (PSMA), which is markedly overexpressed by prostate cancer cells, including at metastatic sites, but have low normal tissue expression, has emerged as an important theranostic target for these diseases. Both beta-emitting and alpha-emitting PSMA-targeted radionuclide therapy (RNT) are in clinical development. Several of these agents have already shown promising activity, however, a subset of patients have primary resistant disease and secondary resistance invariably occurs. Further, the effect of these therapies on healthy organs limit their therapeutic window. Elucidating the biology of PSMA, characterising the pharmacokinetic and pharmacodynamic properties of PSMA-targeted RNT, as well as mechanisms radio-resistance will facilitate therapeutic approaches aimed at improving efficacy and safety. In this review, we provide an overview of existing PSMA-targeting RNT and an update on novel combinatorial approaches, such as those with PARP inhibitors and immunotherapy, currently under investigation.

NOTEWORTHY POINTS

- Numerous PSMA-targeting radionuclides, which include alpha and beta emitters, are currently under clinical development. (page 4)
- The ^{177}Lu conjugated small molecule peptide, ^{177}Lu-PSMA-617, is the most developed PSMA-targeted RNT with phase 3 outcome data. (page 5)
- Elucidating the biology of PSMA expression, regulation, and function, will facilitate the development of novel, sequential and combinatorial approaches aimed at improving response and safety. (page 7)

INTRODUCTION

Prostate cancer is one of the most common causes of male cancer mortality globally (*1*). Whilst localised disease is curable with surgery or radiotherapy, a third of patients present with or develop lethal metastatic disease, which is invariably fatal (*2,3*). Prostate specific membrane antigen (PSMA) has emerged as a promising theranostic target for prostate cancer and PSMA-targeted therapies are rapidly changing the therapeutic landscape. Clinical trials of PSMA-targeted therapies have demonstrated anti-tumor activity in patients with advanced disease, and clinical evaluation in earlier stages of disease and larger randomized studies is ongoing(*4*). To date, several clinical trials have shown that PSMA-targeting radionuclides (RNT) is tolerable and effective, however, a subset of patients have primary resistant disease, and secondary resistance is inevitable. In this review, we provide an overview of existing PSMA-targeting RNT and an update on therapeutic strategies aimed at minimising toxicity and improving the efficacy of PSMA-targeting RNT both as monotherapy and in combination with other agents.

Biology Of PSMA

PSMA, also known as hydrolase 1 (FOLH1), is a cell-surface, transmembrane aminopeptidase. It consists of a large extracellular domain, a small transmembrane domain, and cytoplasmic tail (*5*). PSMA is overexpressed on most prostate cancer cells compared with normal prostatic epithelium, and further increased in metastatic and high Gleason score disease(*6*). However, some PSMA expression is also observed on proximal renal tubules, duodenum, salivary and lacrimal glands, and non-myelinated ganglia (*7*). On-target, off-tumor effects at these sites account for some of the treatment-related toxicities of PSMA-targeted RNT.

The function of PSMA on prostate cancer cells remains incompletely understood. PSMA has multiple established physiologic functions. In the duodenum, PSMA is involved in the processing and uptake of dietary folates; it cleaves gamma-linked glutamates from polyglutamated folates (known as folate hydrolase activity) (*8*). Prostate cancer cells also demonstrate increased glutamine utilisation, therefore, may in part depend on PSMA for nucleotide biosynthesis and metabolism, which in turn impacts cell proliferation and invasiveness (*9*).

PSMA targeting radionuclide (RNT)

PSMA-targeting tracers can be labelled with different radionuclides for diagnostic purposes or therapeutic purposes. These consist of a PSMA-binding domain, a linker and a chelator labelled with various radionuclides. The PSMA-targeting small molecules are divided into three types – urea-based, phosphorous-based and thiol-based – with the urea-based compounds preferentially used for its superior PSMA binding affinity (*10*). Changing the linker or chelator structure can influence PSMA binding efficacy and pharmacokinetics. In addition, adding an albumin binding domain, which effectively increases the

agent's size, has been explored to increase circulation time within tumor vasculature and reduce healthy organ circulation time with the goal of reducing on-target, off-tumor associated toxicities (*11*). Different approaches to modify components of PSMA-RNT have been extensively reviewed elsewhere (*12*).

Upon accumulation of the PSMA-targeting tracer at the tumor site, radioactive decay of the alpha or beta-emitting radionuclides induce DNA strand breaks and cause cell death. Alpha radiation reaches shorter range (40-100 μm) compared with beta particles (50-12,000 μm), but have significantly higher linear energy transfer of 5-9 MeV compared with linear energy transfer of 0.1-2.2 MeV of the beta particles (*12*). Alpha-emitting radionuclides therefore lead to several ionizing events resulting in DNA double strand breaks (DSBs) in a short range(*13*). This results in more effective tumor kill which is largely independent of cell cycle or oxygenation and potentially has less off-target toxicity(*14*). However, these properties also mean that alpha emitting RNT are associated with greater on-target damage to healthy tissue including irreversible damage to lacrimal and salivary glands (*15*). The effectiveness of alpha particles was demonstrated by the bone-targeting alpha emitter, radium-223 chloride, which in a phase 3 randomised trial was shown to improve overall survival (OS) and delay time to first symptomatic skeletal event in patients with metastatic prostate cancer with bone metastases (*16*). Both PSMA-targeted alpha and beta-emitting RNT are now in clinical development (*17-22*).

CLINICAL DEVELOPMENT OF PSMA-TARGETED RNT

Clinical development of beta-emitting PSMA-targeting RNT

The Lutetium-177 conjugated small molecule peptide, ^{177}Lu-PSMA-617, is the furthest PSMA-targeted RNT currently in clinical development. ^{177}Lu has favourable physical characteristics with a short-range medium-energy β particle for crossfire to surrounding tumor cells, relatively long half-life of 6.7 days and low energy γ emission that enables RNT administration in an ambulatory setting as well as post treatment dosimetry. Promising antitumor activity and modest toxicity was first reported in retrospective case series (*18*). A meta-analysis of 10 PSMA RNT trials in patients with metastatic castration-resistant prostate cancer (mCRPC) treated with prior enzalutamide and/or abiraterone showed a >50% PSA decline in 51% (123/238) of patients (*23*) (Table 1).

A prospective phase 2 trial conducted in Australia evaluated up to four cycles of ^{177}Lu-PSMA-617 (mean radioactivity was 7.5 GBq per cycle) delivered every 6 weeks in mCRPC patients who had progressed after most conventional therapies. Thirty-two (64%) of 50 patients achieved the primary endpoint of having PSA responses ≥ 50%. Importantly, treatment with ^{177}Lu-PSMA-617 improved quality of life (*17,24*). Fifteen patients (30%) who had attained a response initially and subsequently developed disease progression received further cycles of ^{177}Lu-PSMA-617 therapy upon confirmation of the presence of adequate PSMA-expressing metastatic deposit and adequate organ function prior to retreatment. Eleven (73%) of these patients had PSA responses ≥ 50%, although the duration of response with retreatment was shorter and disease recurrence eventually occurred in all patients (*24*). Expectedly, PSA decline of ≥ 50% predicted

improved OS. Given the OS on this study was 13.3 months, data on longer term effects of LuPSMA on marrow and renal function are required especially if this treatment is to be implemented in earlier stages of disease.

The randomised phase II TheraP trial compared ^{177}Lu-PSMA-617 (8.5 GBq decreasing by 0.5 GBq per cycle, for up to 6 cycles) versus cabazitaxel (20 mg/m^2 every 3 weeks) in 200 mCRPC patients with PSMA-positive scans who had prior treatment with docetaxel. Ninety-one percent also had prior treatment with an AR-targeted inhibitor(*25*). Patients were selected using PSMA and FDG-PET/CT requiring SUVmax ≥20 at a site of disease, SUVmax ≥10 at sites of measurable soft tissue metastasis and no site with FDG positive, PSMA negative disease. Notably, 28% of patients were ineligible based on these stringent imaging criteria (*25,26*). ^{177}Lu-PSMA-617 led to a significant improvement in PSA responses ≥ 50% compared to cabazitaxel (66% vs. 37%). ^{177}Lu-PSMA-617 had a higher rate of thrombocytopenia (Grade 1-2: 18% vs 5%; Grade 3-4: 11% vs. 0%), xerostomia (Grade 1-2 only: 60% vs. 21%), and dry eyes (Grade 1-2: 30% vs. 4%), although the rate of grade 3-4 toxicity was higher with cabazitaxel (54% vs. 35%). ^{177}Lu-PSMA-617 delayed radiographic and PSA progression compared to Cabazitaxel (hazard ratio 0.63). At 12 months, 19% had not progressed with ^{177}Lu-PSMA-617 compared to 3% with cabazitaxel, although the median progression-free survival was similar at 5.1 months, with a greater benefit for ^{177}Lu-PSMA-617 emerging after 6 months. The objective response rate defined by RECIST 1.1 was higher with ^{177}Lu-PSMA-617 (49% vs. 24%). Patient-reported outcomes favoured ^{177}Lu-PSMA-617 with significantly less diarrhoea, fatigue, hair loss, skin rash, sore hands/feet, dizziness, insomnia and urinary symptoms reported in the patients receiving ^{177}Lu-PSMA-617 compared with those receiving cabazitaxel (*25,26*).

The phase 3 VISION study (NCT03511664) randomized patients who had progressed on at least one novel anti-androgen therapy (NAAT) and one or two taxane(s) to receive ^{177}Lu-PSMA-617 (7.4 GBq) (every 6 weeks for up to 6 cycles) plus standard-of-care (SOC) or SOC alone (1:1 randomization). The study reported an improvement in its two alternate primary endpoints, OS (median: 11.3 to 15.3 months, hazard ratio: 0.62) and radiologic progression-free survival (median: 3.4 to 8.7 months; hazard ratio: 0.4), in the ^{177}Lu-PSMA-617 arm. Cabazitaxel and radium-223 were not permitted in the SOC arm and the study initially reported a high dropout rate from the control arm that was subsequently improved following site education. The side effect profile of ^{177}Lu-PSMA-617 was as expected, with common serious side effects being bone marrow suppression (all grade: 47%; grade 3-5: 23%), common low-grade toxicities being xerostomia (grade 1-2 only: 39%), nausea and vomiting (all grade: 39%; grade 3-5: 1.5%), and five deaths (1%) on study(*27*). The study will likely see ^{177}Lu-PSMA-617 become part of the mCRPC treatment paradigm, initially to be sequenced after chemotherapy and NAAT.

Since a dose-escalation study was never performed for ^{177}Lu-PSMA-617, a phase 1/2 study exploring the benefit of a fractionated (2 doses, 2 weeks apart), higher cumulative dose of ^{177}Lu-PSMA-617 (3.75-11.1

GBq per fractionated dose) which enrolled 44 men is ongoing. Preliminary results show no dose-limiting toxicity and 61% had PSA decline >50% (*28*).^{177}Lu-labeled DOTAGA-based PSMA ligand, PSMA-I&T, has demonstrated a similar biodistribution to ^{177}Lu-PSMA-617 with comparable response and safety profile in a retrospective case series of 100 patients who had received prior NAAT and taxane chemotherapy and have PSMA-avid disease. PSA decline of ≥50% was achieved in 38 patients and median OS was 12.9 months(*29*). Likewise, PSA response of ≥50% was associated with improved OS (median 16.7 vs 7.4 months) (*29*). Prospective studies in earlier disease settings, including for the management of neoadjuvant disease, and as an earlier line of treatment for mCRPC and HSPC, are ongoing (NCT04297410, NCT04647526, NCT04689828, NCT04720157).

PSMA-targeting alpha-emitting radionuclides

The antitumor activity of alpha-emitting RNT was first demonstrated by Radium-223 dichloride, an alpha-emitting RNT that binds areas of increased bone turnover, which showed an OS benefit and reduced time to first symptomatic skeletal event in patients with mCRPC involving bone (*16*). Several alpha-emitting PSMA-targeted RNT, including an antibody-based RNT, ^{225}Ac-J591, and the small molecules, ^{225}Ac-PSMA-617 and PSMA-targeted thorium-227 conjugate (BAY2315497) are in clinical development. The high energy, short range (<0.01mm) alpha emissions enables pinpoint tumor targeting which has advantages in patients with marrow infiltration due to limited crossfire effect on surrounding bone marrow reserve, however, also has limitations in the setting of heterogenous cellular PSMA expression within tumor deposits (*30,31*).

Antibody-based RNT have different pharmacokinetics to small molecules, and have been shown to have less uptake in glandular tissue and kidneys but may also have less tumor uptake(*32*). In a phase 1 clinical trial, 22 men (55% had previously received ^{177}Lu-PSMA) received a single dose of ^{225}AC-J591 across 7 dose levels (13.3-93.3 KBq/kg). To date, one patient receiving 80 KBq/kg had DLT's with grade 4 thrombocytopenia and anaemia in the context of prior treatment with ^{177}Lu-PSMA. Thirty-five percent of patients to date have had PSA decline >50% and whilst PSMA uptake was not a selection criteria, the vast majority of patients had PSMA uptake with SUV_{max} greater than that seen in the liver (*33*). This trial has recently begun the phase 2a expansion.

Retrospective case series of patients treated with ^{225}Ac-PSMA-617, showed anti-tumor activity in 60-70%, including in some patients who had progressed on ^{177}Lu-PSMA. Xerostomia and weight loss were clinically significant (*34*). A recent study evaluating ^{225}Ac-PSMA-617 in 26 mCRPC patients who had progressed on abiraterone or enzalutamide, taxane-based chemotherapy, and ^{177}Lu-PSMA and demonstrate PSMA-ligand uptake on imaging, reported a PSA decline of ≥50% in 17/26 (65%) patients. Median OS of 7.7 months. Grade 3/4 myelosuppression was seen in a 35%, grade 1/2 renal impairment in 19% and all patients had grade 1/2 xerostomia (*35*). The clinical context in which alpha-emitting RNT will be used, is

yet to be defined. Given the distinct physical properties between alpha-particle and beta-particle emitters, these therapies may have specific indications based on disease pattern and biology and should be compared prospectively. Moreover, given their distinct properties and emerging evidence of antitumor activity when alpha and beta-emitting PSMA-targeted therapies are given sequentially, rational combination of these radioisotopes may serve a complementary role when delivered concurrently or sequentially.

COMBINATION STRATEGIES WITH PSMA-TARGETING RNT

Despite antitumor activity being observed in patients treated with PSMA-targeted RNT, all patients invariably develop recurrence or acquired resistance (see Figure 1). In the phase II TheraP trial where patients were selected on the basis of PSMA expression on PSMA PET and the absence of discordant FDG-PET avid disease, 17/99 (17%) of patients had primary resistance with no decline in PSA(*25*). Purported acquired resistance mechanisms include heterogeneity in or loss of PSMA expression or a failure to deliver a sustained lethal dose to the target. Potential strategies to improve PSMA-targeted therapies include combining PSMA-targeted therapies with agents that: i) upregulate PSMA expression, ii) increase tumor radio-sensitivity, iii) target different PSMA binding sites, iv) or utilize complementary antitumor effects(*36*). To this end, several potential combinations are currently being explored in ongoing clinical studies. These include the combination of PSMA-targeting RNT with AR-targeted agents, DNA repair inhibitors, immunotherapies, chemotherapy, or a combination of different PSMA-targeting RNT (Table 2).

Combining androgen receptor blockade and RNT

There are several mechanisms through which androgen receptor (AR) blockade may synergize with PSMA-RNT. First, AR blockade has been reported to sensitize to radiotherapy by delaying DNA repair through temporal prolongation of repair factor complexes and halting the cell cycle (*37*). Second, AR blockade has been shown to modulate the expression of PSMA, although the exact effect of AR blockade on PSMA expression likely hinges on the state of castration. In a study of 20 paired, prostate tumor samples collected before and after castration, the expression of PSMA increased in 55% of post-treatment primary tissues and 100% of post-treatment metastatic specimens (*38*). A recent study indicated that AR blockade appear to have dichotomous effects on PSMA expression in patients at different disease states (*39*); In patients with castration-sensitive prostate cancer (CSPC), a significant reduction in ^{68}Ga-PSMA intensity occurred in 86% of men as early as nine days after starting androgen blockade, while in patients with CRPC, AR-blockade caused an increase in PSMA expression (*39*). Third, treatment with enzalutamide, dutasteride, and rapamycin have all been shown to increase the uptake and internalization of ^{177}Lu-PSMA in prostate cancer cell lines(*40*). These studies provide rationale for combining PSMA-targeted RNT and drugs that block AR signalling in the castration-resistant setting. They also highlight the need to carefully consider the differential impact of AR-blockade on PSMA at different disease stages when designing combinatorial approaches.

Given existing reported trials of PSMA-targeting RNT are conducted in the castration-resistant setting, the effect of hormone sensitivity and androgen deprivation on response to PSMA-targeting therapy is unclear. Enza-P (NCT04419402) is a phase II randomized control trial of the combination of enzalutamide and ^{177}Lu-PSMA versus enzalutamide alone in patients with mCRPC who have previously progressed on docetaxel in the setting of hormone sensitive disease but are naive to an androgen-signalling-targeted inhibitor. This trial is recruiting 160 mCRPC patients with risk factors that predict for early treatment failure with single agent enzalutamide. Patients will be randomised 1:1 to receive either concurrent enzalutamide with up to 4 doses of ^{177}Lu-PSMA or enzalutamide alone. This study embeds multi-time point PSMA PET/CT and translational biomarkers to investigate the longitudinal effects of enzalutamide on PSMA receptor expression on PET scans and circulating tumor cells.

PSMA RNT plus inhibitors of DNA damage repair and radioresistance mechanisms

Since RNT cause cell damage through DNA strand breaks, the combination of PSMA-targeting RNT with inhibitors of DNA repair or DNA damaging agents are likely to be synergistic (*41*). RNT induces both DNA single strand breaks (SSB) and DSB. Depending on the type of DNA damage incurred, different DNA damage sensing and repair mediators such as PARP, ATR and ATM, are triggered. Specifically, poly-[ADP-ribose]-polymerase 1 (PARP-1) and PARP-2 are involved in the detection and repair of SSB. DSB are either repaired by homologous recombination (key mediators include: BRCA1/2, PALB2, ATM, ATR, RAD51, CHEK2, MRE11, XRCC2/3) or error-prone non-homologous end-joining. PARP-1 inhibitors, such as olaparib, impair DNA SSB repair; this leads to replication fork arrest and conversion to DSB, which require homologous recombination for repair and continued replication(*42*). Suboptimal repair of DSB in the setting of defective homologous recombination in mCRPC tumors with germline (approximately 12%) or somatic DNA repair gene defects (approximately 20-25%), lead to vulnerability to PARP inhibition (*43*). Drugs targeting ATR have also shown activity in tumors with ATM loss in preclinical models, and are synergistic with PARP inhibition in these models(*44*). Other likely targets that could sensitize RNT include ATM and DNA-PK inhibitors(*45*). As such, targeting the various mediators of these repair mechanisms in combination of PSMA-targeting RNT can impair repair of DNA strand breaks, mediate radiosensitization and improved cell death. Targeting other mechanisms of radioresistance, such as AKT activation, should also be considered(*46*). Since a key mechanism of radiotherapy induced cell death is apoptosis, pro- and anti-apoptotic pathways may also be targeted to overcome radioresistance. As such, potential synergy between PSMA-targeting RNT and drugs targeting potential radioresistance mechanisms warrant further evaluation.

The first clinical evidence for combining agents which impair DNA repair and RNT comes from studies of Radium-223 dichloride, an alpha emitter. A retrospective analysis showed that the presence of defective DNR repair (somatic and/or germline) was associated with improved OS and prolonged reduction in alkaline phosphatase(*47*). Synergy between an ATR inhibitor and Radium-223 has also been demonstrated(*48*). A

phase 1 dose-escalation study is currently ongoing to evaluate the safety and antitumor activity of ^{177}Lu-PSMA-617 plus olaparib in mCRPC patients who have previously progressed on NAAT and docetaxel (NCT03874884). Other DNA repair inhibitors (e.g., targeting ATR kinase, DNA-PK, Pol 1) may also synergize with ^{177}Lu-PSMA-617. Strategies to overcome mechanisms of radioresistance have also been explored. A non-randomised phase II study combined idronoxil, a synthetic flavonoid derivative with radiosensitising properties mediated through the inhibition of NADH oxidase 2 to induce apoptosis and cell cycle arrest, with ^{177}Lu-PSMA-617 in mCRPC patients with PSMA-PET avid metastatic disease and no discordant FDG-avid disease. This study showed that 63% of patients had a PSA response of >50%. Twenty-eight percent of patients experienced anal inflammation attributable to the mode of delivery of idronoxil, and other side effects were as expected with ^{177}Lu-PSMA-617(*49*). Whilst the aforementioned strategies appear overall tolerable when combined with PSMA-targeting RNT, whether they truly improve efficacy would require randomised clinical evaluation.

Combining ^{177}Lu-PSMA-617 with immune checkpoint inhibitors

Whilst prostate cancer outcomes have not been significantly impacted by recent advances in cancer immunotherapy, there is substantial preclinical and clinical evidence that radiotherapy is immunostimulatory. The abscopal effect, in which non-irradiated tumors have been observed to shrink in some patients following radiation therapy targeted to other tumor sites, is hypothesized to be mediated by the generation of systemic anti-tumor immune responses following immune recognition at the irradiated site (*50*). Mechanistically, genomic instability in the context of DNA damage and suboptimal repair is associated with increased neoantigen formation, antigen presentation, immune recognition and immunogenic cell death (*51*). DNA damage and the release of cytosolic DNA also causes activation of the cGAS-STING, an innate immune signalling pathway, which results in the release of type 1 interferon and other pro-inflammatory cytokines, thereby facilitating an adaptive immune response (*52*). Moreover, immunogenic cell death accompanied by the release of danger associated molecular patterns can induce further immune cell recruitment (*53*). Finally, in a mouse model, PD-L1 in the tumor microenvironment was upregulated following treatment with ionizing radiation(*54*). Specifically, alpha-emitters have been to elicit immunogenic response (*55*), although there is no published study directly comparing of the relative immunogenicity of different RNTs in prostate cancer. Taken together these findings suggest that PSMA-targeted RNT may have the potential to synergize with immune checkpoint inhibitors.

The phase Ib/II PRINCE trial (NCT03658447) is testing the combination of pembrolizumab with ^{177}Lu-PSMA-617 in mCRPC patients who have progressed on second generation anti-androgens. Patients will receive continual dosing with pembrolizumab for up to two years (35 cycles given every 3 weeks) and up to 6 doses of ^{177}Lu-PSMA-617 (6 weeks apart). The co-primary objectives of this study are PSA response rate and safety and tolerability of the treatment combination. This hypothesis is currently being evaluated in another phase 1 study in a similar patient population (NCT03805594). This trial embeds three different

sequences, a single cycle of ^{177}Lu-PSMA-617 followed by pembrolizumab, both drugs given on the same day, and pembrolizumab 21 days prior to ^{177}Lu-PSMA-617. First results report a median radiographic PFS of 6.5 months after and a PSA ≥ 50% response rate of 28% (ASCO 21).

Combining RNT and chemotherapy

Taxane chemotherapy can radiosensitize tumors to the effect of radiation by impairing DNA damage repair, improving tumor reoxygenation which in turn sensitizes cells to the effects of radiation and preventing accelerated repopulation during radiotherapy(*56*). Theoretically, chemotherapy can target areas of PSMA non-avid disease to prevent their outgrowth after selective killing of PSMA-expressing tumors by PSMA-targeting RNT. However, the antiproliferative effect of chemotherapy may also reduce cellular sensitivity to radiation, although this is less likely to be an issue with alpha-emitting RNT(*34*).

The UpFrontPSMA study (NCT04343885) is a phase II randomised study comparing 2 sequential doses of ^{177}Lu-PSMA delivered 6 weeks apart followed by 6 cycles of docetaxel versus docetaxel alone in patients with newly diagnosed high-volume metastatic CSPC (i.e. within 12 weeks of diagnosis, and within 4 weeks of commencing ADT) based on PSMA-PET scans. This study will shed light on the impact of castration sensitivity and ^{177}Lu-PSMA-617 in addition to docetaxel standard-of-care. In this study, a PSMA PET/CT will be performed at baseline, within 4 weeks of commencing ADT, then at 12 weeks after commencing treatment. The study will enable evaluation of the effect of ADT on PSMA expression by comparing the PSMA PET/CT performed prior to ADT commencement and that performed at study entry. Separately, a phase 1 trial is evaluating the combination of standard docetaxel (75 mg/m2 every 3 weeks) administered with 2 fractionated doses of ^{177}Lu-J591 (initial dose 20 mCi/m2 x2 up to max of 40 mCi/m2 x2) with cycle 3 in patients with mCRPC. Among the 15 patients enrolled, 11 (73.3%) patients had PSA decline > 50% and no dose-limiting toxicity was seen, although three (20%) of patients experienced grade 4 neutropenia and two patients experienced grade 4 thrombocytopenia (*57*).

Combining different RNTs

Since different PSMA-targeting antibodies and small molecules may have different extracellular PSMA binding sites, co-targeting with multiple agents could result in additive benefit (*58*). Additionally, given different PSMA-RNT have different biodistribution and different toxicity profiles, a combination of different agents at lower doses may improve their collective therapeutic window.

In a retrospective analysis of “tandem” PSMA-targeting RNT combining a single lower dose of ^{225}Ac-PSMA-617 (median: 5.3 MBq) with ^{177}Lu-PSMA-617 (median: 6.9 GBq) in 20 mCRPC patients with inadequate responses to ^{177}Lu-PSMA-617, 13/20 (65%) patients had PSA decline > 50%. Xerostomia was generally mild (grade 1-2) although formal studies comparing this approach to RNT monotherapy is required (*59*). In

another retrospective case series, 15 patients with confirmed PSMA expression on PSMA PET received ^{225}Ac-PSMA-617 after disease progression on ^{177}Lu-PSMA. Five had a PSA decline of > 50%. The treatment was poorly tolerated with five patients discontinuing treatment due to xerostomia, two patients developing grade 2 renal impairment, four patients developing grade 3-4 anaemia, and two patients developing grade 3 thrombocytopenia (*60*). Currently, there are no published results from prospective studies of a combination of different PSMA-targeting RNT.

BIOMARKER SELECTION IN THE ERA OF COMBINATORIAL THERAPIES

Evaluating intensity of PSMA expression across metastatic sites with PSMA PET offers the advantage of selecting patients most likely to respond to PSMA-targeting RNT. Several studies select patients based on PSMA expression on PSMA PET and the absence of discordant FDG-PET avid disease(*25,61*). The relationship between PSMA uptake on imaging and immunohistochemical analysis is also of interest since imaging does not capture the heterogeneity of PSMA expression at a cellular level. For example, a study showed that the lack of PSMA protein expression on immunohistochemistry predicted for lack of avidity on PSMA PET although it is unclear whether a subset of patients who have PSMA-PET negative disease may have some level of PSMA expression at a cellular level (*62*). At the same time, given the heterogeneity of PSMA expression at different metastatic sites and discordance between primary and metastatic disease, it would be difficult to predict response based on PSMA immunohistochemistry using tissue from a single metastatic site (*30,62*). Moreover, PSMA expression evolves with treatments thereby limiting the utility of archival, diagnostic tumor samples for assessing PSMA expression. Finally, the clinical utility of patient selection based on PSMA expression in the setting of combination therapies with other efficacious systemic therapies is yet to be defined.

Low-energy γ-rays radiation from ^{177}Lu allows for post-treatment scintigraphic imaging that provides information including the presence of residual targetable disease, monitoring response and dosimetry ascertainment. Dosimetry analysis from our Phase II study established a mean “whole body” tumor dose of 14.7 Gy was associated with a PSA response of ≥ 50% at 12 weeks compared with doses of 10.4 Gy ($p<0.01$) as well as a strong correlation between the mean standardized uptake value (SUV) of PSMA expression and ≥ 50% PSA reduction although the SUV threshold for optimal PSMA avidity remains to be defined (*63*).

As expected, PSA response of ≥ 50% has been shown in several studies to associated with improved OS in a median range of 16.7 to 18 months compared with 7.4 to 8.7 months in patients with PSA declines of <50% (*29*). Beyond PSA response, FDG-positive molecular tumor volume and PSMA intensity (mean SUV) in patients receiving ^{177}Lu-PSMA-617 were most prognostic of OS, followed by LDH, ALP, and bone scan index (*64*). Patients with FDG-positive PSMA-negative that were excluded from the Peter Mac phase 2 trial had a poor prognosis with a median OS 2.5 months(*65*).

Combinatorial therapies add further to the complexity. If treatments such as AR blockade can induce PSMA expression, then combinatorial studies may be effective in patients with PSMA-negative disease and on-treatment evaluation of PSMA expression, for example, after AR inhibition alone, may dictate the sequential use of PSMA-targeted treatment. Additionally, it is unclear whether treatments such as the combination of DNA damage repair inhibitors and PSMA-targeting RNT are only effective in tumors with defective DNA repair or are more broadly synergistic. Finally, the patient's disease profile may also impact the modality chosen, with for example, a preference for treatments with less marrow toxicity in patients who have a heavy burden of bone disease. In summary, clinical, molecular and imaging biomarkers should form a part of future studies of PSMA-RNT combinations.

CONCLUSION

The field of PSMA-targeting RNT has seen rapid progress with promising antitumor activity being observed across a number of agents. However, strategies to achieve more durable response and better understanding of optimal patient selection and therapeutic resistance remain key ongoing challenges. Refinement of the PSMA-RNT molecules to achieve even better targeting is ongoing. Multiple biologically rational combinations, including the combination of PSMA-targeting RNT with immunotherapy, DNA damaging agents, AR-targeted therapy, and radiosensitisers, are at various stages of clinical development and will careful thought about patient selection and dose-schedules. The overall aim is that these combinations can be utilized in a biomarker-driven manner to overcome resistance, improve disease control, quality of life and OS in patients with lethal prostate cancers.

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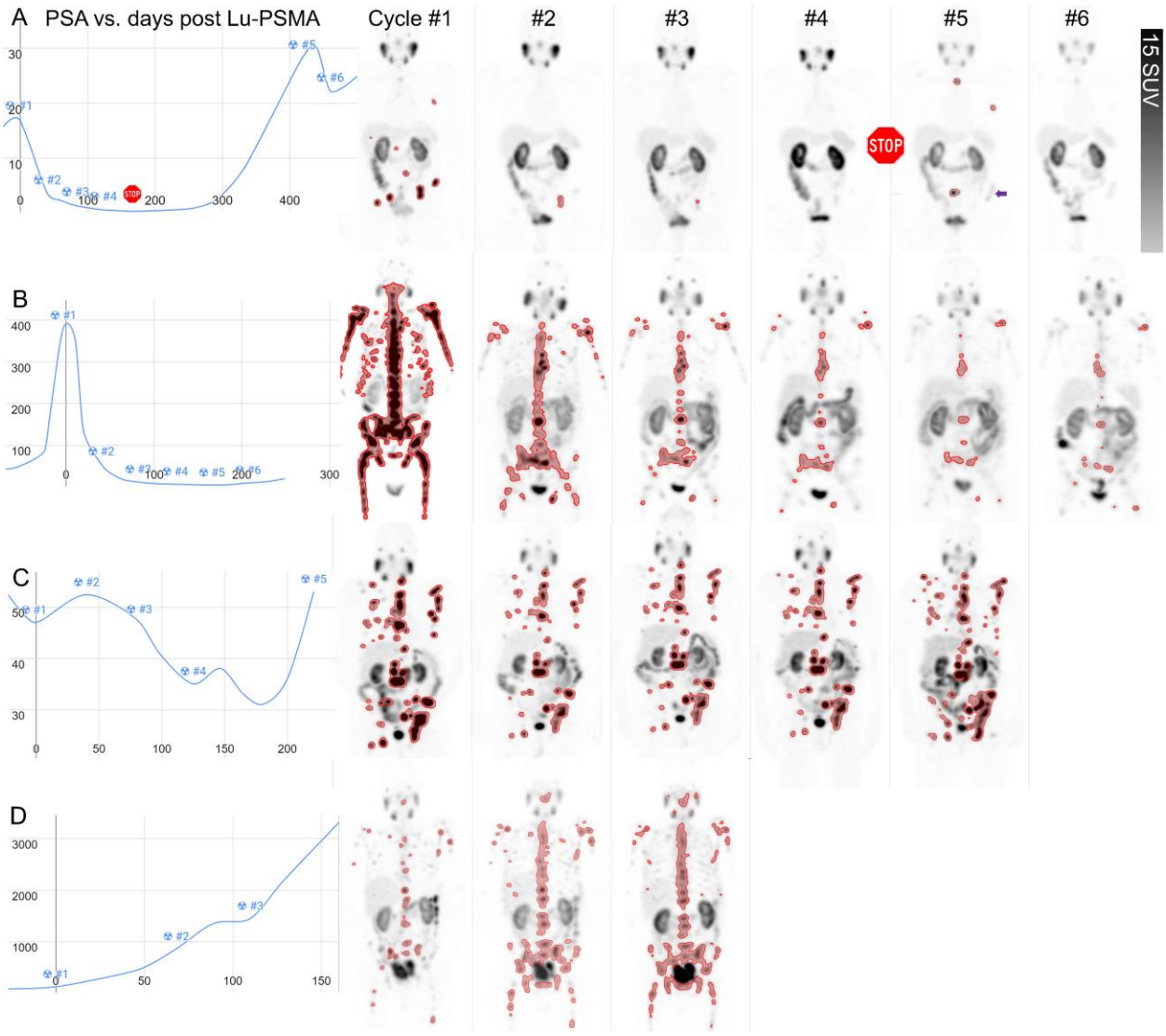

FIGURE 1: Examples of patterns of progression in men with mCRPC undergoing ^{177}Lu-PSMA-617 therapy. PSA over time and post-therapy quantitative SPECT/CT imaging after each cycle of treatment. A) Patient with relatively small volume disease with a complete response. At time of progression, some disease has low PSMA expression (arrow: left pelvis) with and response to further cycles is limited. B) Patient with PSMA-superscan and exceptional response to treatment. Disease eventually recurred (not shown) with diffuse marrow involvement and leucoerythroblastic phenotype. C) Rise in PSA after cycle #1 but subsequent response cycles #2-4, with subsequent progressive PSMA-avid disease but limited response. D) Primary progression. After cycle #3 patient was switched to cabazitaxel with good response (not shown).

Table 1: Key Prospective Clinical Trials of PSMA-targeting radionuclides in prostate cancer

	Trial ID (name)	***Setting***	***Treatment***	***Phase***	***Primary endpoint***	***Outcome***
Neoadjuvant	NCT04430192 (Lutectomy)(*66*)	High-risk localized or locoregional advanced prostate cancer with high PSMA uptake	^{177}Lu-PSMA-617 (1-2 cycles)	Phase 1/2 single arm	Radiation absorbed dose in the prostate and involved lymph nodes	Recruiting
	NCT04297410	Locally advanced prostate cancer with PSMA uptake	^{177}Lu-PSMA-I&T	Feasibility	Surgical safety. Surgical histology. Post-operative PSA	Recruiting
Metastatic hormone sensitive prostate cancer	NCT04443062 (Bullseye)	Oligo-metastatic hormone sensitive prostate cancer with high PSMA uptake	^{177}Lu-PSMA-I&T vs standard-of-care (SOC)	Phase 2 randomised	Disease progression at 6 months	Recruiting (SOC=deferred androgen deprivation therapy)
	NCT04343885 (UpFrontPSMA)(*67*)	Newly diagnosed high-volume metastatic hormone-naive prostate cancer	^{177}Lu-PSMA-617 followed by docetaxel vs. docetaxel	Phase 2 randomized	Undetectable PSA rate at 12 months	Recruiting
	NCT04720157 (PSMAddition)	Metastatic hormone-sensitive prostate cancer	^{177}Lu-PSMA-617 + SOC vs. SOC	Phase 3	rPFS	Recruiting
Metastatic castration-resistant prostate cancer	NCT03042468 (*68*)	mCRPC (n=44). Prior taxane chemotherapy and at least one line of prior NAAT	^{177}Lu-PSMA-617 2 weeks apart	Phase 1/2 single arm	DLT, MTD , R2PD	No DLT at any pre-planned dose. RP2D: 22.2 GBq per cycle
	ANZCTR 12615000912583 Published (*17,61*)	mCRPC (n=50; 30 in initial; 20 in expansion). At least one line of prior taxane chemotherapy.	^{177}Lu-PSMA-617	Phase 2 single arm	% patients with ≥50% PSA decline	≥50% PSA decline: 64%
	NCT03392428 (TheraP)	mCRPC (n = 200) for whom cabazitaxel was considered the appropriate next line of treatment. Previous treatment with NAAT permitted.	^{177}Lu-PSMA-617 vs. cabazitaxel	Phase 2 randomised	% patients with ≥50% PSA decline	PSA decrease ≥50% from baseline: 66% of vs. 37%, favouring ^{177}Lu-PSMA-617, p<0.0001.
	NCT03511664 (VISION)	mCRPC (n=831). Prior taxane chemotherapy and NAAT	^{177}Lu-PSMA-617 + SOC vs. SOC (2:1)	3	OS, rPFS	OS: 15.3 vs. 11.3 months favouring ^{177}Lu-PSMA-617; HR: 0.62, p<0.001.
	NCT04689828 (PSMAfore)	mCRP (n=495). Prior NAAT,	^{177}Lu-PSMA-617 vs. abiraterone or enzalutamide (2:1)	3	rPFS	Recruiting
	NCT04647526 (SPLASH)	mCRPC with PSMA PET positive disease. Prior NAAT & chemotherapy, except for in the HSPC setting, is not permitted.	^{177}Lu-PSMA-I&T vs abiraterone or enzalutamide (2:1)	3	rPFS	Recruiting

mCRPC = metastatic castration resistant prostate cancer; NAAT = novel antiandrogen; OS = overall survival; rPFS = radiologic progression-free survival; OS = overall survival; DLT = dose limiting toxicity; MTD = maximum tolerated dose; RP2D = recommended phase 2 dose

Table 2: Key PSMA-targeting RNT combination studies

Combination strategy	Trial ID (name)	Setting	Treatment	Phase
RNT plus immune checkpoint inhibitor	NCT03805594	mCRPC PSMA PET positive at at least 3 metastatic sites Prior treatment with NAAT Prior chemotherapy, except for in the HSPC setting, is not permitted.	^{177}Lu-PSMA-617 and pembrolizumab	1
	NCT03658447 (PRINCE)	mCRPC Prior treatment with NAAT Prior docetaxel permitted	^{177}Lu-PSMA-617 and pembrolizumab	1/2
RNT plus radiosensitiser	NCT03511664 (LuPIN)	mCRPC Prior treatment with taxane and NAAT	^{177}Lu-PSMA-617 and Idronoxil	1/2
RNT plus PARP inhibitor	NCT03874884 (LuPARP)	mCRPC Prior treatment with NAAT and taxane chemotherapy	^{177}Lu-PSMA-617 plus Olaparib	1
RNT plus novel anti-androgen therapy	NCT04419402 (ENZA-p)	mCRPC with PSMA positive disease previously treated with abiraterone. Prior chemotherapy, except for in the HSPC setting, is not permitted.	^{177}Lu-PSMA-617 plus enzalutamide vs. enzalutamide	2

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