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Phase I/II Trial of the Combination of ^{177}Lutetium Prostate specific Membrane Antigen 617 and Idronoxil (NOX66) in Men with End-stage Metastatic Castration-resistant Prostate Cancer (LuPIN)

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Abstract

Background: Trials of lutetium prostate specific membrane antigen (PSMA) in men with metastatic castration-resistant prostate cancer (mCRPC) have demonstrated good safety and efficacy, but combination strategies may improve outcomes. Idronoxil is a synthetic flavonoid derivative with radiosensitising properties.

Objective: To evaluate the safety and activity of ^{177}Lu PSMA 617 (LuPSMA-617) in combination with idronoxil suppositories (NOX66) in patients with end-stage mCRPC.

Design, setting, and participants: Thirty-two men with progressive mCRPC previously treated with taxane-based chemotherapy (91% treated with both docetaxel and cabazitaxel) and abiraterone and/or enzalutamide were enrolled in this phase I dose escalation study with phase II dose expansion.

Intervention: Screening with ^{68}Ga PSMA and ^{18}F-fludeoxyglucose positron emission tomography (PET)/computed tomography (CT) was performed. Men received up to six cycles of LuPSMA-617 (7.5 GBq) on day 1, with escalating doses of NOX66 on days 1–10 of a 6-wk cycle. Cohort 1 (n = 8) received 400 mg and cohort 2 (n = 24) 800 mg of NOX66.

Outcome measurements and statistical analysis: Adverse events (AEs), pain inventory scores, prostate-specific antigen (PSA) response, progression-free survival, and overall survival were evaluated.

Results and limitations: Fifty-six men were screened and 32 (57%) were enrolled with a screen failure rate of 21% for PET imaging criteria. Dosing was as follows: 97% (31/32) received two or more doses and 47% (15/32) completed six doses. Common

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AEs included xerostomia, fatigue, and anaemia. Anal irritation attributable to NOX66 occurred in 28%. PSA responses were as follows: 91% (29/32) had any PSA response (median –74%; 95% confidence interval [CI] 76–97) and 62.5% (20/32) had a PSA fall of >50% (95% CI 45–77). The median PSA progression-free survival was 6.1 mo (95% CI 2.8–9.2) and median overall survival was 17.1 mo (95% CI 6.5–27.1).

Conclusions: NOX66 with LuPSMA-617 is a safe and feasible therapeutic strategy in men treated with third-line therapy and beyond for mCRPC.

Patient summary: Addition of NOX66 to ^{177}Lu prostate-specific membrane antigen 617 is safe, and further studies are needed to assess its potential to augment the anticancer effects of LuPSMA-617.

1. Introduction

Despite recent advances, metastatic castration-resistant prostate cancer (mCRPC) remains incurable, and its treatment is associated with significant morbidity. Treatment resistance is an intractable problem, and new effective treatments that improve outcomes while maintaining quality of life are needed. Lutetium-177 prostate-specific membrane antigen 617 (LuPSMA-617) is a radiolabelled small-molecule peptide that targets the prostate-specific membrane antigen (PSMA) receptor, which is highly expressed on prostate cancer (PCa) cells, to deliver targeted beta-particle therapy. Single-centre studies of LuPSMA-617 have demonstrated good safety and efficacy [1–3]. Larger prospective trials are currently underway (TheraP [4], VISION [NCT03511664]). Acquired and de novo resistance to LuPSMA-617 occurs in a subset of men, but synergistic combinations may improve treatment responses.

Idronoxil is a synthetic flavonoid derivative of genistein that inhibits external NADH oxidase 2 (ENOX2) to induce apoptosis and cell cycle arrest, and inhibit DNA topoisomerase 2 [5–10]. ENOX2 inhibition activates the sphingomyelinase pathway and deactivates the antiapoptotic protein kinase B (Akt) pathway [10], a key driver of radiotherapy resistance [11]. The targeted effect on ENOX2 has the potential to limit toxicity to noncancer cells, which preferentially express ENOX1 [12]. Previous studies have demonstrated improved radiation sensitivity in PCa with flavonoid derivatives [13–16]. Early studies of intravenous and oral formulations of idronoxil were hampered by limited bioavailability. NOX66 is a suppository formulation of idronoxil, minimising phase 2 metabolism in the liver via rectal administration [17]. The safety of the combination of NOX66 and external beam radiotherapy has been tested in men with symptomatic mCRPC [18]. We hypothesised that the addition of NOX66 to LuPSMA-617 may act as a radiosensitiser, which could improve treatment responses while contributing minimal additional toxicity. The trial was designed to assess safety of the combination.

This novel phase I/II study evaluated the safety and efficacy of NOX66 in combination with LuPSMA-617 in men with progressive mCRPC following treatment with taxane chemotherapy and enzalutamide and/or abiraterone.

2. Patients and methods

2.1. Study design

This was a phase I/II, open-label, single-centre, study. The study protocol was approved by the St. Vincent's Hospital institutional review board (HREC/17/SVH/19, ACTRN12618001073291), and all patients provided informed written consent. The study population included men with progressive mCRPC despite prior docetaxel and cabazitaxel in addition to abiraterone and/or enzalutamide. Disease progression was required with either progression on conventional imaging (computed tomography [CT] and bone scan) or a rising prostate-specific antigen (PSA) level based on the PCa Working Group 3 (PCWG3) criteria. Additional key eligibility criteria included a baseline platelet count of ≥100 10^9/l, haemoglobin ≥100 g/l, and estimated glomerular filtration rate ≥40 ml/min. Life expectancy must have been estimated at >12 wk with a World Health Organization Eastern Cooperative Oncology Group performance status of ≤2.

2.1.1. Screening

Men underwent screening with ^{18}F-fludeoxyglucose (FDG) and ^{68}Ga-HBEDD-PSMA-11 (PSMA) positron emission tomography (PET)/CT, bone scan, and CT of the chest, abdomen, and pelvis. For PET scans, patients were injected with 2.0 MBq/kg ^{68}Ga-PSMA (HBEDD CC-11) and 3.5 MBq/kg FDG. PET scans were analysed semiquantitatively (MIM) to derive maximum standardised uptake value (SUV max), SUV mean, and total metabolic volume. Men were eligible if they had an SUV max of >15 on PSMA PET at one or more sites, an SUV max of >10 at all measurable sites, and no FDG avidity without corresponding PSMA uptake.

2.1.2. Study treatments

A PSMA-617 precursor (AAA Novartis) was radiolabelled to no-carrier-added ^{177}Lu chloride according to the manufacturer's instructions. LuPSMA-617 was administered by slow intravenous (IV) injection.

A cohort of eight patients (cohort 1) received 7.5 GBq of LuPSMA-617 IV on day 1 and 400 mg NOX66 suppositories on days 1–10 of a 6-wk cycle (Supplementary material). Recruitment was paused for data safety review prior to recruitment of the second cohort (cohort 2) of eight patients receiving the same dose of LuPSMA-617 in combination with 800 mg NOX66 on the same schedule. Following further safety review, cohort 2 was expanded to include 24 patients. Eligible patients could receive a maximum of six cycles of treatment.

2.2. Endpoints

2.2.1. Primary endpoints

Safety and tolerability were assessed using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version

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5.0 every 2 wk during each 6-wk cycle, until 6 wk following the final dose of study treatment. All adverse events (AEs), whether treatment or disease related, are reported.

2.2.2. Secondary endpoints

Efficacy was evaluated by PSA decline from baseline (any and ≥50% [PSA50]) at any time point and PCWG3 PSA progression-free survival (PFS). Overall survival (OS) was defined as the time from day 1 of treatment to death. Patient-reported outcomes were assessed for each cycle and follow-up using the Brief Pain Inventory—short form (BPI-SF) [19] and University of Michigan Xerostomia-related Quality of Life Scale (XeQoLS) [20].

2.2.3. Exploratory endpoints

Blood was prospectively collected for exploratory analyses of potential biomarkers, including androgen receptor splice variant 7 (ARV7) expression [21] and cell-free DNA alterations (Supplementary material). Germline DNA sequencing results were collected from patients with available data from peripheral blood analysis.

2.3. Statistical analyses

The planned enrolment was eight patients in two dosing cohorts with expansion of phase II cohort to 24 patients. This study was designed to assess safety and antitumour activity of the combination of LuPSMA-617 and NOX66. Sample sizes were based on a pragmatic design taking into account a risk assessment of the agents, relevant AEs, and activities. All patients who received one or more cycles of study treatment were considered in the safety analysis and for evaluation of PSA response and PSA progression. A two-sided exact binomial 95% confidence interval (CI) was calculated for PSA response rates. Time-to-event outcomes (PSA PFS and OS) were analysed using the Kaplan-Meier method and 95% CIs were calculated (SPSS Software).

The mean composite scores of four pain items for pain severity on the BPI-SF and seven pain interference (PI) items were collated in addition to the single worst pain question response. Clinically significant pain criteria are outlined in the Supplementary material. Scores from the XeQoLS questionnaire at baseline, before cycle 3, and at 1-mo follow-up were compared. A two-tailed paired *t* test was used to assess for a deterioration in scores. Descriptive statistics were used for treatment responses based on germline DNA repair gene alteration status, ctDNA findings, and ARV7 expression.

3. Results

3.1. Baseline patient characteristics

Of 56 men who were screened during November 2017–June 2019, 32 (57%) were enrolled, with 12 (21%) being ineligible based on PET imaging criteria (either low PSMA intensity disease or sites of FDG/PSMA mismatch on screening PET; Fig. 1). The remaining 12 screen failures were due to anaemia (n = 11) and a concomitant active cancer on PET imaging (n = 1). Baseline characteristics are summarised in Table 1. All men enrolled had received one or more lines of taxane chemotherapy and androgen signalling inhibition therapy, and 91% (29/32) had received two lines of taxane chemotherapy. Eighteen (56%) patients had grade 1 anaemia at baseline, while two (6%) had grade 1 thrombocytopenia.

3.2. Safety and tolerability

The most frequent any-grade AEs were anaemia (88%), xerostomia (59%), and fatigue (69%) (Table 2). Anal

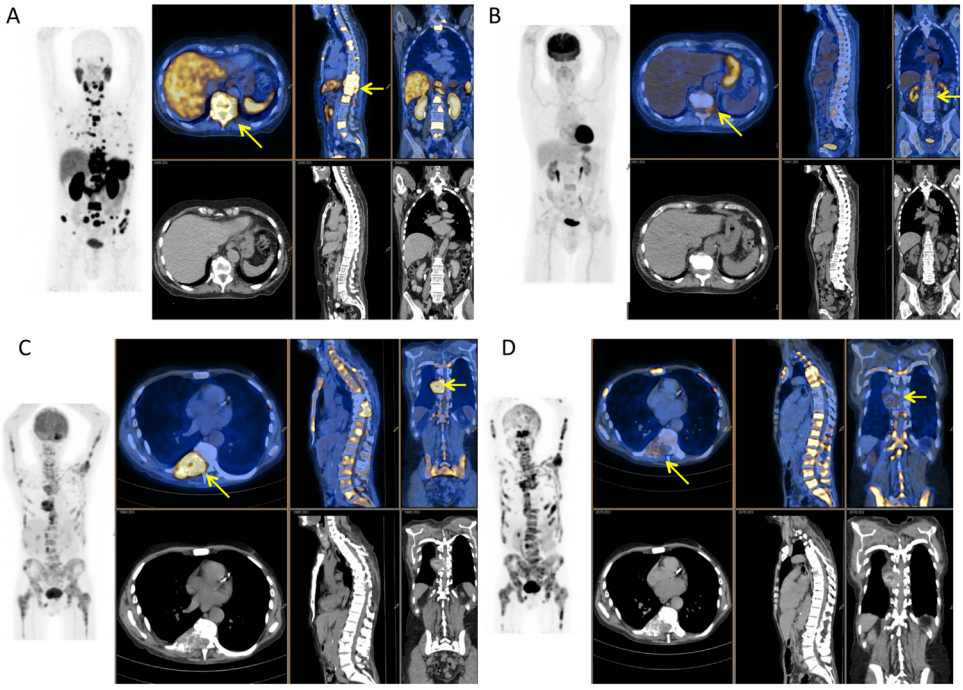

Fig. 1 – The patient was classified as a screen imaging pass (A) with multiple sites of PSMA-avid disease, (B) with no regions of PSMA SUV max <10 at sites of measurable disease and no regions of FDG avidity in the absence of PSMA avidity. The patient was classified as an imaging screen failure (C) with a large mixed soft tissue/bone metastasis in the mid thoracic spine that is highly FDG avid, (D) with no significant PSMA avidity. This metastasis was very symptomatic and at a site of prior radiotherapy. FDG = ^{18}F-fludeoxyglucose; PSMA = prostate-specific membrane antigen; SUV = standardised uptake value.

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Table 1 – Patient characteristics.

Characteristics	*N* = 32
Age (yr), median (IQR)	69 (64–73.5)
ECOG, *n* (%)	
0 or 1	25 (78)
2	7 (22)
PSA in C1 (μg/l), median (IQR)	115 (61–439)
Haemoglobin (normal range [28] 130–180 g/l), median (IQR)	119 (110–131)
Alkaline phosphatase (NR 30–100 U/l), median (IQR)	106 (88–295)
Albumin (NR 36–52 g/l), median (IQR)	37 (34–41)
Disease volume, *n* (%)	
<20 metastases	19 (59)
≥20 metastases	13 (41)
Sites of disease, *n* (%)	
Bone	19 (59)
Lymph node	10 (31)
Visceral	3 (9)
PSMA PET	
SUV mean (IQR)	8.7 (7.5–11)
SUV max (IQR)	35 (25–57)
Volume (IQR)	300 (86–837)
FDG PET	
SUV max (IQR)	8 (1.1–10)
SUV mean (IQR)	4.8(0.7–5.3
Volume (IQR)	32 (0.4–134)
Prostate cancer history, *n* (%)	
Gleason score	
≤7	15 (47)
8–10	7 (22)
Unknown/not available	10 (31)
Prior systemic treatments	
LHRH agonist/antagonist	32 (100)
Chemotherapy	32 (100)
Docetaxel	32 (100)
Cabazitaxel	29 (91)
Other chemotherapy	3 (9)
Androgen signalling inhibitor	32 (100)
Enzalutamide only	15 (47)
Abiraterone only	6 (19)
Abiraterone + enzalutamide	11 (34)
Trial medication	3 (9)

C1 = cohort 1; CI = confidence interval; ECOG = Eastern Cooperative Oncology Group; FDG = ^{18}F-fludeoxyglucose; IQR = interquartile range; LHRH = luteinising hormone-releasing hormone; NR = normal range; PET = positron emission tomography; PSA = prostate-specific antigen; PSMA = prostate-specific membrane antigen; SUV = standardised uptake value.

inflammation related to NOX66 suppository occurred in 28%. This was predominately mild, but required topical treatment in two patients, one of whom ceased NOX66 after four cycles. Three grade 3 toxicities, including fatigue (n = 1) and anaemia (n = 2), were reported. No grade 4–5 AEs or treatment-related deaths occurred.

3.3. Exposure

Patients completed a median of five cycles (interquartile range 3–6). Of 32 patients, 31 (97%) received two or more cycles and 15 (47%) completed six cycles. Of those who ceased treatment prior to completing six cycles, one ceased after three cycles following an exceptional response that lasted >6 mo, one withdrew consent following two cycles over personal concerns, and 15 ceased due to progressive disease. No patient ceased LuPSMA-617 due to an AE or toxicity. One patient ceased NOX66 due to grade 2 anal toxicity, and one patient had a dose reduction of LuPSMA-617 for worsening anaemia but ceased treatment following this cycle for radiologically progressive disease.

3.4. Efficacy

At a median follow-up of 16.3 mo, any decline in PSA from baseline occurred in 29/32 (91%, 95% CI 76–97) and a PSA50 in 20/32 (63%, 95% CI 45–77) patients. The best PSA responses at any time point are summarised in Figure 2. The median PSA PFS was 6.1 mo (95% CI 2.8–9.2; Fig. 3A), and five of 32 (16%) patients have not yet progressed by either PSA or radiographic criteria. Seventeen patients have died since enrolment, and the median OS is 17.1 mo (95% CI 6.5–27.1; Fig. 3B).

3.5. Quality of life

Baseline BPI-SF results were available for 31/32 (97%) patients. Baseline mean composite PI scores were 3.06 (range 0.14–9.14, standard deviation [SD] 2.376). Twenty-one (68%) patients had a significantly elevated mean PI score (≥1.25) at baseline (Supplementary Fig. S1), of whom 11 (52%) had significant reductions in their PI. The mean worst pain score was 4.1 (range 1–9, SD 2.45) at baseline.

Baseline XeQoLS scores were available for 31/32 (97%) patients, with matched results for 25 patients at cycle 3 and 15 patients at 1-mo follow-up. There was no difference in XeQoLS scores between baseline and cycle 3; however, there was deterioration in scores from baseline to 1 mo following treatment ($p < 0.005$; Supplementary Fig. S1).

3.6. Molecular characteristics

Germline data were available for 26/32 (81%) patients, of whom four (15%) had a pathogenic germline variant in PCa-related genes (*BRCA1* [n = 2], *BRCA2*, and *RAD51C*). These four patients completed a median of 4.5 cycles, and two (50%) achieved a PSA response of >50%. Cell-free DNA was extracted from 18 patients, and 16 had an adequate yield for analysis (Supplementary Tables S1–3). All the three patients with the highest DNA yields per nanogram of plasma responded poorly to treatment, completing only two cycles each with short PFS (<2 mo) and OS (<7 mo). Among these poor responders, two of three (67%) had copy number (CN) loss in *RB1* and one had a germline *BRCA2* mutation in conjunction with *BRCA2* CN loss. None of the four exceptional responders (PFS > 10 mo, OS > 20 mo) had deleterious mutations in key DNA repair genes. *CHEK2* amplifications occurred in three (75%) of these men, with concomitant *BRCA1* gains in two (50%).

ARV7 status was assessed in 16/32 patients at baseline and before cycle 3 (Supplementary Table S4). Four (25%) patients had detectable ARV7 at baseline, one remained positive after two cycles of treatment, while a further two became positive on treatment. The patient with persistent positivity did not have any PSA response.

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Table 2 – Summary of common and therapeutically relevant AEs in the overall cohort (*N* = 32).

Toxicity	Grade 1, *n* (%)	Grade 2, *n* (%)	Grade 3, *n* (%)	All grades, *n* (%)
Anaemia	17 (53)	9 (28)	2 (6)	28 (88)
Xerostomia	17 (53)	2 (6)	0 (0)	19 (59)
Fatigue	14 (44)	7 (6)	1 (3)	18 (69)
Anal inflammation	7 (22)	2 (6)	0 (0)	9 (28)
Nausea	8 (25)	0 (0)	0 (0)	8 (25)
Thrombocytopenia	8 (25)	3 (9)	0 (0)	11 (34)
Pneumonitis[a]	0 (0)	1 (3)	0 (0)	1 (3)
Neutropenia	3 (9)	0 (0)	0 (0)	3 (9)

AE = adverse event.
[a] Pneumonitis was attributed to radiation therapy administered prior to enrolment.

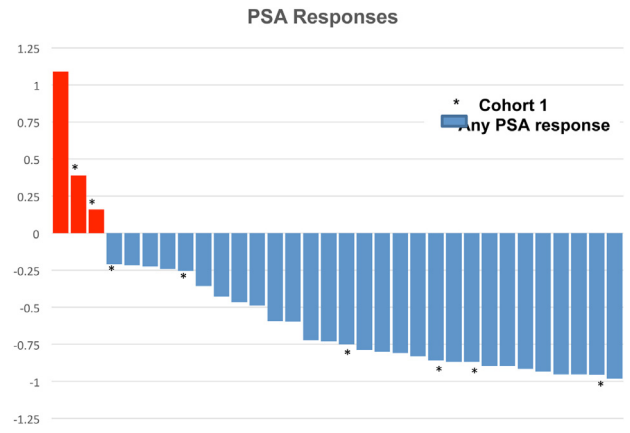

Fig. 2 – Wawaterfall plot of best PSA responses at any time point in maximum % change from baseline. PSA = prostate-specific antigen.

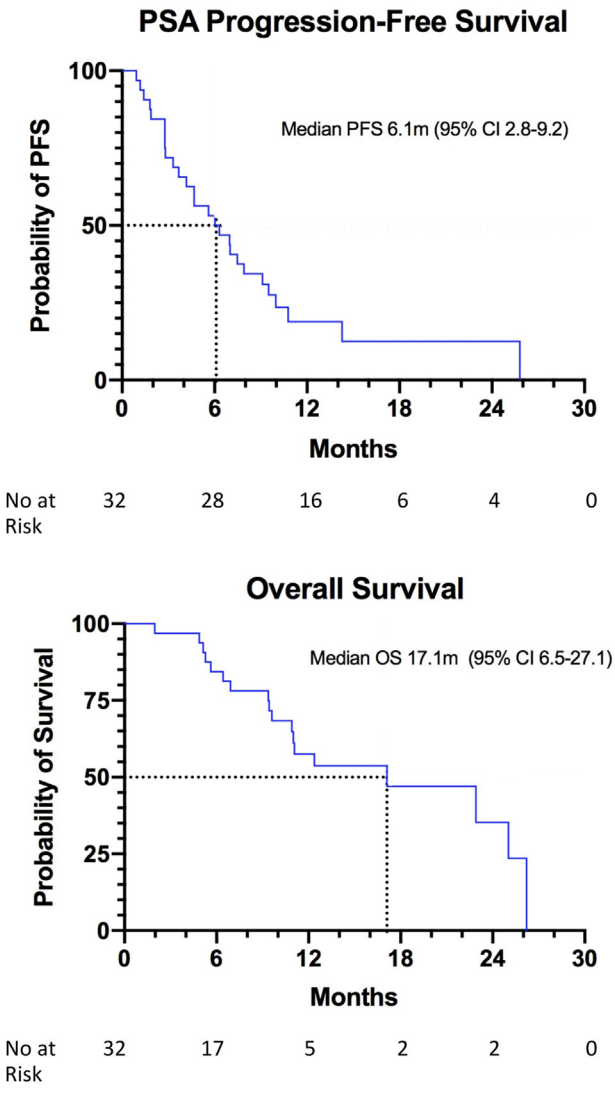

Fig. 3 – Kaplan-Meier curves for (A) PSA progression-free survival and (B) overall survival. CI = confidence interval; OS = overall survival; PFS = progression-free survival; PSA = prostate-specific antigen.

4. Discussion

Lutetium PSMA utilising small-molecule peptides has emerged as a promising treatment option for men with mCRPC, with a number of retrospective and prospective single-site trials demonstrating its safety and efficacy [1,2,22–24]. However, similar to all treatments in mCRPC, primary and acquired treatment resistance are an on-going problem. Combination therapies may be required to improve and extend treatment responses. This phase I/II dose escalation/expansion study of the combination of LuPSMA-617 and NOX66 has demonstrated that the combination is safe and well tolerated, resulting in high treatment response rates and improved pain scores in men with end-stage mCRPC.

LuPSMA-617 beta radiation induces cell death via mitotic catastrophe through direct ionisation and the generation of oxidative free radicals that induce single- and double-strand DNA breaks [25]. We have previously demonstrated that a proportion of men on LuPSMA-617 therapy progressed despite having persistently high PSMA expression, suggesting that acquired resistance may be, at least in part, due to radiation resistance [1]. We hypothesised that combining a tumour-specific radiation sensitiser with LuPSMA-617 would reduce radiation resistance, prolonging and deepening treatment responses compared with LuPSMA-617 alone. We proposed that idronoxil, via its effects on apoptosis, cell cycle arrest, and topoisomerase II, may impair DNA repair mechanisms and improve sensitivity to LuPSMA-617 [9,10,26]. Furthermore, we postulated

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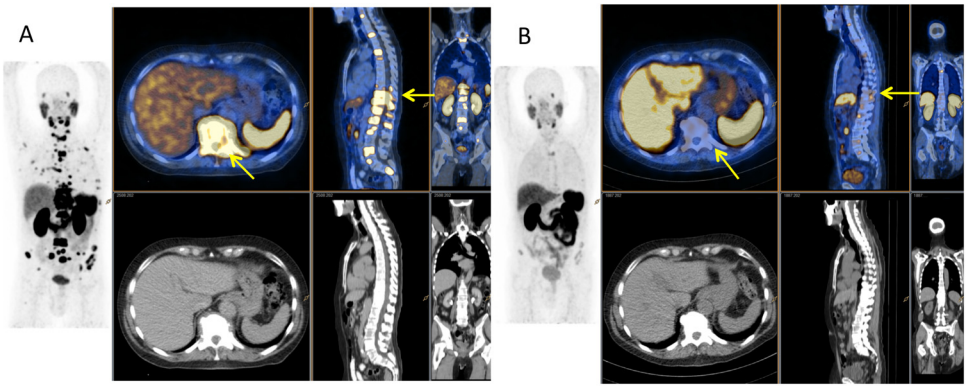

Fig. 4 – A 76-yr-old male with progressive symptomatic bone metastases after abiraterone and both docetaxel and cabazitaxel was enrolled. The (A) screening PSMA PET scan and (B) post-trial (42 wk) PSMA PET demonstrate a significant reduction in PSMA avidity at all sites of disease with no new sites identified. PSA progression occurred at 20 mo following enrolment. PET = positron emission tomography; PSA = prostate-specific antigen; PSMA = prostate-specific membrane antigen.

that the specific action of NOX66 on ENOX2, which is preferentially expressed on tumour cells [12], would lead to relative sparing of nontarget organs, limiting AEs such as marrow and salivary gland toxicities.

The combination of LuPSMA-617 and NOX66 was well tolerated, with most AEs being of grade 1 severity and no serious AEs being reported. Xerostomia was common, although the incidence of xerostomia was not higher than that reported in single-agent LuPSMA-617 prospective trials [1,2] and grade 1 severity in most cases. Similarly, anaemia was a common event seen in 88%, although it was predominantly of grade 1 (53%) and, in most, pre-existing (56%). Haematological toxicity was within the range reported with previous studies of single-agent LuPSMA-617. The only AE solely attributable to NOX66 was anal inflammation. There was no difference in reported AEs between the two dose levels of NOX66 (400 and 800 mg). As a radiosensitising strategy, the study did not attempt to determine a dose-limiting toxicity for NOX66.

Efficacy endpoints, including PSA response rates and OS outcomes, from this phase I/II trial are promising: 63% experienced a >50% PSA decline in response to treatment and the median OS was 17.1 mo. These findings are supported by the pain reduction in more than half of the men with significant pain at baseline. Often these symptomatic improvements occurred in parallel to metabolic responses (Fig. 4). The PSA response rate is comparable with the rates in a recently published prospective study of LuPSMA-617 in 30 (56%) men and the randomised TheraP trial (66%) [2,4]. However, there is a difference in the rates of previous cabazitaxel between this trial, that of Hofman et al [2,4], and TheraP (91%, 47%, and 0%, respectively). These findings compare well with published treatment responses to chemotherapy in a comparable patient population. Buonerba et al [27] undertook a phase II study of carboplatin and etoposide in a similar patient population (after two lines of chemotherapy and novel androgen signalling inhibitors). They reported OS of 18 wk (95% CI 12–26) [27], shorter than the 17.1 mo reported here. The median OS in our study is comparable with that in previously published studies with single-agent LuPSMA-617 therapy [1,2]. Violet et al [3] reported median OS of 13.3 mo (95% CI 10.5–18.7) in 50 men treated with LuPSMA-617.

Mechanisms of acquired resistance to LuPSMA-617 have not yet been resolved. PCa progression is characterised by an accumulation of genomic alterations, and DNA analysis may identify predictors of treatment response or resistance. This study included liquid biopsies in a subgroup to explore whether specific genetic alterations or detectable levels of ARV7 RNA were predictive of outcomes. Somatic and germline DNA repair defects were not predictive of response, but conclusions are limited by the small number of deleterious events identified. However, *RB1* CN losses were enriched in poor responders (2/3) and the third poor responder had a deleterious alteration in the PI3K pathway, which is associated with radiation resistance [28]. We previously published a case of dramatic response to LuPSMA-617 in a man with a germline *BRCA2* alteration [29]. However, instead of loss of function alterations in DNA repair genes, amplifications were common in exceptional responders in this cohort, including CN gains in *CHEK2*, *BRCA1*, and *MSH6*. Men with detectable ARV7 RNA completed fewer cycles of treatment, but it is unclear whether this is a predictive biomarker or simply prognostic. These findings suggest that larger studies with correlative molecular and imaging biomarkers are merited.

5. Conclusions

This study demonstrates that LuPSMA-617 in combination with NOX66 is a safe treatment for heavily pretreated mCRPC. Efficacy appears similar to that in previously reported LuPSMA-617 studies. Further research is required to better evaluate the efficacy of this combination.

***Author contributions*:** Louise Emmett 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: Emmett.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.euo.2020.07.002.

References

[1] Emmett L, et al. Results of a prospective phase 2 pilot trial of (177) Lu-PSMA-617 therapy for metastatic castration-resistant prostate cancer including imaging predictors of treatment response and patterns of progression. Clin Genitourin Cancer 2019;17:15–22.
[2] Hofman MS, et al. [(177)Lu]-PSMA-617 radionuclide treatment in patients with metastatic castration-resistant prostate cancer (LuPSMA trial): a single-centre, single-arm, phase 2 study. Lancet Oncol 2018;19:825–33.
[3] Violet J, et al. Long term follow-up and outcomes of re-treatment in an expanded 50 patient single-center phase II prospective trial of Lutetium-177 ((177)Lu) PSMA-617 theranostics in metastatic castrate-resistant prostate cancer. J Nucl Med 2020;61:857–65.
[4] Hofman MS, et al. TheraP: a randomised phase II trial of 177Lu-PSMA-617 (LuPSMA) theranostic versus cabazitaxel in metastatic castration resistant prostate cancer (mCRPC) progressing after docetaxel: initial results (ANZUP protocol 1603). J Clin Oncol 2020;38 (15_suppl):5500.
[5] Kamsteeg M, et al. Phenoxodiol—an isoflavone analog—induces apoptosis in chemoresistant ovarian cancer cells. Oncogene 2003;22:2611–20.
[6] Kelly GE, Husband AJ. Flavonoid compounds in the prevention and treatment of prostate cancer. Methods Mol Med 2003;81:377–94.
[7] Liu XX, et al. Genistein sensitizes sarcoma cells in vitro and in vivo by enhancing apoptosis and by inhibiting DSB repair pathways. J Radiat Res 2016;57:227–37.
[8] Mahoney S, et al. Cytotoxic effects of the novel isoflavone, phenoxodiol, on prostate cancer cell lines. J Biosci 2012;37:73–84.
[9] Constantinou AI, Husband A. Phenoxodiol (2H-1-benzopyran-7-0,1,3-(4-hydroxyphenyl)), a novel isoflavone derivative, inhibits DNA topoisomerase II by stabilizing the cleavable complex. Anticancer Res 2002;22:2581–5.
[10] De Luca T, et al. Downstream targets of altered sphingolipid metabolism in response to inhibition of ENOX2 by phenoxodiol. Biofactors 2008;34:253–60.
[11] Chang L, et al. PI3K/Akt/mTOR pathway inhibitors enhance radiosensitivity in radioresistant prostate cancer cells through inducing apoptosis, reducing autophagy, suppressing NHEJ and HR repair pathways. Cell Death Dis 2014;5:e1437.
[12] Hostetler B, et al. Cancer site-specific isoforms of ENOX2 (tNOX), a cancer-specific cell surface oxidase. Clin Proteomics 2009;5:46–51.
[13] Hillman GG, et al. Genistein potentiates the radiation effect on prostate carcinoma cells. Clin Cancer Res 2001;7:382–90.
[14] Hillman GG, et al. Genistein potentiates inhibition of tumor growth by radiation in a prostate cancer orthotopic model. Mol Cancer Ther 2004;3:1271–9.
[15] Raffoul JJ, et al. Genistein inhibits radiation-induced activation of NF-kappaB in prostate cancer cells promoting apoptosis and G2/M cell cycle arrest. BMC Cancer 2006;6:107.
[16] Tang Q, et al. Genistein and AG1024 synergistically increase the radiosensitivity of prostate cancer cells. Oncol Rep 2018;40:579–88.
[17] Porter K, et al. Idronoxil as an anticancer agent: activity and mechanisms. Curr Cancer Drug Targets 2020;20(5):341–54.
[18] de Souza PL, et al. Phase I study of a novel S1P inhibitor, NOX66, in combination with radiotherapy in patients with metastatic castration-resistant prostate cancer. J Clin Oncol 2020;38 (15_suppl):5533.
[19] Cleeland CS. The measurement of pain from metastatic bone disease: capturing the patient's experience. Clin Cancer Res 2006;12 (20 Pt 2), 6236s–42s.
[20] Henson BS, et al. Preserved salivary output and xerostomia-related quality of life in head and neck cancer patients receiving parotid-sparing radiotherapy. Oral Oncol 2001;37:84–93.

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[21] To SQ, et al. Expression of androgen receptor splice variant 7 or 9 in whole blood does not predict response to androgen-axis-targeting agents in metastatic castration-resistant prostate cancer. Eur Urol 2018;73:818–21.
[22] Heck MM, et al. PSMA-targeted radioligand therapy in prostate cancer. Urologe A 2017;56:32–9.
[23] Rahbar K, et al. Response and tolerability of a single dose of 177Lu-PSMA-617 in patients with metastatic castration-resistant prostate cancer: a multicenter retrospective analysis. J Nucl Med 2016;57:1334–8.
[24] Yadav MP, et al. 177Lu-DKFZ-PSMA-617 therapy in metastatic castration resistant prostate cancer: safety, efficacy, and quality of life assessment. Eur J Nucl Med Mol Imaging 2017;44:81–91.
[25] Kassis AI. Therapeutic radionuclides: biophysical and radiobiologic principles. Semin Nucl Med 2008;38:358–66.
[26] Mahoney S, et al. The effects of phenoxodiol on the cell cycle of prostate cancer cell lines. Cancer Cell Int 2014;14:110.
[27] Buonerba C, et al. Carboplatin plus etoposide in heavily pretreated castration-resistant prostate cancer patients. Future Oncol 2014;10:1353–60.
[28] Schuurbiers OCJ, et al. The PI3-K/AKT-pathway and radiation resistance mechanisms in non-small cell lung cancer. J Thorac Oncol 2009;4:761–7.
[29] Crumbaker M, et al. Exceptional response to 177Lutetium prostate-specific membrane antigen in prostate cancer harboring DNA repair defects. JCO Precis Oncol 2019;3:1–5.

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