Prostate Specific Membrane Antigen as a Diagnostic and Therapeutic Target

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A thesis submitted to fulfil requirements for the degree of

Doctor of Philosophy, The University of Sydney

February 2025

The research reported in this thesis was supported by the award of a Research Training Program scholarship to the PhD Candidate.

Statement of originality

This is to certify that to the best of my knowledge, the content of this thesis is my own work. This thesis has not been submitted for any degree or other purposes.

I certify that the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and sources have been acknowledged.

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Thesis abstract

177Lutetium-PSMA-617 is an effective treatment in metastatic castration resistant prostate cancer (mCRPC), but approximately a third of apparently suitable patients do not respond to this treatment. Prostate specific membrane antigen (PSMA) based imaging has the potential to improve clinical outcomes through better patient selection and response assessment. The aims of the work described in this thesis were to identify imaging biomarkers from pre-treatment 68Ga-PSMA and 18F-fluorodeoxygluose (18F-FDG) positron emission tomography (PET) imaging that could be used to optimise patient selection for 177Lu-PSMA-617 therapy; and, to characterise responses to treatment with 177Lu-PSMA-617 on imaging with both PET and 177Lu SPECT (single photon emission computed tomography).

Imaging studies including 68Ga-PSMA, 18F-FDG PET, and 177Lu SPECT were acquired as part of a prospective single arm trial (LuPIN trial) and used for analyses reported in four studies. Semiautomated quantitative imaging analysis workflows were used to derive imaging biomarkers including measures of total tumour volume, and of intensity of 68Ga-PSMA and 18F-FDG uptake.

The baseline 68Ga-PSMA and 18F-FDG imaging and clinical characteristics associated with PSA response and overall survival were identified. In multivariable analyses, higher PSMA SUVmean was associated with increased odds of PSA response (OR 1.57, 95% CI 1.12 to 2.19) and higher PSMA total tumour volume was associated with decreased odds of PSA response (OR 0.41, 95% CI 0.19 to 0.87). Higher PSMA total tumour volume was associated with worse overall survival (HR, 2.18 95% CI 1.36 to 3.51), while duration of prior treatment with androgen-signalling

inhibitors for greater than 12 months was associated with improved overall survival (HR 0.45 95% CI 0.22 to 0.91).

Quantitative analysis of post-treatment 68Ga-PSMA and 18F-FDG PET imaging demonstrated that 32% (12/37) of participants had an increase in post-treatment PSMA total tumour volume, and this was significantly associated with poorer overall survival (HR 5.1 95% CI 1.5 to 17). All participants with an increase in total tumour volume also had persistently high PSMA expression.

Patterns of treatment response and progression were explored further using a novel approach (Traffic Light Workflow, TLW) to characterise heterogeneous treatment responses seen on 68Ga-PSMA PET following 177Lu-PSMA-617 therapy. Only 9/37 participants demonstrated a treatment response at all sites of disease. Heterogeneous and complex treatment responses were common, with 16/37 participants demonstrating a reduction in PSMA total tumour volume despite the presence of low-volume disease progression. TLW overall response category was prognostic for overall survival.

Finally, the feasibility of quantitative analysis of interim imaging with 177Lu SPECT was examined. Quantitative analysis of post cycle 1 177Lu SPECT and post cycle 3 177Lu SPECT was performed, demonstrating that an increase in 177Lu SPECT total tumour volume was associated with worse PSA PFS (HR 4.1 95% CI 1.5 to 11.2).

The findings reported in this thesis support the use of quantitative imaging methods to extract clinically relevant information from PSMA based imaging to personalise the use of 177Lu-PSMA-617 therapy and improve clinical outcomes. This work raises the hypotheses that 1) patient selection should involve consideration of pretreatment PSMA SUVmean and PSMA total tumour volume, and 2) that criteria for response-

assessment after treatment with 177Lu-PSMA-617 should incorporate both changes in PSMA total tumour volume and the characterisation of lesional treatment responses using the novel TLW, to accurately stratify treatment response and guide subsequent therapy. The innovative use of interim 177Lu SPECT response assessment could be practice-changing by promptly identifying patients with tumours that are responding poorly to 177Lu-PSMA-617 and might benefit from treatment escalation.

Authorship attribution statement

I undertook this PhD under the supervision of Professor Martin Stockler, Professor Andrew Martin, and Professor Louise Emmett.

This is a hybrid thesis, combining 4 traditional chapters (chapters 1, 2, 3 and 8), 3 papers published in peer-reviewed journals (chapters 4, 5, 7), and 1 manuscript submitted for publication in a peer-reviewed journal (chapter 6). I was principally responsible for the development of the research proposals, review of existing literature, selection of research methods, collection and analysis of data, interpretation of findings, and drafting and revising the thesis.

For chapters 1, 2, 3 and 8, I was responsible for the literature review, data synthesis, interpretation of the findings and drafting and revision of these chapters.

The data presented in the studies in chapters 4, 5, 6, 7 were collected as part of a clinical trial that completed recruitment before the start of my PhD candidature (ACTRN12618001073291). Human Research Ethics Committee / Institutional Review Board approval was obtained, and all participants provided written informed consent. The primary results of the trial have been published separately. For chapters 4, 5, 6, and 7, I was responsible for the development of the research proposals for the sub-studies, selection of research methods, data collection, data analysis, interpretation of findings, and drafting and revision of these manuscripts.

Supervisor Statement

As the corresponding author for the publications in chapter 4 and 7, I give permission for the inclusion of the published works in this thesis, and I confirm that the authorship attribution statements above are correct.

Professor Louise Emmett

As supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements above are correct.

Professor Martin Stockler

Publications arising from the thesis

Published

Pathmanandavel, S., Crumbaker, M., Yam, A. O., Nguyen, A., Rofe, C., Hovey, E., Gedye, C., Kwan, E. M., Hauser, C., Azad, A. A., Eu, P., Martin, A. J., Stockler, M.R, Joshua, A. M., & Emmett, L. (2022). 177Lu-PSMA-617 and Idronoxil in Men with End-Stage Metastatic Castration-Resistant Prostate Cancer (LuPIN): Patient Outcomes and Predictors of Treatment Response in a Phase I/II Trial [Article]. Journal of Nuclear Medicine, 63(4), 560-566. https://doi.org/10.2967/jnumed.121.262552

Pathmanandavel, S., Crumbaker, M., Nguyen, A., Yam, A. O. W., Wilson, P., Niman, R., Ayers, M., Sharma, S., Eu, P., Martin, A. J., Stockler, M. R., Joshua, A. M., & Emmett, L. (2022). The prognostic value of post-treatment PSMA and FDG PET/CT in metastatic, castration-resistant prostate cancer treated with 177Lu-PSMA-617 and NOX66 in a phase I/II trial (LuPIN). Journal of Nuclear Medicine 2023 Vol. 64 Issue 1 Pages 69-74. https://doi.org/10.2967/jnumed.122.264104

Pathmanandavel, S., Crumbaker, M., Ho, B., Yam, A. O., Wilson, P., Niman, R., Ayers, M., Sharma, S., Hickey, A., Eu, P., Stockler, M., Martin, A. J., Joshua, A. M., Nguyen, A., & Emmett, L. (2023). Evaluation of 177Lu-PSMA-617 SPECT/CT Quantitation as a Response Biomarker Within a Prospective 177Lu-PSMA-617 and NOX66 Combination Trial (LuPIN). Journal of Nuclear Medicine, 64(2), 221-226. https://doi.org/https://dx.doi.org/10.2967/jnumed.122.264398

Under submission

Pathmanandavel, S., Crumbaker, M., Nguyen, A., Yam, A. O. W., Wilson, P., Niman, R., Ayers, M., Sharma, S., Eu, P., Martin, A. J., Stockler, M. R., Joshua, A. M., & Emmett, L. Quantifying patterns of response and progression on PSMA PET/CT using a novel Traffic Light Workflow analysis method in men with metastatic castration-resistant prostate cancer within a prospective phase I/II combination trial of 177Lu-PSMA-617 and NOX66 (LuPIN).

Acknowledgements

I would like to thank Professor Louise Emmett, for her mentorship over the past four years. I am so grateful to her for generously sharing her time and insights, and for the research and career opportunities she has made possible.

I would like to thank Professor Martin Stockler, for sharing his expertise and experience. His patient guidance and support during this journey has been invaluable.

I would like to thank Professor Andrew Martin for sharing his time and knowledge. His thoughtful advice has been most appreciated.

I would like to thank the patients who participated in the clinical trials, without whom this work would not be possible. I have also been fortunate to work with many oncology and nuclear medicine physicians on these studies, and I would like to thank them all for sharing their time and expertise with me.

I gratefully acknowledge the Research Training Program funding I received, and the Cancer Institute of NSW grant that supported my research.

My sincerest thanks to my colleagues and fellow PhD candidates for their encouragement and camaraderie.

I would like to thank my parents and my sister for their practical support and encouragement, I could never have completed this work without you all. My most heartfelt thanks to my two daughters, Liyaani and Nishanthi, who keep me grounded and fill my life with joy; and to my husband Rueben, for supporting me in every way to achieve this goal.

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List of frequently used abbreviations

177Lu-PSMA-617	177Lutetium vipivotide tetraxetan; also known as Pluvicto®
177-Lu-PSMA-I&T	177Lutetitum zadavotide guraxetan or 177Lu-PNT2002
177Lu SPECT	177Lutetium single photon emission computed tomography
18F-FDG	18Fluorine-fluorodeoxyglucose
68Ga-PSMA	68Gallium-PSMA-HBED-CC; also known as 68Ga-PSMA-11
ASI	Androgen signalling inhibitor
CI	Confidence interval
CT	Computer tomography
mCRPC	Metastatic, castration-resistant prostate carcinoma
NOX66	Radiation sensitiser idronoxil (3-(4-hydroxyphenyl)-2H-1-benzopyran-7-ol)
OS	Overall survival
PET	Positron emission tomography
PSA	Prostate specific antigen
PSA PFS	PSA progression free survival
PSA50	PSA decline > 50% at any time point
PSMA	Prostate specific membrane antigen
rPFS	Radiographic progression free survival
SPECT	Single photon emission computed tomography
SUV	Standardised uptake value
SUVmax	The pixel with the highest SUV in a region of interest
SUVmean	The average SUV in a region of interest
TTV	Total tumour volume

1 Introduction

1.1 Rationale for the thesis

177Lu-PSMA-617 is a novel and efficacious treatment for metastatic castration resistant prostate cancer. However, approximately one third of patients do not respond to treatment, and duration of responses can be short lived (*1,2*). Clinical outcomes for patients treated with 177Lu-PSMA-617 may be improved by better patient selection, and accurate response assessment.

68Ga-PSMA and 18F-FDG positron emission tomography (PET) imaging are integral to evaluating patients prior to treatment with 177Lu-PSMA-617. PET imaging provides both structural (extent and location of disease) and functional (PSMA expression or degree of glucose metabolism) information about the tumour. The existing selection criteria for 177Lu-PSMA-617 treatment may be improved by the application of quantitative PET imaging analysis methods to identify imaging factors that are associated with improved clinical outcomes.

There is a paucity of information about patterns of response and disease progression following treatment with 177Lu-PSMA-617. Quantitative analysis of post-treatment PET and 177Lu SPECT imaging may provide more detailed information about treatment response and tumour heterogeneity compared with conventional imaging modalities such as computed tomography (CT) and whole body bone scintigraphy.

The work in this thesis has the overarching goal of optimising the selection and response assessment of patients undergoing 177Lu-PSMA-617 therapy to maximise clinical benefit.

1.2 Selection of studies for the thesis

This hybrid thesis comprises of four manuscripts accepted or under submission in peer reviewed journals (chapters 4-7), that are linked by traditional chapters 2 (the background), chapter 3 (methods) and chapter 8 (the discussion).

The studies in this thesis assess different aspects of 177Lu-PSMA-617 patient selection and response assessment, including: the baseline clinical and PET imaging factors associated with tumour response and survival; and the interim (mid-treatment) and post-treatment imaging findings associated with survival. Post-treatment PET imaging analysis examined the whole body and lesional changes, while interim (mid-treatment) imaging response was assessed using 177Lu SPECT (single photon emission tomography).

The studies in this thesis contribute original information to the current body of evidence regarding 177Lu-PSMA-617.

1.3 Aims and objectives

The overarching aims of the thesis were to identify potential imaging biomarkers which could optimise patient selection; and to describe the PET and 177Lu SPECT imaging responses to 177Lu-PSMA-617 therapy.

The specific objectives were to:

1. Identify baseline clinical and PET imaging markers which are associated with treatment response and survival
2. Identify quantitative changes in post treatment whole body PET imaging and assess their association with survival

3. Identify lesional changes in post treatment PET imaging and describe patterns of treatment response and progression

4. Assess the feasibility of quantitative 177Lu SPECT imaging analysis and assess the association of interim 177Lu SPECT imaging markers with survival

1.4 References

1. Hofman MS, Emmett L, Sandhu S, et al. [177Lu]Lu-PSMA-617 versus cabazitaxel in patients with metastatic castration-resistant prostate cancer (TheraP): a randomised, open-label, phase 2 trial. *The Lancet.* 2021.

2. Sartor O, De Bono J, Chi KN, et al. Lutetium-177-PSMA-617 for metastatic castration-resistant prostate cancer. *New England Journal of Medicine.* 2021;385:1091-1103.

2 Background

This chapter provides a background for the work in this thesis. It will describe the landscape of therapeutic options for metastatic, castration-resistant prostate cancer (mCRPC) and the known prognostic and predictive factors associated with overall survival in men with mCRPC. This is followed by a summary of the current understanding of the biological role of the prostate specific membrane antigen (PSMA) receptor in mCRPC, and the role of 68Ga-PSMA and 18F-FDG imaging in assessing prostate cancer. Finally, the evidence for 177Lu-PSMA-617 radioligand therapy as a treatment for mCRPC, and the current understanding prognostic and predictive factors associated with response to 177Lu-PSMA-617 and survival are summarised.

This section summarises the existing knowledge at the time of commencing the thesis work. Evidence which emerged during the course of the thesis will be discussed in Chapter 8 (Discussion).

2.1 Metastatic, castration-resistant, prostate cancer

mCRPC is defined as clinical or biochemical disease progression of metastatic prostate carcinoma in the setting of very low testosterone (*1*). Almost all patients with metastatic prostate carcinoma will eventually develop castration resistance, and 10-20% of those develop mCRPC within five years (*2*). mCRPC carries a poor prognosis, with a median overall survival of approximately two years based on US and Swedish health registry data (*3,4*). Methods to optimise the use of existing and

new treatments that maximise benefits and minimise toxicities are critical for improving overall survival and quality of life.

2.2 Current therapies for mCRPC

Initial treatment for mCRPC includes continuation of androgen deprivation therapy (ADT), with the addition of either chemotherapy with a taxane, or a novel androgen receptor signalling inhibitor (e.g. abiraterone acetate, enzalutamide; ASI).

In the first line setting, chemotherapy with docetaxel showed superior survival compared with mitoxantrone (HR 0.76 95% CI 0.62 to 0.94) (*5*). First line enzalutamide demonstrated superior radiographic progression free survival and overall survival compared with placebo (HR 0.19 95%CI 0.15 to 0.23 and HR 0.71 95% CI 0.60 to 0.84 respectively) (*6*).First line abiraterone acetate plus prednisone demonstrated superior overall survival compared with prednisone alone (HR 0.81 95% CI 0.70 to 0.93) (*7,8*).

In the post chemotherapy setting, enzalutamide demonstrated superior survival compared with placebo (HR 0.63 95% CI 0.53 to 0.75) (*9*). Similarly, abiraterone acetate plus prednisone demonstrated superior overall survival compared with prednisone alone (HR 0.65 95% CI 0.54 to 0.77) (*10*).

The choice and sequencing of therapies is guided by prior treatment in the hormone sensitive setting, disease burden, and comorbidities (*11,12*). For patients who have progressed on these therapies, chemotherapy with cabazitaxel demonstrated superior overall survival compared with mitoxantrone as a third line treatment (HR 0.70 95% CI 0.59 to 0.83) (*13*).

The poly (ADP-ribose) polymerase (PARP) inhibitor olaparib was compared with physician's choice of enzalutamide or abiraterone in participants with tumours that had mutations in homologous recombination repair genes (BRCA1, BRCA2 and ATM), and who had been previously treated with either enzalutamide or abiraterone. 75% of the study cohort also had prior treatment with taxane chemotherapy. The study showed superior imaging based progression free survival and overall survival with olaparib (HR 0.34 95% CI 0.25 to 0.47, and HR 0.69 95% CI 0.50 to 0.97, respectively) (*14,15*).

Sipuleucel-T, an autologous dendritic cell vaccine, demonstrated a modest survival benefit compared with placebo in participants previously treated with androgen deprivation therapy in a single, randomized phase 3 trial, however use of this treatment is limited (*16*).

Radium-223 is an alpha emitter, which acts as a calcium mimetic and binds to bone stroma. In the setting of mCRPC with bony metastatic disease, this has a cytotoxic effect by causing double stranded DNA breaks, leading to cell death. Radium-223 was compared with placebo in a randomised trial in men who had previously been treated with androgen deprivation therapy. 57% had also been treated with docetaxel chemotherapy. Patients with nodal disease measuring >3cm in short axis or visceral metastatic disease were excluded from the trial. The study showed superior overall survival with radium-223 compared to placebo (HR 0.70 95% CI 0.58 to 0.83) (*17,18*).

177Lu-PSMA-617 is currently considered a third or fourth line treatment, following disease progression after ADT, chemotherapy, and a novel androgen signalling inhibitor. 177Lu-PSMA-617 will be discussed in detail in section 2.8.

2.3 Known prognostic and predictive factors in mCRPC

Prognosis is the estimation the risk of a future outcome in individuals based on clinical and non-clinical factors, known as prognostic factors (*19*). Prognostic factors are associated with the outcome in the absence of treatment, or with the use of a standard treatment across the cohort. A cohort study or the control arm of a randomised study may be used to identify prognostic factors. Multivariable prognostic models assign relative weights to multiple prognostic factors to estimate an overall risk (*20*). By contrast, a predictive factor is a marker that is associated with a response (or absence of response) to a specific treatment. A randomised clinical trial is required to identify a predictive factor (*21*). This section will discuss the prognostic factors and multivariable prognostic models which have been developed utilising clinical and biochemical indices to estimate the risk of death in men with metastatic CRPC.

In patients receiving first line treatment for mCRPC, an externally validated prognostic model incorporated opioid analgesia use, sites of metastatic disease, Eastern Cooperative Oncology Group (ECOG) performance status, and serum concentrations of albumin, haemoglobin, alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and prostate specific antigen (PSA) to predict overall survival. The model categorised patients into low, intermediate and high risk groups. The median overall survival time in each group was: low risk 33 months, intermediate risk 20 months, and high risk 12 months in non-ASI therapy trials, and 43 months, 28 months and 15 months in ASI therapy trials (*22,23*).

In the second line setting, a validated model was developed incorporating ECOG performance status, time since last docetaxel use, presence of measurable disease, presence of visceral disease, presence of pain, duration of hormonal use, serum concentrations of haemoglobin, PSA, and ALP (*24*).

Prognostic factors for patients commencing third line therapy have also been reported. In one retrospective cohort study of 3616 patients, multivariable analysis identified ECOG performance status, opioid analgesia use, presence of visceral metastases, serum concentrations of haemoglobin, PSA, ALP and LDH as independently significant prognostic factors (*25*).

The prognostic value of cellular and molecular biomarkers has also been evaluated. In a prospective cohort study, baseline and post-treatment circulating tumour cell counts were prognostic for overall survival in multivariable models (*26,27*). In another prospective cohort, gene expression analysis of circulating tumour cells demonstrated that presence of androgen receptor splice variant 7 (AR-V7) expression was independently associated with worse progression free and overall survival in men being treated with an ASI. AR-V7 is a splice variant which encodes an androgen receptor which is constitutively active, and conveys resistance to androgen deprivation and ASI treatment (*27*). Assessment of circulating tumour cells is not in routine clinical practice in Australia.

68Ga-PSMA and 18F-FDG PET imaging have not yet been incorporated into existing prognostic models. Their potential as prognostic factors will be discussed below in section 2.10.

2.4 Prostate specific membrane antigen

Prostate specific membrane antigen (PSMA) is a transmembrane glycoprotein, also known as glutamate carboxypeptidase II or folate hydrolase I (*28*). It is expressed at higher levels in prostate adenocarcinoma compared to normal prostate cells. Higher levels of PSMA expression in localised prostate carcinoma tissue samples correlates with higher Gleason score, and shorter metastasis-free survival and disease-free survival (*29,30*). Thus, PSMA expression appears to be associated with more aggressive disease biology.

PSMA expression on prostate cancer cells appears to promote cancer growth. In prostate cancer cell lines, cells expressing PSMA have a proliferative advantage in low or normal folate environments (*31*). In experiments using cell lines and xenograft models in mice, Kaitannis et al determined that in prostate cancer cells, PSMA cleaves glutamated folate to release free glutamate, causing downstream activation of the PI3K-Akt-mTOR pathway, which could promote cancer progression. In a separate study, they performed preoperative 68Ga-PSMA PET and MRI in patients undergoing radical prostatectomy and showed strong correlation between preoperative Ga68-PSMA PET avidity and quantitative immunofluorescence staining for phosphorylated AKT in the subsequently resected specimens. This raises the hypothesis that that PSMA avidity on 68Ga-PSMA PET imaging might be an indicator of PI3K-Akt pathway activation in prostate cancer (*29*).

PI3K pathway activation is thought to have a reciprocal negative feedback loop with androgen receptor pathway activation. Blocking the androgen receptor pathway upregulates PI3K-Akt signalling. This may explain why PI3K signalling is activated in patients with mCRPC and establishes a relationship between the androgen receptor pathway and PSMA (*32*). This relationship is also evident in 68Ga-PSMA PET

imaging, as androgen deprivation therapy and androgen signalling inhibitors have been shown to alter PSMA avidity on 68Ga-PSMA PET (discussed in section 2.5).

2.5 68Ga-PSMA imaging

A monoclonal antibody targeting the intracellular domain of PSMA (known as 7E11-C5) was developed in 1987. In-111 capromab pendetide was developed using this antibody to be the first prostate cancer imaging agent. This agent had low sensitivity because it required a disrupted cell membrane to reach the intracellular binding site and predominantly identified necrotic cells. An extracellular antibody, J591, was subsequently developed with improved performance (*33*). PSMA targeting antibodies have limited utility in imaging due to their relatively long half-life and poor tissue penetrability. This led to interest in peptide inhibitors of PSMA, which have lower molecular weight and smaller size, and are rapidly internalised and cleared from the blood pool, improving the signal to noise ratio (*34*).

Several PSMA peptide inhibitors are in clinical use, the most commonly used are 68Ga-PSMA-11, 18F-DCFPyl, and 18F-PSMA-1007. This work focuses on 68Ga-PSMA-11, a urea-based, peptide inhibitor of PSMA first used for imaging in humans in 2013 (*35*). Since 2013, 68Ga-PSMA has become one of the most commonly used radiotracers for diagnostic imaging in prostate cancer.

68Ga-PSMA imaging is potentially useful in all stages of prostate cancer. 68Ga-PSMA PET is superior to conventional imaging (computerised tomography (CT) and whole body radio-isotope bone scanning) in staging of men with high risk localised prostate cancer, with greater accuracy, sensitivity, and specificity in detecting nodal

and distant metastases (*36*). In men with biochemical recurrence after local treatment, conventional imaging (CT and whole body bone scintigraphy) has lower sensitivity and specificity than 68Ga-PSMA PET, particularly when serum PSA levels are low. The recurrence detection rate with 68Ga-PSMA PET was greater than 80% in men with serum PSA greater than 1.0ng/mL (*37*). 68Ga-PSMA PET findings have important clinical implications in prostate cancer. For example, a prospective study of 312 men with biochemical recurrence and negative or equivocal conventional imaging, found that the addition of 68Ga-PSMA PET lead to a change in management in 62% of cases (*38*).

As discussed in section 2.4, PSMA indirectly interacts with the androgen receptor (AR) pathway. Therefore, treatments which modulate the androgen receptor pathway may also modulate PSMA expression. Limited data suggest that interactions between PSMA expression and AR blockade differ in hormone-sensitive versus castration-resistant prostate cancer. These studies will be summarised below.

In vitro studies reported by Meller et al found that androgen deprivation therapy (ADT) increased PSMA mRNA expression in both androgen-sensitive and castration-resistant cell lines (*39*). Animal studies using hormone-sensitive xenografts demonstrated that treatment with testosterone reduced uptake of 64Cu-J591 (an antibody to PSMA), while treatment with a novel androgen signalling inhibitor (enzalutamide) increased uptake of 64Cu-J591(*40*).

A study in 4 participants with metastatic hormone sensitive prostate carcinoma (mHSPC) starting ADT and 4 participants with mCRPC starting enzalutamide examined changes in uptake in individual lesions. 22 out of 45 lesions demonstrated an increase (‘flare’) in PSMA uptake, however there was considerable interpatient

and intrapatient heterogeneity of the changes in PSMA avidity in both the mHSPC and mCRPC groups (*41*).

A study in 8 participants with mHSPC commencing ADT and 7 participants with mCRPC commencing ASI used serial imaging with PSMA PET at days 9, 18, and 28 to monitor changes in PSMA uptake. In the participants with mHSPC, a median reduction in SUVmax of 30% occurred at day 9, followed by variable change in PSMA intensity on subsequent scans: 3/8 participants had persistent or increased SUVmax, while 5/8 had a reduction in SUVmax. In contrast, in participants with mCRPC commencing ASI, there was a median increase of 45% in SUVmax on day 9, and this remained stable on subsequent scans. The reduction in PSMA uptake in mHSPC may reflect a rapid response to ADT resulting in reduced tumour volume. The increase in PSMA uptake in mCRPC may reflect the reciprocal relationship between the activity of the androgen pathway and the PI3K pathway (*42*).

2.6 68Ga-PSMA for assessing response to treatment

The use of 18F-FDG PET imaging for response assessment is common in other malignancies, with well established response criteria such as PERCIST for solid tumours, and Lugano classification for Hodgkin and non-Hodgkin lymphoma (*43,44*). In addition, studies in advanced Hodgkin lymphoma demonstrated that a negative (defined as Deauville score 1-3) interim 18F-FDG PET after two cycles of treatment is prognostic for progression free survival (*45,46*). Subsequently, trials have examined the role of interim 18F-FDG PET in guiding treatment escalation or de-escalation in advanced Hodgkin lymphoma (*47-49*).

In a similar way, 68Ga-PSMA may be used for monitoring response and guiding treatment in mCRPC. Consensus guidelines for response assessment have been proposed, however the evidence basis for this is currently limited (*50,51*). In small, retrospective studies of HSPC treated with either ADT, radiotherapy, or chemotherapy, indices from pre-treatment and post-treatment 68Ga-PSMA PET (e.g. PSMA avid tumour volume PSMA and total lesional PSMA), correlated with changes in PSA (*52,53*). Another study of 28 participants with HSPC treated with docetaxel found that a complete response on 68Ga-PSMA PET was highly correlated with a reduction in serum PSA to <0.02 (*54*). Further prospective evidence is needed to define 68Ga-PSMA PET response assessment criteria in the context of 177Lu-PSMA-617 treatment.

2.7 18F-FDG imaging

18Fluorine-2-deoxy-2-fluoro-D-glucose (18F-FDG) was first synthesised in 1978 (Ido et al 1978). It is transported across the cell membrane by glucose transporters and phosphorylated by hexokinase enzymes. Increased glucose uptake and increased metabolic activity is a hallmark of cancer cells. Therefore, 18F-FDG PET is useful in the assessment and monitoring of many types of cancer (*55*).

18F-FDG PET has limited use in the setting of early prostate cancer or biochemical relapse, as prostate cancer tends to have lower metabolic activity compared with other cancer types. However, in metastatic prostate cancer, particularly in the castration-resistant setting, metabolic activity tends to be higher, and 18F-FDG PET may be useful as a prognostic marker, and for assessing response to treatment (*56*). In a prospective study of 87 participants with mCRPC commencing chemotherapy, a

higher sum of the SUVmax of each lesion (derived from baseline 18F-FDG PET) was associated with poorer overall survival in multivariable analysis (*57*). Another retrospective study of 114 participants with mCRPC commencing either chemotherapy or androgen receptor signalling inhibitor, identified that a higher total lesional glycolysis (SUVmean multiplied by metabolic tumour volume) was associated with poorer overall survival (*58*).

18F-FDG PET may also be useful in identifying dedifferentiated prostate cancer, and prostate cancer with neuroendocrine differentiation. Case reports and case series data support the hypothesis that prostate cancer with neuroendocrine differentiation often shows high uptake of 18F-FDG (*59-61*).

Thus 18F-FDG PET may be useful for characterising disease phenotypes in mCRPC before and after treatment with 177Lu-PSMA-617.

2.8 177Lu-PSMA therapy

The high tracer uptake of 68Ga-PSMA in prostate cancer makes it is well-suited for theranostic drug development by replacing the imaging radioisotope (68Ga) with a therapeutic radioisotope (e.g. 177Lu).

177Lutetium is a beta-emitter that can be bound to the peptide inhibitor of PSMA. It is administered intravenously, and binds to PSMA, which is overexpressed by prostate cancer cells. It is then internalised, where 177Lu-PSMA causes cell death via single and double stranded DNA breaks. 177Lutetium has a half-life of approximately 1 week (6.7 days), and a tissue penetration of 2 mm, therefore ‘crossfire’ to adjacent cells can also cause cell death, however normal tissues are largely spared (*62,63*). The two commonly used theranostic agents are 177Lutetium-

PSMA-617 and 177Lutetium-PSMA-I&T (*64,65*). The two agents differ in structure, but have shown similar treatment outcomes (which will be discussed in section 2.9). No randomised comparison of the two radioligands has been undertaken.

Optimal criteria for selecting candidates for treatment with 177Lu-PSMA-617 have not been established. Clinical trials have used measures of 68Ga-PSMA avidity such as SUVmax to guide eligibility. For example the TheraP trial required a minimum SUVmax of 20 at one or more sites of disease, and SUVmax > 10 at all sites of measurable disease ≥10 mm (*66*). This was based on the rationale that pre-treatment PSMA avidity on imaging is associated with therapeutic uptake of 177Lu-PSMA-617, and therefore with the absorbed dose of radiation (*67*). However, the phase 3 VISION trial has included participants with PSMA avidity in metastatic lesions which is greater than physiological liver activity, a relatively lower cutoff than in TheraP (*68*).

Some studies of 177Lu-PSMA-617 have excluded patients with sites of 'discordant disease', defined as lesions which demonstrate 18F-FDG avidity above physiological background activity, but do not demonstrate 68Ga-PSMA avidity above physiological background activity. Due to limited PSMA expression, these sites of disease are less likely to respond to treatment with 177Lu-PSMA (*66,69*).

2.9 Current evidence for efficacy of 177Lu-PSMA-617 therapy

The evidence supporting the use of 177Lu-PSMA in mCRPC is derived from a variety of prospective and retrospective studies and a phase II randomised trial. These will be discussed in this section.

A retrospective multicentre study by Rahbar et al published the results of 145 participants with mCRPC undergoing 1-4 cycles of 177-LuPSMA-617. They reported a decline in serum PSA concentration of >50% in 45% of participants. Common adverse events were anaemia, leukopaenia, thrombocytopaenia, elevated liver enzymes (ALT or AST), xerostomia, and nausea (*70*).

A 2019 meta-analysis of 17 studies (14 prospective, 3 retrospective) included 744 participants with mCRPC treated with either 177Lu-PSMA-617 or 177Lu-PSMA-I&T. There was marked heterogeneity in patient characteristics, 177Lu-PSMA dosing, and number of cycles of treatment. They reported a serum PSA decline >50% in 46%, and a median overall survival of 14 months (IQR 8 to 14months) (*71*).

The single arm phase 2 LuPSMA trial of 30 participants with mCRPC, previously treated with an ASI and at least one line of taxane chemotherapy, admininstered up to 4 cycles of 177Lu-PSMA-617. A PSA decline of >50% was observed in 57%, and median overall survival was 14 months (*72*). In the expanded cohort of 50 participants, PSA decline of >50% was observed in 64%, and median overall survival was 13.3 months (*73*).

177Lu-PSMA-617 was compared to third line therapy with cabazitaxel in a prospective, randomised, phase 2 trial including 200 participants with mCRPC. 66% of men in the 177Lu-PSMA-617 group achieved the primary endpoint of PSA response >50%, compared with 37% in the cabazitaxel group ($p<0.0001$). There were fewer grade 3-4 toxicities in the 177Lu-PSMA-617 arm, indicating that 177Lu-PSMA-617 was less toxic and better tolerated. There was no significant difference in overall survival between the two groups (*74,75*).

VISION, a phase 3 trial of 750 participants with mCRPC previously treated with chemotherapy and ASI, randomised to either six cycles of 177Lu-PSMA-617 or protocol-permitted best supportive care, will be discussed in chapter 8 (NCT03511664).

2.10 Current understanding of prognostic factors with 177Lu-PSMA-617 therapy

Despite promising outcomes in mCRPC, 30% of patients with mCRPC previously treated with an ASI and chemotherapy do not respond to 177Lu-PSMA-617 (*72,76*). Pre-treatment clinical, biochemical and imaging factors associated with treatment response and survival have been examined in retrospective studies.

Baseline biochemical markers of response have been examined in retrospective analyses. A retrospective multivariate analysis of 100 participants after at least one line of therapy for metastatic prostate cancer identified that serum concentrations of albumin > 38g/L, AST < 24U/L, haemoglobin > 104g/L, and the absence of hepatic metastases were independently associated with overall survival (*77*). Two other retrospective studies found that the presence of visceral metastases and an elevated serum LDH were associated with shorter overall survival (*70,78*).

A retrospective analysis of 50 participants with mCRPC treated with up to 4 cycles of 177Lu-PSMA-617 found that higher PSMA SUVmean was associated with longer overall survival (HR 0.89 95% CI 0.8–0.98) while higher FDG-positive tumour volume was associated with shorter survival (HR 2.6 95% CI 1.4–4.8) (*79*).

Seifert et al performed semiautomated quantitation of 68Ga-PSMA imaging markers including tumour volume, SUVmax, SUVmean in 40 participants treated with 177Lu-

PSMA-617 therapy. In multivariable analysis, only tumour volume was independently associated with overall survival (HR 1.004 95%CI 1.001-1.006) although the effect size was small. A study of 110 participants by the same group found that PSMA total lesion quotient (defined as the sum of each tumour lesion volume divided by its SUVmean) and serum concentration of LDH were associated with shorter overall survival (HR 1.24, 95%CI 1.01–1.54 and HR 1.64, 95%CI 1.11–2.42 respectively) (*80,81*).

Molecular markers have been examined in one study of 19 patients. The study analysed PSMA PET, baseline biochemical markers, in addition to circulating tumour cell expression of androgen receptor (full length and androgen receptor splice variant 7), PSA, PSMA and neuroendocrine markers. Global correlation analysis showed that PSA response and PSMA avid tumour volume correlated with overall survival. No molecular markers were associated with survival in this small study (*82*).

2.11 Summary

This chapter has described the current understanding of 177Lu-PSMA-617 in the treatment of mCRPC, including the currently used patient selection criteria and known prognostic markers. Thus far, most data have been obtained from retrospective analyses of heterogeneous patient groups, and no clear predictors of response or resistance have emerged. Further study and prospective data are needed in this area.

Chapters 4-7 of this thesis aim to further our understanding of the role of imaging biomarkers in selecting patients for treatment with 177Lu-PSMA-617 and monitoring response to treatment, using data from a phase I/II prospective clinical trial. Chapter

4 will describe the pre-treatment clinical and imaging indices associated with PSA response and survival with 177Lu-PSMA-617 treatment. Chapter 5 and 6 will examine the changes on post-treatment PET imaging and their association with survival. Chapter 7 will examine the novel use of 177Lu SPECT imaging in assessing response to 177Lu-PSMA-617.

2.12 References

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3 Methods

The specific methodology for each research project is described in each chapter. This section will discuss the overall methodological concepts and considerations that apply to all the projects.

3.1 Imaging procedures and acquisition protocols

Radiotracer manufacture and quality assurance was undertaken according to standard clinical procedures. 68Ga-HBEDD-CC-PSMA-11 was produced on-site according to Good Laboratory Practice procedure using a TRASIS automated radio-pharmacy cassette. Patients were injected with 2.0MBq/kg 68Ga-PSMA, with identical imaging parameters (dose, time post injection and imaging protocols) for each patient.

18F-FDG was produced off-site commercially. Radio-pharmacy quality control was undertaken using a high-pressure liquid chromatography method. Patients were injected with 3.5 MBq/kg 18F-FDG, with identical imaging parameters (dose, time post injection and imaging protocols) for each patient.

Known factors affecting image quantitation were controlled for according to standard clinical procedures (*1*). All PET/CT imaging was undertaken using a Phillips Ingenuity TOF-PET/64 slice CT scanner at a single site. A non-contrast low dose computed tomography (CT) scan was performed 60 minutes post tracer injection for attenuation correction and co-localisation using the following parameters: 2mm slice thickness soft tissue reconstruction kernel, 120 keV and 50mAs, pitch of 0.828, 600mm FOV and a 512 matrix. Immediately after CT, a vertex to mid-thigh PET scan

was acquired for 2 minutes per bed position. The emission data were corrected for randoms, scatter and decay using the standard clinical Philips® blob-basis function ordered subsets time of flight (Blob-OS-TF) reconstruction protocol with 3 iterations and 33 subsets.

3.2 18F-FDG PET analysis workflow

An 18F-FDG PET quantitative analysis workflow was applied to all 18F-FDG PET scans, using a standardised uptake value (SUV) cutoff of bloodpool intensity + 1.5 x standard deviation and size >1mL to identify lesions (MIM Software, Cleveland, USA). A semiautomated workflow was used to reduce potential variability which may be introduced with manual lesion selection. The conventional SUV threshold for defining solid tumour lesions is 1.5 x liver SUV + 2 standard deviations (when liver SUV is measured in a 3-cm spherical ROI in normal right lobe of liver) (*2,3*). However, as prostate cancer tends to have lower 18F-FDG avidity compared with most solid cancers, the minimum SUV threshold was reduced to be greater than bloodpool SUV + 1.5 standard deviations (*4,5*). After the physician manually selected reference regions for mediastinal bloodpool, liver and gluteus maximus, the workflow identified regions of interest (ROIs) defined as all voxels above the SUV threshold. The automatically segmented ROIs were reviewed by a physician, and incorrectly identified lesions and normal physiologic activity was removed. Then, total metabolic tumour volume, standardised uptake values (SUVmax, SUVmean) and total lesional glycolysis (metabolic tumour volume x total tumour SUVmean) were calculated (*2*).

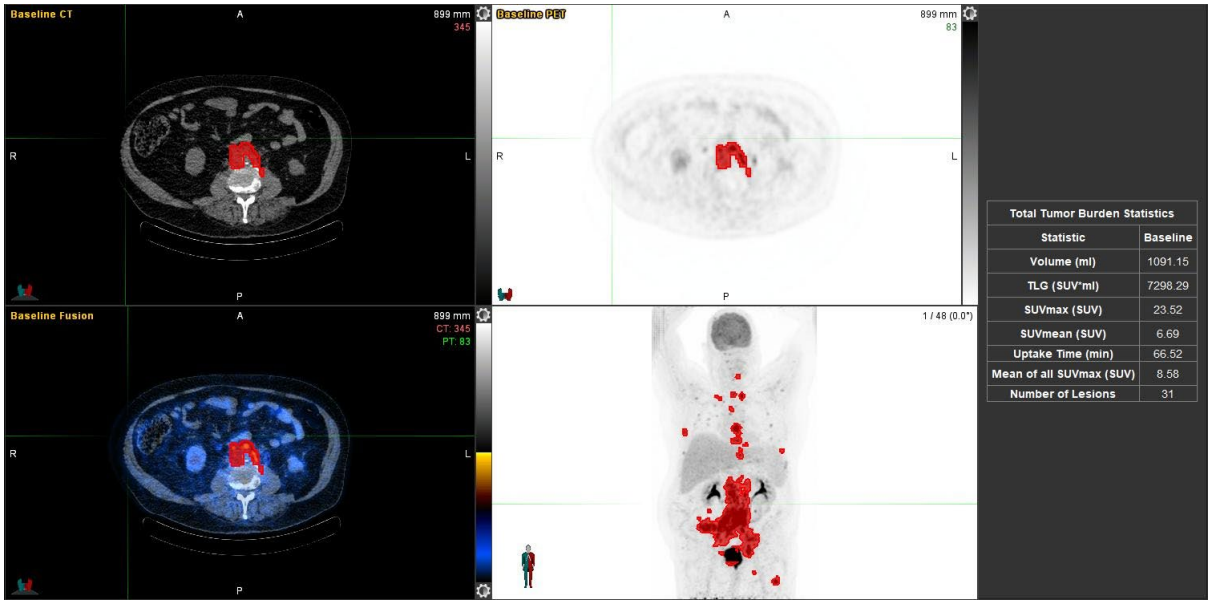


Figure 3-1. Summary output of 18F-FDG quantitative analysis workflow.

3.3 68Ga-PSMA PET analysis workflow

A 68Ga-PSMA PET quantitative analysis workflow was applied to all 68Ga-PSMA PET scans, using a minimum SUV > 3 and volume > 1mL to identify lesions (MIM software, Cleveland, USA). The SUV threshold was selected based on clinical expertise and prior publications (*6-8*). As above, the automatically segmented ROIs were reviewed by a physician and non-tumour lesions were removed. As liver background activity was typically greater than SUV 3, hepatic lesions were manually segmented using a gradient based tool (MIM PET Edge™). Total PSMA tumour volume, SUVmax, SUVmean and total lesional activity (metabolic tumour volume x total tumour SUVmean) were derived (*2*).

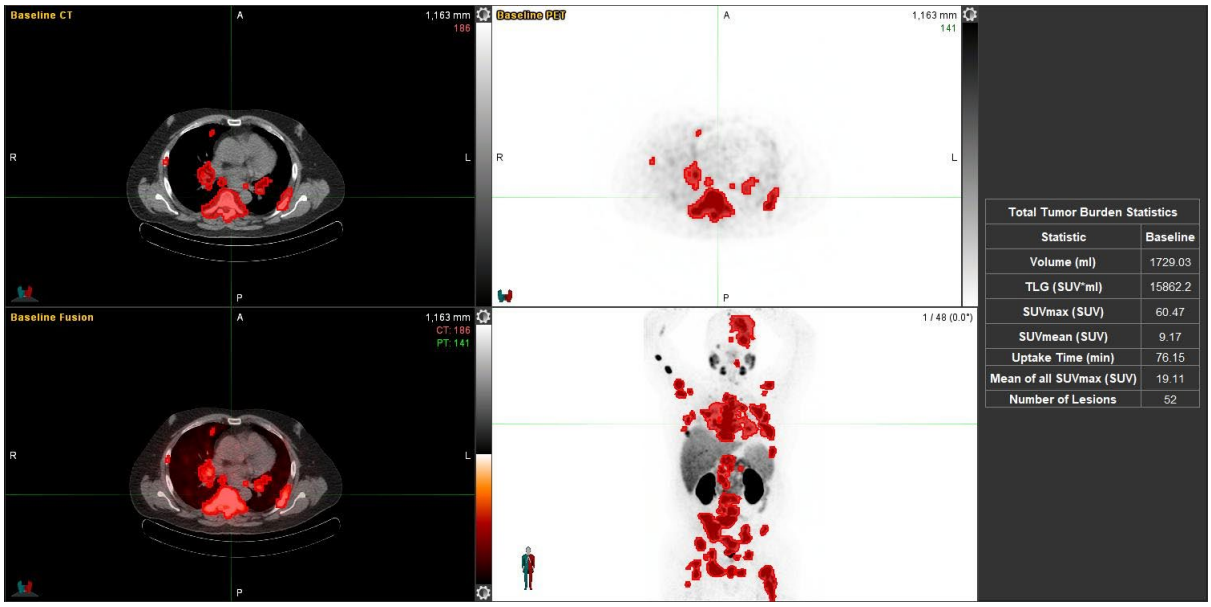


Figure 3-2. Summary output of 68Ga-PSMA quantitative analysis workflow.

3.4 New Lesion analysis workflow

This workflow was developed by MIM Software to compare two timepoint PET/CT scans (either 68Ga-PSMA or 18F-FDG) and identify new lesions on the post-treatment scan. The 68Ga-PSMA or 18F-FDG PET analysis workflow was applied to both baseline and post-treatment PET scans to delineate tumour regions of interest as described in section 3.2 and 3.3. The New Lesion analysis workflow used the regions of interest identified on baseline and post-treatment PET to compare and identify new lesions. The lesions categorised as ‘new’ were reviewed and adjusted by the physician. The new lesion tumour volume, SUVmax, SUVmean and total lesional activity (metabolic tumour volume x total tumour SUVmean) were derived.

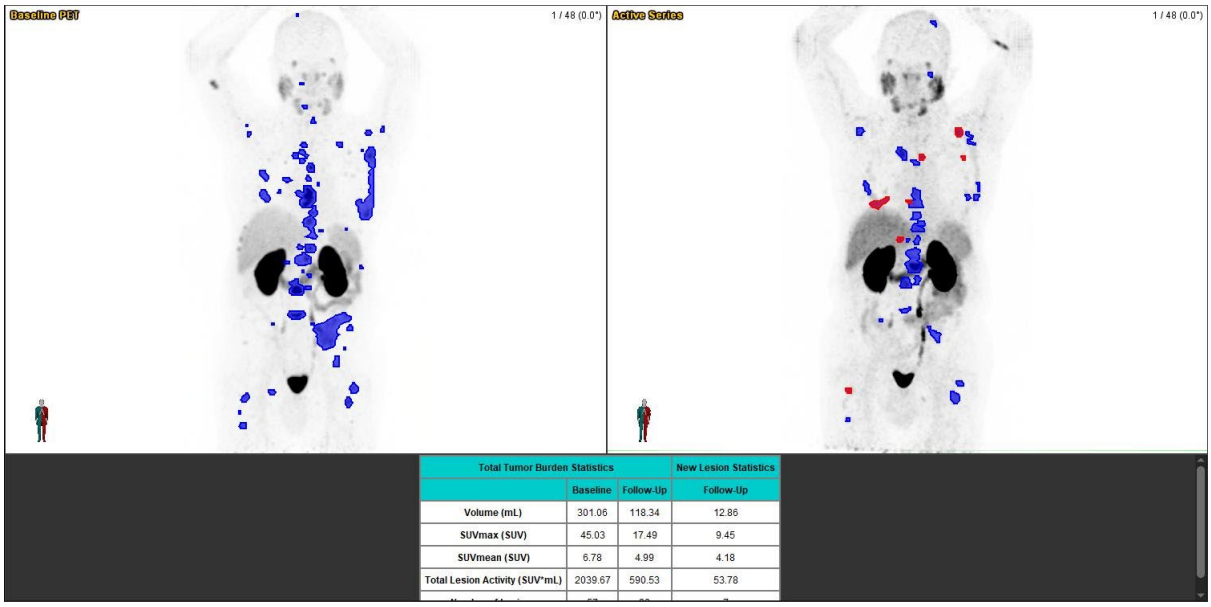


Figure 3-3. Summary output of New Lesion quantitative analysis workflow.

3.5 Traffic Light workflow

Building on the New Lesion analysis workflow, the Traffic Light workflow was developed by MIM software. With this workflow, we sought to characterize individual lesion response to treatment. Lesion responses were categorised as:

Lesion type	**Definition**	**Colour**
Resolved	The lesion is absent on post treatment PET	Green
Reducing	≥30% decrease in volume	Green
Stable	<30% change in volume/SUVmax	Yellow
Increasing	≥30% increase in volume/SUVmax	Red
New	The lesion was not present on the baseline PET and is considered to represent disease	Red

Table 3-1. Lesion response categories as defined in the Traffic Light Workflow analysis.

The 30% cutoff for defining a lesion as reducing or increasing was based on limited available repeatability data for PSMA based imaging. The repeatability coefficient is “a threshold such that 95% of the normal variability between 2 SUV measurements will be within these numbers, so larger differences in scans are likely attributable to true disease progression or regression“ (*9*).

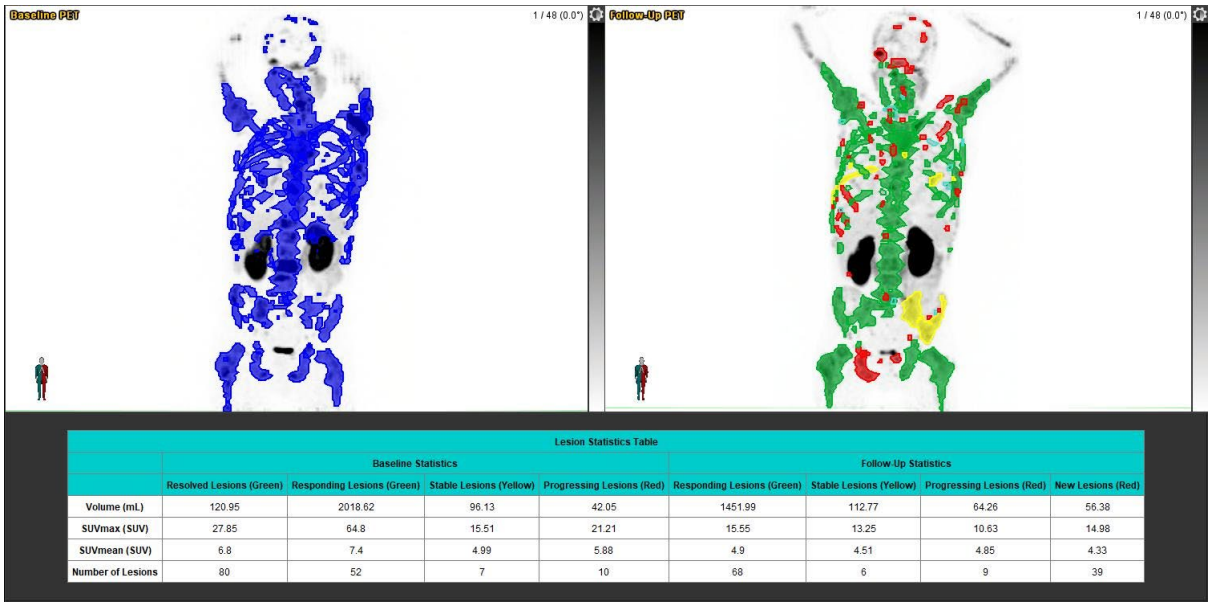
Data from PSMA based imaging using 18F-DCFPyl demonstrated repeatability coefficient of 17% for patient level tumour volume and 28% for lesion level tumour volume. The repeatability coefficient for lesion level SUVmax was 31% and SUVmean was 24% (*10*).

Analysis of 68Ga-PSMA imaging data demonstrated repeatability coefficient of 35% for semiautomated total tumour volume, and 77% for manually delineated lesion level tumour volume. Another study examining measures of PSMA intensity found a repeatability coefficient of 33% in bone lesions and 38% in nodal lesions for SUVmax and 31% for bone lesions and 37% for nodal lesions for SUVmean (*9,11*). The high repeatability coefficient for lesion level tumour volume may be related to the use of manual techniques to delineate lesions, which is likely to introduce greater variability.

The use of 30% cutoff for changes in lesion tumour volume and PSMA uptake in the TLW is in alignment with proposed response assessment guidelines which defined progression as an increase in size or PSMA uptake of 30% or greater in a lesion (*12,13*).

The Traffic Light workflow allows the tumour lesions delineated on baseline and post treatment PET to be matched. The physician could additionally identify lesions which

had ‘merged’ (coalesced together, most likely representing an increase in size of at least one of the lesions) or ‘split’ (most likely representing a reduction in size of the lesion). Once completed, the Traffic Light Workflow categorises each lesion into either ‘reducing’, ‘stable’ or ‘increasing’ based on the criteria defined above.



Lesion Statistics Table

	Baseline Statistics				Follow-Up Statistics			
	Resolved Lesions (Green)	Responding Lesions (Green)	Stable Lesions (Yellow)	Progressing Lesions (Red)	Responding Lesions (Green)	Stable Lesions (Yellow)	Progressing Lesions (Red)	New Lesions (Red)
Volume (mL)	120.95	2018.62	96.13	42.05	1451.99	112.77	64.26	56.38
SUVmax (SUV)	27.85	64.8	15.51	21.21	15.55	13.25	10.63	14.98
SUVmean (SUV)	6.8	7.4	4.99	5.88	4.9	4.51	4.85	4.33
Number of Lesions	80	52	7	10	68	6	9	39

Figure 3-4. Summary output of Traffic Light Workflow quantitative analysis.

3.6 177Lu SPECT analysis

Quantitative SPECT/CT reconstruction was performed using commercially available software (MIM SPECTRA Quant, MIM Software Inc.) (*14*). Imaging analysis was performed using a similar semiautomated workflow to the 68Ga-PSMA imaging analysis workflow described in section 3.3, with a SUV cutoff > 3 for defining regions of interest. As above, the automatically segmented ROIs were reviewed, and non-

tumour lesions were removed. 177LuSPECT total tumour volume, SUVmax and SUVmean were then derived.

There was no existing data to guide the definition of disease progression in 177LuSPECT imaging. We examined any change in total tumour volume and >30% change in tumour volume, in line with current guidelines for PET imaging (*12,13*).

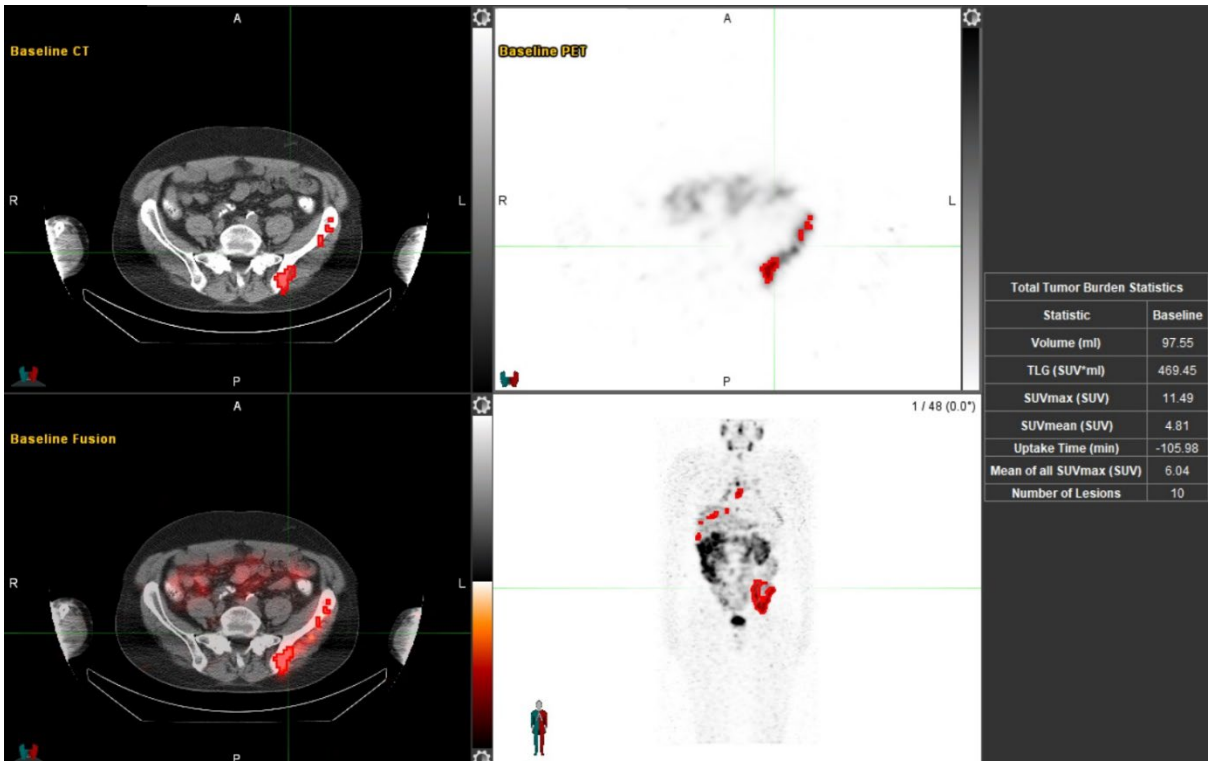


Figure 3-5. Summary output of 177Lu SPECT quantitative analysis workflow.

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4 177Lu-PSMA-617 and Idronoxil in Men with End-Stage Metastatic Castration-Resistant Prostate Cancer (LuPIN): Patient Outcomes and Predictors of Treatment Response in a Phase I/II Trial.

4.1 Overview

This chapter is a published manuscript aimed at nuclear medicine and oncology clinicians and researchers. The study used clinical and PET imaging data collected as part of a phase I/II trial. The baseline clinical characteristics and quantitative 68Ga-PSMA and 18F-FDG PET imaging parameters were described. The association between PSA PFS and overall survival, and baseline PET and clinical parameters was assessed using a multivariable model.

This research was originally published in the Journal of Nuclear Medicine.

Publication details

Pathmanandavel, S., Crumbaker, M., Yam, A. O., Nguyen, A., Rofe, C., Hovey, E., Gedye, C., Kwan, E. M., Hauser, C., Azad, A. A., Eu, P., Martin, A. J., Stockler, M.R., Joshua, A. M., & Emmett, L. (2022). 177Lu-PSMA-617 and Idronoxil in Men with End-Stage Metastatic Castration-Resistant Prostate Cancer (LuPIN): Patient Outcomes and Predictors of Treatment Response in a Phase I/II Trial. Journal of Nuclear Medicine, 63(4), 560-566. https://doi.org/10.2967/jnumed.121.262552

Contributions of the authors

SP: research proposal development, data acquisition, data analysis, interpretation of results, drafted and revised the manuscript.

MC: contributed to the data acquisition, ctDNA data analysis and interpretation of results, helped revise the manuscript.

AY, AN, CR, EH, CG, EK, CH, AA, PE, AMJ: contributed to the data acquisition, and helped revise the manuscript.

AJM, MS: data analysis, interpretation of results, and helped revise the manuscript.

LE: conceived the research proposal, concept development, data analysis, interpretation of results, and helped revise the manuscript.

4.2 Manuscript

ABSTRACT

Background: 177Lu-PSMA-617 is an effective therapy for metastatic castration-resistant prostate cancer (mCRPC). However, treatment resistance occurs frequently, and combination therapies may improve outcomes. We report the final safety and efficacy results of a phase I/II study combining 177Lu-PSMA-617 with idronoxil (NOX66), a radiosensitizer, and examine potential clinical, blood-based, and imaging biomarkers.

Methods: Fifty-six men with progressive mCRPC previously treated with taxane chemotherapy and novel androgen signaling inhibitor (ASI) were enrolled. Patients received up to 6 doses of 177Lu-PSMA- 617 (7.5 GBq) on day 1 in combination with a NOX66 suppository on days 1–10 of each 6-wk cycle. Cohort 1 (n = 8) received 400 mg of NOX66, cohort 2 (n = 24) received 800 mg, and cohort 3 (n = 24) received 1,200 mg. 68Ga-PSMA and 18F-FDG PET/CT were performed at study entry, and semiquantitative imaging analysis was undertaken. Blood samples were collected for analysis of blood-based biomarkers, including androgen receptor splice variant 7 expression. The primary outcomes were safety and tolerability; secondary outcomes included efficacy, pain scores, and xerostomia. Regression analyses were performed to explore the prognostic value of baseline clinical, blood-based, and imaging parameters.

Results: Fifty-six of the 100 men screened were enrolled (56%), with a screening failure rate of 26% (26/100) for PET imaging criteria. All men had received prior treatment with ASI and docetaxel, and 95% (53/56) had received cabazitaxel. Ninety-

six percent (54/56) of patients received at least 2 cycles of combination NOX66 and 177Lu-PSMA-617, and 46% (26/56) completed 6 cycles. Common adverse events were anemia, fatigue, and xerostomia. Anal irritation attributable to NOX66 occurred in 38%. Forty-eight of 56 had a reduction in prostate-specific antigen (PSA) level (86%; 95% CI, 74%–94%); 34 of 56 (61%; 95% CI, 47%–74%) had a PSA reduction of at least 50%. Median PSA progression-free survival was 7.5 mo (95% CI, 5.9–9 mo), and median overall survival was 19.7 mo (95% CI, 9.5–30 mo). A higher PSMA SUVmean correlated with treatment response, whereas a higher PSMA tumor volume and prior treatment with ASI for less than 12 mo were associated with worse overall survival.

Conclusion: NOX66 with 177Lu-PSMA-617 is a safe and feasible strategy in men being treated with third-line therapy and beyond for mCRPC. PSMA SUVmean, PSMA-avid tumor volume, and duration of treatment with ASI were independently associated with outcome.

BACKGROUND

Metastatic castration-resistant prostate cancer (mCRPC) is a lethal disease, and treatment options remain limited. 177Lu-prostate-specific membrane antigen-617 (177Lu-PSMA-617) is a radioligand therapy that targets prostate-specific membrane antigen (PSMA), a receptor highly expressed on prostate cancer cells (*1*). 177Lu-PSMA-617 has shown promising results in prospective single-center studies, the phase II TheraP trial, and the phase III VISION trial (*2-5*). However, secondary treatment resistance hinders longer-term outcomes for many men (*2,4,6*).

Combination therapies may overcome resistance mechanisms and improve clinical outcomes. Idronoxil (NOX66) is a derivative of the flavonoid genistein that binds to external NADH oxidase 2, a tumor-specific enzyme that induces apoptosis and inhibits topoisomerase II. It has shown potential as a radiation sensitizer in prostate cancer (*7-9*). We hypothesized that combining NOX66 with 177Lu-PSMA-617 may improve treatment responses, with a minimal increase in toxicity.

Improving treatment response with targeted radionuclide therapy involves not only optimizing treatment responses through effective combinations but also improving patient selection. Quantitative parameters on 68Ga-HBEDD-PSMA-11 and 18F-FDG PET/CT have shown potential as predictive and prognostic biomarkers for 177Lu-PSMA-617 therapy (*6,10-13*). The duration of prior treatments and other markers of treatment resistance, such as androgen receptor splice variant 7, may also have prognostic utility (*11,14,15*). We report the results of a trial of combination NOX66 and 177Lu-PSMA-617. Additionally, we evaluate the predictive and prognostic potential of blood-based markers, clinical factors, and molecular imaging.

MATERIALS AND METHODS

Study Design

This was a prospective single-center phase I/II dose escalation/expansion trial of combination 177Lu-PSMA-617 and NOX66. The St. Vincent's Hospital institutional review board approved the study protocol (HREC/17/SVH/ 19 and ACTRN12618001073291), and all patients provided written informed consent.

Screening

Men with mCRPC experiencing progression on conventional imaging (CT and bone scanning) or a rising level of prostate-specific antigen (PSA) based on Prostate Cancer Working group 3 criteria (*16*), and previously treated with at least 1 line of taxane chemotherapy (docetaxel or cabazitaxel) and at least 1 androgen signalling inhibitor (ASI) (abiraterone or enzalutamide), were screened. All patients had adequate organ function (baseline hemoglobin > 100 g/L, platelet count > 100 x 109/L, and estimated glomerular filtration rate > 40 mL/min), an estimated life expectancy of more than 12 wk, and a World Health Organization Eastern Cooperative Oncology Group performance status of no more than 2.

Men underwent screening with 18F-FDG and PSMA PET/CT, bone scanning, and CT and were eligible if they had an SUVmax of more than 15 on PSMA PET at 1 or more sites, an SUVmax of more than 10 at all measurable sites, and no 18F-FDG avidity without corresponding PSMA uptake (Fig. 1).

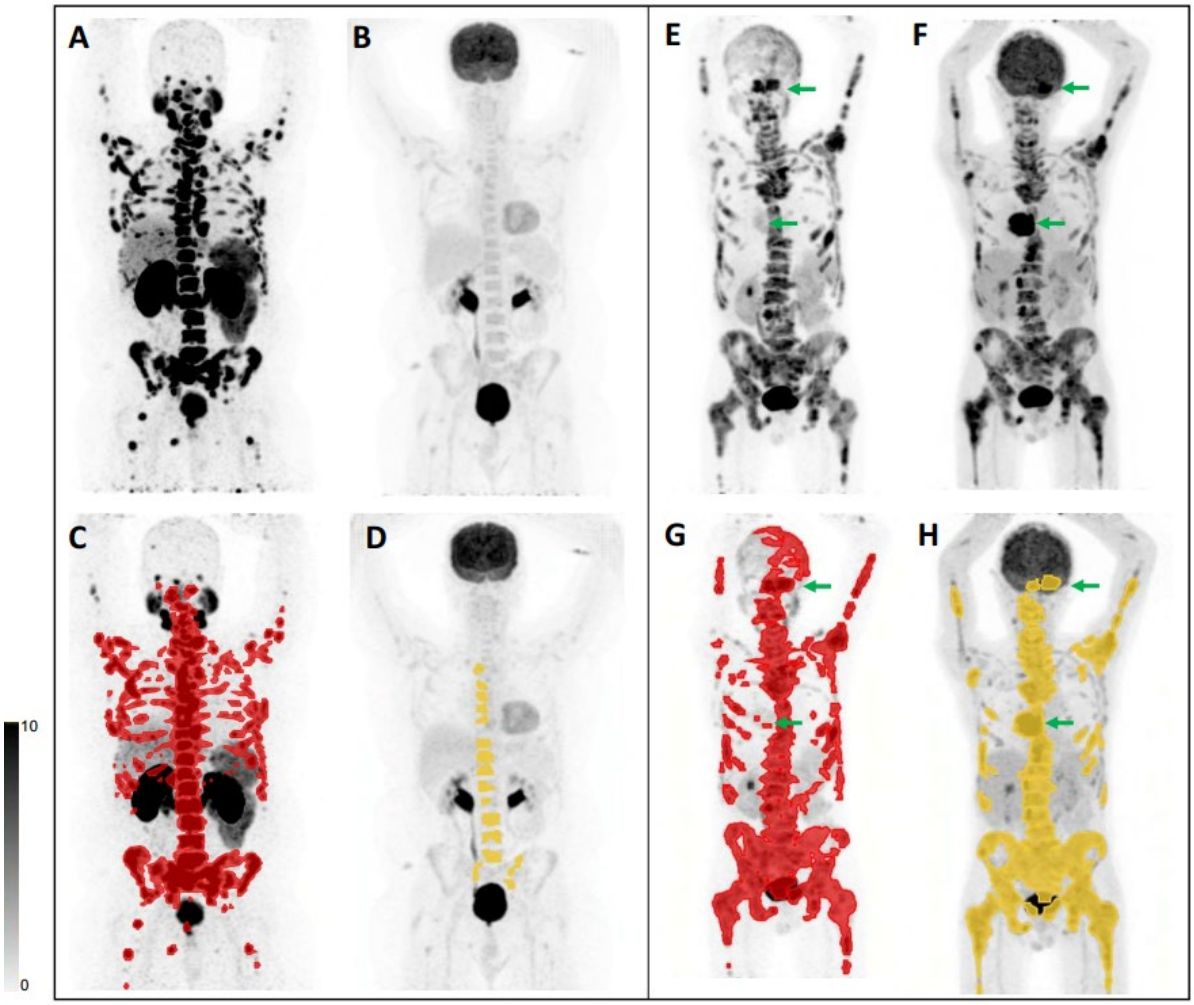

Figure 4-1. (A–D) Patient who was eligible on the basis of imaging with PSMA-avid disease (A) and no sites of discordant 18F-FDG (B). Quantitative PSMA tumor volume (C) and 18F-FDG tumor volume (D) are also shown. (E–H) Patient who was ineligible on the basis of imaging showing 2 sites (arrows) with higher 18F-FDG avidity (F) than PSMA avidity (E). Quantitative PSMA tumor volume (G) and 18F-FDG tumor volume (H) are also shown.

Study Treatment

All men received up to 6 cycles of 177Lu-PSMA-617 at 6-wk intervals in combination with 1 of 3 doses of NOX66 (400, 800, and 1,200 mg). NOX66 was administered via suppository on days 1–10 after each 177Lu-PSMA-617 injection. All cohorts were administered 7.5 GBq of 177Lu-PSMA-617 on day 1 via a slow intravenous injection. In addition, participants in cohort 1 (n = 8) received 400 mg of NOX66. After interim safety data reviews, the dose of NOX66 was escalated to 800 mg for cohort 2 (n = 24) and 1,200 mg for cohort 3 (n = 24).

The PSMA-617 precursor (AAA, Novartis) was radiolabeled to no-carrier-added 177Lu-chloride according to the manufacturer's instructions by a qualified radiopharmacist or radiochemist. Quality control tests for radionuclidic and radiochemical purity were performed using high-pressure liquid chromatography and thin-layer chromatography. NOX66 (Noxopharm Ltd.) was commercially produced.

Imaging Procedures and Analysis

68Ga-HBEDD-CC-PSMA-11 was produced on-site in compliance with good laboratory practices using a Trasis automated radiopharmacy cassette. 18F-FDG was produced off-site commercially. Radiopharmacy quality control testing used a high-pressure liquid chromatography method. Patients were injected with a 2.0 MBq/kg dose of PSMA and a 3.5 MBq/kg dose of 18F-FDG, with identical imaging parameters (dose, time after injection, and imaging protocols) for each patient. All PET/ CT imaging was undertaken using a Phillips Ingenuity TOF-PET/64- slice CT scanner. An unenhanced low-dose CT scan was performed 60 min after tracer

injection. Immediately after CT, a whole-body PET scan was acquired for 2 min per bed position.

PET/CT scans were analyzed semiquantitatively using MIM software and a standardized semiautomated workflow to delineate regions of interest with a minimum SUVmax cutoff of 3 for PSMA and blood pool intensity, plus 1.5 SDs for 18F-FDG (*17*). Quantitation derived total metabolic tumor volume, SUVmax, SUVmean, and total lesional activity for both 18F-FDG and PSMA (MIM Software).

Study Endpoints

Safety and tolerability were assessed using the National Cancer Institute Common Terminology Criteria for Adverse Events (version 5.0) every 2 wk during each 6-wk cycle until 6 wk after the final study treatment. To assess efficacy, we measured the PSA decline from baseline (absolute and >50% [PSA50]) at any time point, PSA progression-free survival (PFS), and overall survival (OS). Time-to-event endpoints (PSA PFS and OS) were defined as the interval from the date of enrollment to the event date, or the date last known to be event-free (at which point the observation was censored). Patient-reported outcomes were measured on day 1 of each cycle and during follow-up using the University of Michigan xerostomia-related quality-of-life scale (XeQoLS) (*18*) and the short form of the brief pain inventory (*19*). Pain palliation was defined as a reduction by at least 30% in the worst pain intensity score over the last 24 h observed at 2 consecutive evaluations (*20*).

Clinical, Blood-Based, and Molecular Analysis

Clinical information regarding initial diagnosis, Gleason score, previous lines of therapy, and prior treatment responses was collected. Blood was prospectively

collected for biomarker measurement, including hemoglobin, platelets, alkaline phosphatase, albumin, and PSA. Whole-blood samples were collected at baseline, before cycle 3, and before cycle 6 for analysis of potential molecular biomarkers, including androgen receptor splice variant 7. Androgen receptor splice variant 7 analysis was performed using the method described by To et al. (*21*).

Statistical Analyses

The study sample size was calculated to characterize the toxicity profile of the combination, based on the expectation that an adverse event with a true 5% incidence would be detected with 70% probability in a sample of 24 and detected with over 90% probability in a sample of 56. All patients who received at least 1 cycle of study treatment were included in the safety and efficacy analyses. P values below 5% were considered significant but interpreted cautiously. A 2-sided exact binomial 95% CI was calculated for PSA response rates. Kaplan–Meier method was used to characterize time-to-event endpoints (PSA PFS, and OS) and to estimate medians (presented with 95% CIs). The 3 NOX66 dose levels were compared in terms of adverse events, PSA50, and OS. Cox proportional-hazards regression, and logistic regression, were used to identify prognostic factors for time-to-event (PSA PFS, and OS) and binary endpoints (PSA50), respectively. The covariates investigated included baseline clinical, blood-based, and imaging parameters, including tumor volume and intensity scores (SUVmax and SUVmean). In the absence of compelling evidence of a dose–response effect on PSA50, the cohorts were grouped, and prognostic analyses were performed on the grouped cohort.

We used the relaxed LASSO (least absolute shrinkage and selection operator) regression method to identify covariates for inclusion in a multivariable model (*22*).

These were fitted in a standard multivariable Cox regression model to obtain conventional hazard ratios (HRs), 95% CIs, and P values.

All patients with a worst pain score of at least 4 were included in the analysis, and changes in score between baseline, precycle 3, and end of treatment were compared. Scores from the XeQoLS questionnaire were compared between baseline, pre-cycle 3, and end of treatment. A 2-tailed paired t-test was used to assess for a change in scores. Analyses were performed using R (version 4.0.5) and SPSS (version 25).

RESULTS

Baseline Patient Characteristics

One hundred men were screened, of whom 56 (56%) were enrolled between November 2017 and February 2020. Twenty-six percent were ineligible on the basis of the PET imaging criteria (13% because of low PSMA intensity disease and 13% because of sites with 18F-FDG/PSMA mismatch). Remaining screening fails (18%) were from clinical deterioration (6%), concurrent illness (3%), low hemoglobin (7%), or personal reasons (2%). Baseline characteristics are summarized in Table 1. All patients had prior treatment with at least 1 ASI and taxane chemotherapy, with 95% (53/56) having 2 lines of taxane chemotherapy; 66% (37/56) had at least 20 PSMA-avid metastases, 88% (49/56) had metastases in bone, 55% (31/56) had metastases in lymph nodes, and 19%(11/56) had visceral metastases.

Because of the small numbers in each NOX66 dose cohort, with 177Lu-PSMA-617 as the key treatment, we combined the 3 patient cohorts for reporting of outcomes and for exploratory analysis of biomarkers of response and survival. Analysis of the 3

dose escalation cohorts of NOX66 did not reveal any statistical differences in adverse events, PSA response rate, PSA PFS, or OS.

Characteristic	N=56
Age (years)	69 (64-74)
ECOG	
0 or 1	49 (88)
2	7 (12)
PSA at C1 (ug/L)	115 (46-476)
Haemoglobin (Normal Range 130-180 g/L)	122 (110-131)
Alkaline Phosphatase (NR 30-100 U/L)	113 (86-231)
Albumin (NR 36-52 g/L)	38 (34-41)
De novo metastatic disease	29 (52)
Gleason Score	
≤ 7	9 (16)
8-10	35 (63)
Unknown/Not Available	12 (21)
Prior Systemic treatments	
LHRH agonist/antagonist	56 (100)
Chemotherapy	56 (100)
Docetaxel	56 (100)
Cabazitaxel	53 (91)
Other chemotherapy	5 (9)
Androgen Signalling Inhibitor (ASI)	56 (100)

Enzalutamide only	27 (48)
Abiraterone only	13 (23)
Abiraterone + Enzalutamide	16 (29)
Clinical trial medication	4 (7)
PSMA PET	
SUV_{mean}	8 (7-10)
SUV_{max}	39 (29-61)
Volume in litres	0.64 (0.19-1.21)
FDG PET	
SUV_{mean}	4(3-5)
SUV_{max}	8 (5-10)
Volume in litres	0.07 (0.02-0.31)
Disease Volume	
< 20 metastases	19 (33)
≥ 20 metastases	37 (66)
Sites of Disease on PSMA PET	
Bone	49 (88)
Lymph Node	31 (55)
Visceral	12 (21)

Table 4-1. Patient Characteristics. Numbers are presented as absolute counts (percentage) or median (interquartile range).

Safety and Tolerability

Adverse events were predominantly grade 1 (149/188; 79%). The most common toxicities were anemia (50/56; 89%), fatigue (36/56; 64%), and xerostomia (33/56; 59%) (Table 2). Anal inflammation due to the NOX66 suppository occurred in 38% (21/56), with 27% (15/56) requiring topical treatment for anal inflammation. The rate of grade 1 anal inflammation was higher in cohort 3 (46%) than in cohort 1 or 2 (25% and 21%, respectively). Two men in cohort 2 and 1 man in cohort 3 required dose reduction or omission of NOX66. Four cases of grade 3 anemia were reported. There were no other significant differences in toxicities across the 3 cohorts and no grade 4–5 adverse events or treatment-related deaths.

Adverse event	**Grade 1 (%)**	**Grade 2 (%)**	**Grade 3 (%)**	**All Grades (%)**
Anemia	31 (55)	16 (29)	4 (7)	51 (91)
Xerostomia	30 (54)	3 (5)	0 (0)	33 (59)
Fatigue	27 (48)	8 (14)	0 (0)	35 (63)
Anal Inflammation	18 (32)	3 (5)	0 (0)	21 (38)
Nausea	15 (27)	0 (0)	0 (0)	15 (27)
Thrombocytopenia	12 (21)	3 (5)	0 (0)	15 (27)
Constipation	11 (20)	1 (2)	0 (0)	12 (21)
Neutropaenia	5 (9)	0 (0)	0 (0)	5 (9)
Pneumonitis*	0 (0)	1 (3)	0 (0)	1 (3)

*attributed to radiation therapy administered prior to enrolment

Table 4-2. Summary of common and therapeutically relevant adverse events (n=56).

Treatment Duration

Participants received a median of 5 (interquartile range, 3–6) cycles of 177Lu-PSMA-617 and NOX66; 96% (54/56) received at least 2 cycles, and 46% (26/56) completed all 6 cycles. Of the 30 participants who ceased treatment before completing 6 cycles, 2 participants ceased because of exceptional responses, and the other patients ceased because of progressive disease (46%, n = 26), withdrawal of consent (2%, n =1), or inability to continue the study because of COVID-19 travel restrictions (2%, n = 1). One participant ceased NOX66 because of grade 2 anal inflammation but continued 177Lu-PSMA-617. No participants ceased LuPSMA-617 because of toxicity.

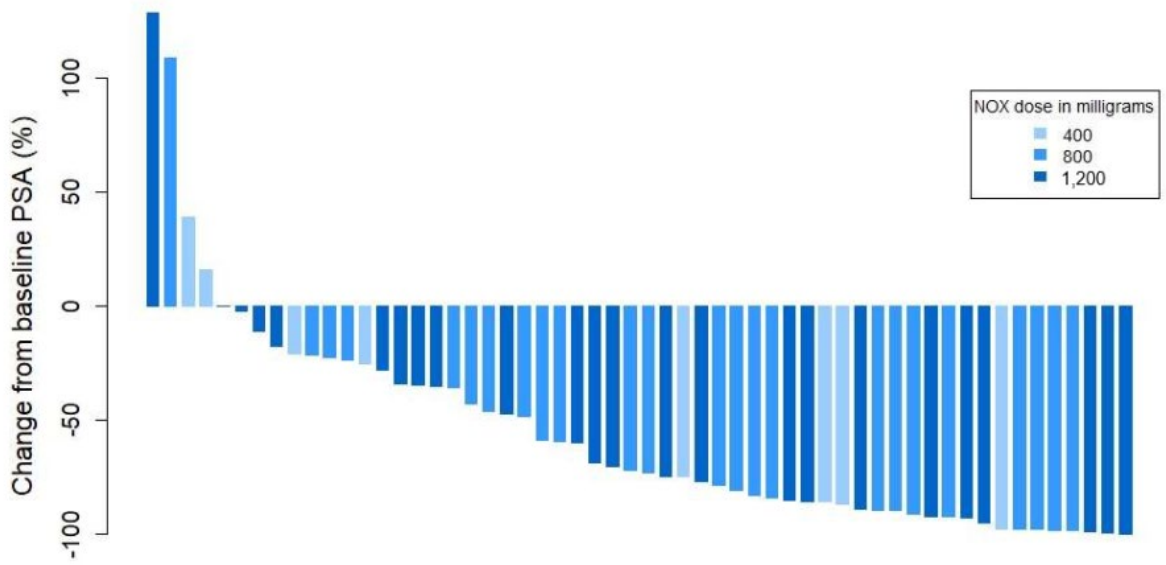

Figure 4-2. Waterfall plot of best PSA responses as indicated by maximum percentage change from baseline at any time point. NOX = NOX66.

Treatment Response

At a median follow-up of 21.8 mo, PSA50 occurred in 61% (34/56; 95% CI, 47%–74%), whereas any decline in PSA occurred in 86% (48/56; 95% CI, 74%–94%). The waterfall plot of best PSA responses at any time point is shown in Figure 2. At the time of this analysis, 91% (51/56) of participants have had PSA progression and 66% (37/56) have died. The median PSA PFS was 7.5 mo (95% CI, 5.9–9.0 mo) (Fig. 3A), and the median OS was 19.7 mo (95% CI, 9.5–30.0 mo) (Fig. 3B).

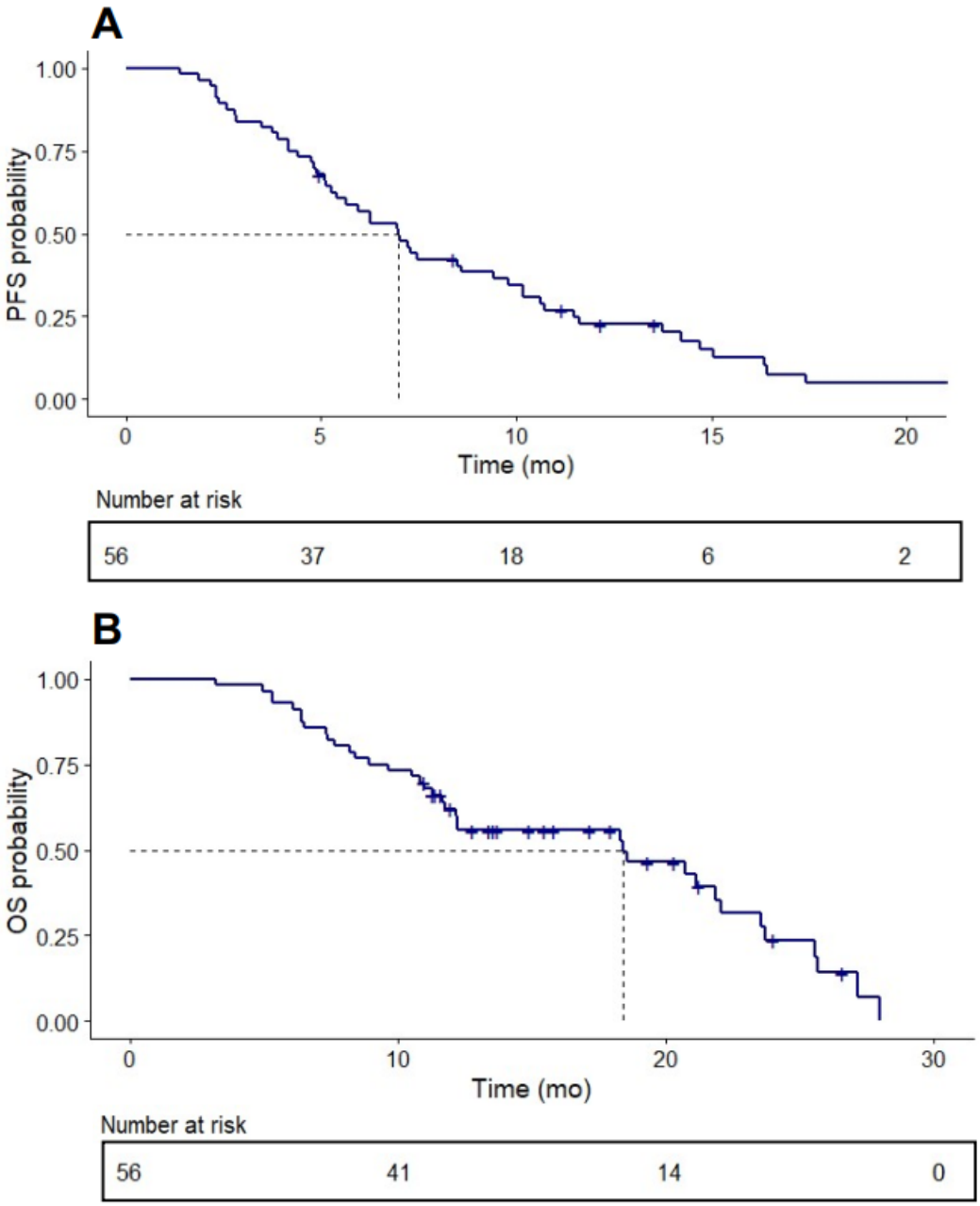

Figure 4-3. Kaplan–Meier curves for PSA progression-free survival (A) and OS (B).

Quality of Life

The baseline brief pain inventory assessment (short form) was completed by 95% (53/56) of the men. The mean worst pain score at baseline was 4.21 (range, 0–10;

SD, 2.99). Fifty-six percent (29/52) of the men recorded a worst pain score of at least 4, and of these, 41% (12/29) experienced pain palliation at any time point.

The baseline XeQoLS assessment was completed by 48 (86%) of 56 men at baseline, with serial results at cycle 3 (48/56) and cycle 6 (26/48). There was no significant difference in XeQoLS scores between baseline and cycle 3, but a statistically significant difference was found between baseline and cycle 6 (P = 0.04). There were no differences in XeQoLS scores among the 3 dose levels.

Potential Prognostic Factors

We performed exploratory univariable analysis to identify potential markers of PSA50 and OS.

Quantitative PET Imaging Markers.

Comparative screening imaging characteristics are detailed in Table 1. A higher tumor volume on PSMA PET was associated with a lower likelihood of achieving PSA50 (odds ratio [OR], 0.41 [95% CI, 0.19–0.87]; P = 0.02) and shorter OS (HR, 2.18 [95% CI, 1.36–3.51]; P = 0.001). PSMA SUVmean was associated with an increased likelihood of achieving PSA50 (OR, 1.57 [95% CI, 1.12–2.19]; P = 0.008). A higher 18F-FDG–avid tumor volume at baseline was associated with a worse OS (HR, 3.02 [95% CI, 1.04–8.79]; P = 0.04). The presence of visceral metastases was also associated with a worse OS (HR, 2.35 [95% CI, 1.06–5.20]; P = 0.04).

Impact of Prior Treatments on Outcome.

The most common treatment immediately before enrollment in the trial was cabazitaxel (70%, 39/56). Receiving either chemotherapy or ASI immediately before

the trial did not predict treatment response or survival. Similarly, the duration of chemotherapy did not predict a response to therapy. However, an ASI treatment duration of more than 12 months was significantly associated with improved OS (HR, 0.45 [95% CI, 0.22–0.91]; P = 0.03).

Blood-Based Markers.

A higher baseline level of hemoglobin was associated with higher odds of PSA50 (OR, 1.05 [95% CI, 1.01–1.10]; P = 0.03) and improved OS (HR, 0.96 [95% CI, 0.93–0.99]; P = 0.004). Other known prognostic markers, including baseline alkaline phosphatase, PSA, and use of opioid analgesia, did not correlate with outcome.

Thirty-five patients had androgen receptor splice variant 7 (ARV7) assessed before cycle 1; of these, 9 (26%) were ARV7-positive. Two patients remained positive at cycle 3, and 2 patients became positive while on treatment. A total of 11 patients had ARV7 detected at any time point. The presence of ARV7 at any time point was not significantly associated with treatment response or survival.

Multivariable Analysis of Potential Prognostic Factors.

A higher PSMA mean intensity score (SUVmean) (OR, 1.61 [95% CI, 1.12–3.32]; P = 0.01) and a lower PSMA tumor volume (OR, 0.42 [95% CI, 0.18–0.94]; P = 0.04) remained predictive of PSA50, whereas PSMA tumor volume (HR, 2.19 [95% CI, 1.38–3.46]; P = 0.001) predicted a worse OS (Fig. 4). The only clinical parameter predictive of survival was treatment with ASI for more than 12 mo (HR, 0.42 [95% CI, 0.20–0.87]; P = 0.02). Baseline 18F-FDG tumor volume, presence of visceral disease, and hemoglobin did not remain independently predictive of outcome (Tables 3 and 4).

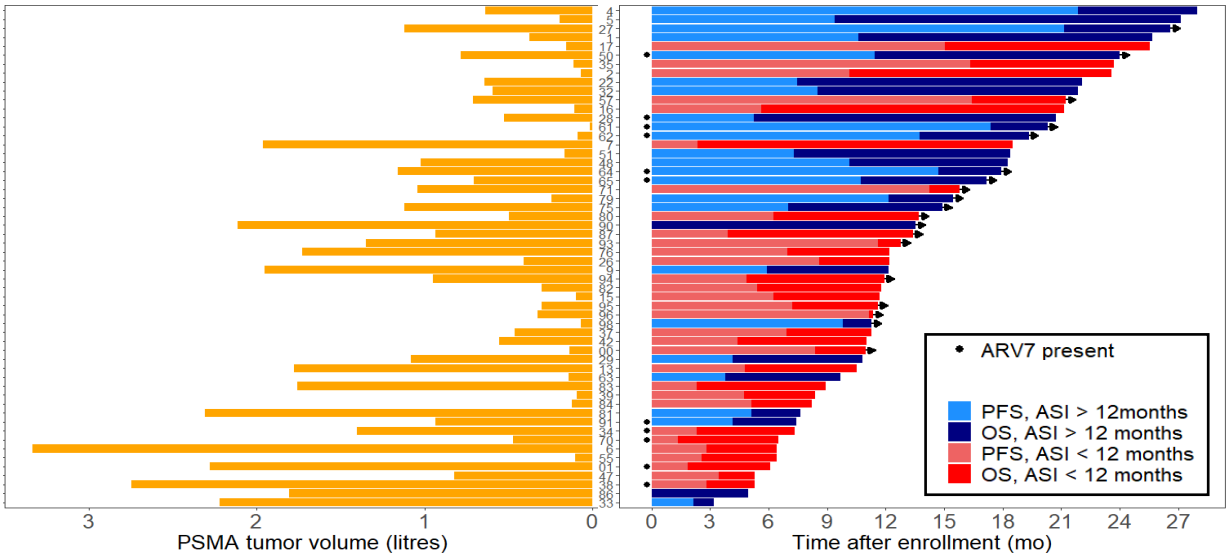

Figure 4-4. Graphical representation of important markers of OS. (Right) Swimmer plot showing individual-patient PFS and OS. (Left) Graph showing corresponding baseline tumor volume for each patient.

Variable	**LASSO Odds ratio**	**Multivariable logistic regression Odds ratio (95% CI) [p-value]**	**Backward elimination model Odds ratio (95% CI) [p-value]**
PSMA TV	0.73	0.47 (0.20-1.09) [0.08]	0.42 (0.18-0.94) [0.04]
PSMA SUV_{mean}	1.20	1.61 (1.10-2.34) [0.01]	1.61 (1.12-2.32) [0.01]
Haemoglobin	1.02	1.04 (0.99-1.10) [0.12]	NA

Table 4-3. Final multivariable model for the association of baseline markers with PSA response ≥ 50% (PSA50).

Variable	LASSO Hazard ratio	Multivariable Cox regression Hazard ratio (95% CI) [p-value]	Backward elimination model Hazard ratio (95% CI) [p-value]
PSMA TV	1.67	2.05 (1.19-3.53) [0.009]	2.19 (1.381-3.463) [0.001]
ASI > 12 months	0.70	0.56 (0.24-1.31) [0.56]	0.42 (0.202-0.869) [0.02]
FDG tumour volume (litres)	NA	0.99 (0.25-3.98) [0.99]	NA
Haemoglobin	0.99	0.98 (0.95-1.02) [0.30]	NA
Presence of visceral disease	1.499	2.01 (0.89-4.53) [0.09]	NA

Table 4-4. Final multivariable model for the association of baseline markers with overall survival

DISCUSSION

PSMA-targeted radionuclide therapy is emerging as a new treatment paradigm in men with mCRPC. The randomized TheraP trial demonstrated a significantly improved treatment response (PSA50), PFS, and quality-of-life parameters for 177Lu-PSMA-617, compared with cabazitaxel chemotherapy, in mCRPC. However, whereas results from TheraP are encouraging, PFS remains short, with a median of

5.1 mo (95% CI, 3.4–5.7) (*3*). We postulate that deepening and prolonging responses to 177Lu-PSMA-617 therapy for men with mCRPC may be possible by targeting intracellular resistance mechanisms to maximize treatment effect. This article reports the results of a dose escalation trial of 177Lu-PSMA-617 with a radiation sensitizer (NOX66) and evaluates potential predictive markers of response to PSMA-targeted therapy.

Treatment response rates to the 177Lu-PSMA-617 and NOX66 combination were high with a 61% PSA50, even though most men in this trial had high-volume disease, baseline anemia, high baseline opiate requirements, and 95% had undergone 2 lines of taxane therapy. Despite these high-risk features, the treatment response rate is in line with previous prospective single-center trials and the TheraP study (range, 36%–66%) (*2-4*). Further, PFS and OS were longer than those reported in previous studies with 177Lu-PSMA- 617 and longer than those reported using alternative treatments after taxane chemotherapy (*4,23*). These results are encouraging for men with ASI and taxane-refractory mCRPC, but a randomized trial—possibly with less stringent imaging enrollment criteria—will be required to determine whether these results are due to the novel treatment combination or to patient selection.

NOX66 was included in the trial as a potential tumor-specific radiation sensitizer that binds to external NADH oxidase 2, a tumor-specific enzyme inducing apoptosis and inhibiting topoisomerase II and demonstrating radiation sensitization in preclinical models (*7*). We did not find an association between an increasing dose of NOX66 and PSA50 or OS. However, the role of NOX66 in the study was as a radiation sensitizer, rather than having a direct effect on therapy, and it may be that either the lower dose of NOX66 was sufficient to induce radiation sensitivity or the impact of

NOX66 was limited. The safety of combination therapy with 177Lu-PSMA-617 and NOX66 has been previously reported for the first 2 cohorts of the LuPIN trial and was confirmed in this study (*24*).

Predictors of treatment response are important to further improve PSMA-targeted therapy. Men were screened for this study with molecular imaging, with a requirement for an SUVmax of at least 15 on PSMA PET and no sites of 18F-FDG/PSMA PET mismatch. An SUVmax of at least 15 has been previously reported to stratify men into responders and nonresponders for 177Lu-PSMA-617 therapy (*2*). Hence it is not surprising that PSMA SUVmax was not predictive of a treatment response in this study. However, PSMA SUVmean was an independent predictor of treatment response in this study and previously (*10*). A relationship between a higher SUVmean and improved clinical outcomes is biologically plausible. Intra- and interlesional heterogeneity of PSMA is common in mCRPC, and high heterogeneity of expression is likely to impact treatment response (*25*). SUVmean is likely a better indicator of lesional heterogeneity than is SUVmax. Further, dosimetry studies have shown that SUVmean correlates with the mean absorbed radiation dose and treatment response (*13*). Although SUVmean requires quantitative analysis, its repeated association with treatment response suggests that it may have a future role as a predictive biomarker for PSMA-targeted radionuclide therapy.

PSMA tumor volume at baseline was the strongest independent predictor of treatment response and was also prognostic for OS. 18F-FDG tumor volume was also prognostic, but not independently of other variables. Essentially, men with higher tumor volumes responded poorly to treatment. This finding agrees with retrospective analyses of men undergoing 177Lu-PSMA-617 therapy (*12,26*) and raises questions about the timing of PSMA- targeted therapy in men with mCRPC,

suggesting that earlier referral for treatment after prior treatment failure may both improve treatment responses and prolong survival.

The duration of response to prior therapies may help predict the treatment response to PSMA-targeted agents and OS. We found that men with a shorter duration of response to ASI (<12 mo) had a worse OS, though the depth of the treatment response was not affected. By contrast, the duration of the response to chemotherapy, or whether the patient received chemotherapy or ASI immediately before the trial, was not predictive of either survival or response.

This study enrolled a population of heavily pretreated men with mCRPC; therefore, the identified prognostic markers may not be generalizable to other stages of prostate cancer. Larger studies are needed to validate the prognostic markers identified in this study.

CONCLUSION

177Lu-PSMA-617 in combination with NOX66 is a safe treatment for heavily pretreated men with mCRPC, with encouraging results that warrant further evaluation. PSMA SUVmean and tumor volume merit further investigation as imaging markers of treatment response and survival.

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5 The Prognostic Value of Posttreatment 68Ga-PSMA-11 PET/CT and 18F-FDG PET/CT in Metastatic Castration-Resistant Prostate Cancer Treated with 177Lu-PSMA-617 and NOX66 in a Phase I/II Trial (LuPIN).

5.1 Overview

This chapter is a published manuscript aimed at nuclear medicine and oncology clinicians and researchers. The study used clinical and PET imaging data collected as part of a phase I/II trial. The study measured quantitative imaging parameters on baseline and post-treatment 68Ga-PSMA and 18F-FDG PET. The association of overall survival with the post-treatment change in imaging indices was examined.

This research was originally published in the Journal of Nuclear Medicine.

Publication details

Pathmanandavel, S., Crumbaker, M., Nguyen, A., Yam, A. O. W., Wilson, P., Niman, R., Ayers, M., Sharma, S., Eu, P., Martin, A. J., Stockler, M. R., Joshua, A. M., & Emmett, L. (2022). The prognostic value of post-treatment PSMA and FDG PET/CT in metastatic, castration-resistant prostate cancer treated with 177Lu-PSMA-617 and NOX66 in a phase I/II trial (LuPIN). Journal of Nuclear Medicine 2023 Vol. 64 Issue 1 Pages 69-74.

Contributions of the authors

SP: research proposal and concept development, data acquisition, data analysis, interpretation of results, drafted and revised the manuscript.

MC: contributed to the data acquisition, data analysis, interpretation of results, helped revise the manuscript.

AY, AN, PW, RN, MA, SS, PE, AMJ: contributed to the data acquisition, and helped revise the manuscript.

AJM, MS: data analysis, interpretation of results, and helped revise the manuscript.

LE: research proposal and concept development, interpretation of results, and helped revise the manuscript.

5.2 Manuscript

ABSTRACT

177Lu-PSMA-617 therapy has shown high prostate-specific antigen (PSA) response rates in men with metastatic castration-resistant prostate cancer. However, early treatment resistance is common. This LuPIN substudy aimed to determine the prognostic value of posttreatment quantitative PET for PSA progression-free survival (PFS) and overall survival (OS) with 177Lu-PSMA-617 therapy.

Methods: Fifty-six men with progressive metastatic castration-resistant prostate cancer were enrolled in the LuPIN trial and received up to 6 doses of 177Lu-PSMA-617 and a radiation sensitizer (NOX66). 68Ga-PSMA-11 and 18F-FDG PET/CT, diagnostic CT, and bone scanning were performed at study entry and exit. Quantitative analysis tracked change in total tumor volume (TTV) and SUV. Univariable and multivariable analyses were conducted to examine the association of change in TTV (continuous and >30%), SUVmax, PSA, and radiographic progression with PSA PFS and OS.

Results: All men (37/56) who underwent both screening and post-treatment molecular imaging were analyzed; 70% (26/37) had a PSA response of more than 50%. Median PSA PFS was 8.6 mo, and median OS was 22 mo. Clinical progression had occurred at trial exit in 54% (20/37). In response to treatment, a reduced PSMA SUVmax was demonstrated in 95% (35/37) and a reduced PSMA TTV in 68% (25/37). An increase in PSMA TTV by at least 30% was associated with worse OS (median, 10.2 vs. 23.6 mo; P = 0.002). Change in PSMA SUVmax was not associated with PSA PFS or OS. 18F-FDG SUVmax was reduced in 51% (18/35)

and 18F-FDG TTV in 67% (22/35). An increased 18F-FDG SUVmax was associated with worse OS (median, 20.7 vs. 25.7 mo; P < 0.01). An 18F-FDG TTV increase by more than 30% was associated with a short PSA PFS (median, 3.5 vs. 8.6 mo; P < 0.001) but not OS. Both PSA and radiographic progression were associated with shorter OS (median, 14.5 vs. 25.7 mo [P < 0.001] and 12.2 vs. 23.6 mo [P = 0.002]). On multivariable analysis, only increased PSMA TTV and PSA progression remained independently prognostic of OS (hazard ratio, 5.1 [95% CI, 1.5–17.1; P = 0.008] and 3.5 [95% CI, 1.1–10.9; P = 0.03], respectively).

Conclusion: Change in quantitative PSMA TTV has strong potential as a prognostic biomarker with 177Lu-PSMA-617 therapy, independent of 18F-FDG PET parameters, PSA, or radiographic progression. Further research into the value of posttreatment PET as an imaging biomarker is warranted.

BACKGROUND

In the VISION trial, 177Lu-PSMA-617–targeted therapy improved overall survival (OS) and progression-free survival (PFS) in metastatic castration-resistant prostate cancer when compared with the standard of care, and in the TheraP trial it yielded a higher prostate-specific antigen (PSA) response rate and PSA PFS than second-line chemotherapy with cabazitaxel (*1,2*). However, further work is needed to deepen treatment response and prolong survival. 68Ga-PSMA-11 PET/CT (68Ga-PSMA PET) and 18F-FDG PET/CT (18F-FDG PET) have been used as screening tools in prospective trials to select patients most likely to respond to 177Lu-PSMA-617–targeted treatments (*1-4*). Less work has been done using molecular imaging to monitor treatment response to 177Lu-PSMA-617 therapy (*5-8*). Preclinical studies have confirmed that there is considerable interpatient and intrapatient heterogeneity of PSMA expression (*9,10*). We hypothesized that an increase in uptake or tumor volume on 68Ga-PSMA PET or 18F-FDG PET might have potential as a prognostic biomarker in men being treated with 177Lu-PSMA-617.

In this study, we aimed to determine whether changes in total tumor volume (TTV) and SUV on both 68Ga-PSMA PET and 18F-FDG PET correlate with clinical outcomes in a prospective trial of treatment with 177Lu-PSMA-617.

MATERIALS AND METHODS

This was an imaging substudy of the LuPIN trial. The LuPIN trial is a prospective single center, phase I/II dose escalation and expansion trial of combining 177Lu-PSMA-617 with NOX66. The study enrolled men with metastatic castration-resistant prostate cancer previously treated with both at least 1 line of taxane chemotherapy

and an androgen signaling inhibitor. The clinical results have been previously published (*11,12*). St. Vincent's Hospital institutional review board approved the study protocol (HREC/ 17/SVH/19 ACTRN12618001073291), and all participants provided written informed consent.

Screening

Men with progressive metastatic castration-resistant prostate cancer, based on either conventional imaging (CT and bone scanning) or a rising serum concentration of PSA based on Prostate Cancer Working Group 3 criteria (*13*), were eligible for screening. The men underwent screening with 18F-FDG PET and 68Ga-PSMA PET, bone scanning, and CT of the chest, abdomen, and pelvis. Patients were eligible if they had an SUVmax of more than 15 on 68Ga-PSMA PET at 1 or more sites, an SUVmax of more than 10 at all measurable sites, and no 18F-FDG PET avidity without corresponding PSMA uptake. All men with 68Ga-PSMA PET and 18F-FDG PET at both baseline and after treatment were included in this substudy.

Study Treatment

All men received up to 6 doses of 177Lu-PSMA-617 at 6-wk intervals, with 3 dose-escalated cohorts of NOX66. NOX66 was provided as a dose-appropriate suppository taken from days 1 to 10 after each 177Lu-PSMA-617 injection. All cohorts were administered 7.5 GBq of 177Lu-PSMA-617 on day 1 via a slow intravenous injection. The PSMA-617 precursor (AAA Novartis) was radiolabeled to no-carrier- added 177Lu-chloride according to the manufacturer's instructions. Quality control tests for radionuclide and radiochemical purity were performed using high-pressure liquid chromatography and thin-layer chromatography. NOX66

suppositories were administered at 400-, 800-, and 1,200-mg doses per a dose escalation protocol (*11*).

Imaging Procedures and Acquisition

68Ga-PSMA PET and 18F-FDG PET were performed at baseline (screening) and after treatment (6 wk after completing all 6 cycles or when treatment ceased earlier because of clinical progression). 68Ga-HBEDD-CC-PSMA-11 was produced on-site in a manner compliant with good-laboratory-practice procedures using a TRASIS automated radio-pharmacy cassette. 18F-FDG was produced off-site commercially under good-manufacturing-practice–compliant conditions. Radiopharmacy quality control was undertaken using a high-pressure liquid chromatography method. Patients were injected with a 2.0 MBq/kg dose of 68GaPSMA-11 and a 3.5 MBq/kg dose of 18F-FDG, with matched imaging parameters (dose, time after injection, and imaging protocols) for each patient. All PET/CT imaging was undertaken using a Phillips Ingenuity time-of-flight PET/64-slice CT scanner. An unenhanced low-dose CT scan was performed 60 min after tracer injection. Immediately after CT scanning, a whole-body PET scan was acquired for 2 min per bed position. The emission data were corrected for randoms, scatter, and decay. Diagnostic contrast-enhanced CT of the chest, abdomen, and pelvis, and a whole-body bone scan, were performed at baseline and after treatment.

Imaging Analysis

All 68Ga-PSMA and 18F-FDG PET scans (screening and after treatment) were analyzed semiquantitatively by a nuclear medicine physician using MIM Software and a standardized semiautomated workflow to delineate regions of interest with a minimum SUV cutoff of 3 for 68Ga-PSMA PET and an SUV cutoff equal to the blood

pool mean intensity plus 1.5 SDs for 18F-FDG PET. All lesions identified quantitatively were manually reviewed and physiologic uptake or scatter removed. Wholebody quantitation derived total metabolic tumor volume, SUVmax, and SUVmean for both 18F-FDG PET and 68Ga-PSMA PET (MIM Software) (*14*). A nuclear medicine physician visually assessed both the quantified and the nonquantified PET images to identify potential sites of 18F-FDG PET–positive/68Ga-PSMA PET–negative progressive disease between the screening and posttreatment scans.

Statistical Analyses

We measured PSA declines from baseline (absolute and >50%) at any time point, PSA PFS as defined by Prostate Cancer Working Group 3 criteria, radiographic progression defined by RECIST 1.1 and Prostate Cancer Working Group 3 criteria, and OS (*13,15*). Time-to-event endpoints (PSA PFS and OS) were defined as the interval from the date of enrolment to the event date, or the date last known to be event-free (at which point the observation was censored). A 2-sided exact binomial 95% CI was calculated for PSA response rates. The Kaplan–Meier method was used to characterize time-to-event endpoints and estimate medians (presented with 95% CIs). We correlated changes in TTV, SUVmax, and SUVmean for 68Ga-PSMA PET and 18F-FDG PET with time-to-event outcomes, using univariable and multivariable Cox proportional-hazards regression models. P values below 5% were considered significant. Analyses were performed using R (version 4.0.5) and SPSS (version 25).

RESULTS

Patient Characteristics

Patient characteristics are summarized in Table 1. Thirty-seven of 56 (66%) men on the LuPIN trial had both baseline screening and posttreatment imaging (6 wk after completion of 6 cycles of treatment, or earlier if the trial was exited because of clinical progression). Of these, 68% (25/37) had posttreatment imaging after completing all 6 cycles of 177Lu-PSMA-617 plus NOX66, 3% (1/37) after 5 cycles, 14% (5/37) after 4 cycles, 14% (5/37) after 3 cycles, and 3% (1/37) after 2 cycles. Nineteen of 56 (34%) did not have exit imaging because of illness (12/19), travel restrictions (3/19), or unknown reasons (3/19).

Patient Characteristics	Sub-study
Age (years)	68 (65-74)
ECOG	
0 or 1	32 (86)
2	5 (14)
PSA at screening (ug/L)	91 (41.3-380)
Haemoglobin (Normal Range 130-180 g/L)	122 (112-131)
Alkaline Phosphatase (NR 30-100 U/L)	124 (83-359)
Prior Systemic treatments	
LHRH agonist/antagonist	37 (100%)
Chemotherapy	37 (100%)
Docetaxel	37 (100%)
Cabazitaxel	34 (92%)
Androgen Signalling Inhibitor	37 (100%)
Cycles of ^{177}LuPSMA-617 administered	6 (4-6)
Exit diagnostic CT and bone scan	34 (92%)

Numbers are presented as absolute counts (percentage) or median (interquartile range).

Table 5-1. Patient characteristics.

Clinical Outcomes

The median reduction in PSA was 77% (interquartile range, 34%–92%), and 70% (26/37) of patients had a PSA response of more than 50%. With a median follow-up of 26 mo, the median PSA PFS was 8.6 mo (95% CI, 5.6–11.6) and the median OS was 22 mo (95% CI, 18.6–25.6). PSA or clinical progression had occurred in 54% (20/37) at the time of exit imaging, whereas 46% (17/37) had no PSA progression after the full 6 cycles of treatment. At exit, 92% (34/37) had conventional imaging (CT and bone scanning). Radiographic progression at the time of exit imaging was identified in 18% (6/34). On univariable analysis, PSA progression and radiographic progression at trial exit were also associated with significantly worse PSA PFS and OS (Table 2).

68Ga-PSMA PET Quantitation

Quantitative 68Ga-PSMA PET SUVmax and SUVmean were reduced in 95% of men independently of whether they had PSA progression at the time of exit imaging (absolute change in PSMA SUVmax: median, -26 [interquartile range, -40 to -13]; absolute change in PSMA SUVmean: median, -3 [interquartile range, -5 to -2]). There was no correlation between an increase in PSMA SUVmax and either PSA PFS or OS (Table 2).

Univariable analysis	OS	PSA-PFS
PSMA-TTV*	6.2 (2.0-19.2) [0.002]	2.9 (1.4-6.1) [0.01]
Increase in PSMA SUVmax †	1.9 (0.2-14.4) [0.56]	1.3 (0.3-5.6) [0.71]
FDG-TTV*	2.7 (1.1-6.2) [0.02]	3.1 (1.4-6.8) [0.005]
Increase in FDG SUVmax †	3.0 (1.2-7.3) [0.02]	3.0 (1.4-6.4) [0.01]
PSA progression †	5.8 (2.1-16.1) [<0.001]	5.0 (2.3-10.7) [<0.001]
Radiographic progression †	5.4 (1.8-16) [0.003]	4.4 (1.6-12) [0.004]

Hazard ratios are presented as HR (95%CI) [p value]. * increase in litres, continuous variable † at trial exit

Table 5-2. Univariable Cox regression analysis for association with PSA-PFS and OS. TTV = total tumour volume

The median change in PSMA TTV was -0.64 liters (interquartile range, -0.29 to +0.07). PSMA TTV was increased in 32% (12/37). Any increase in PSMA TTV was associated with shorter PSA PFS (hazard ratio [HR], 2.9 [95% CI, 1.4–6.1]; P = 0.01) and OS (median, 12.2 vs. 25.5 mo; HR, 6.2 [95% CI, 2.0–19.2]; P < 0.01) (Fig. 1). An increase in PSMA TTV by at least 30% was significantly associated with worse OS (HR, 6.0 [95% CI, 1.9–19.2]; P = 0.002), whereas association with PSA PFS was not significant (Fig. 2).

All 12 patients with an increasing PSMA TTV had an SUVmax of more than 15 at trial exit (above trial entry criteria), compared with 52% (13/25) of those with a reduced PSMA TTV.

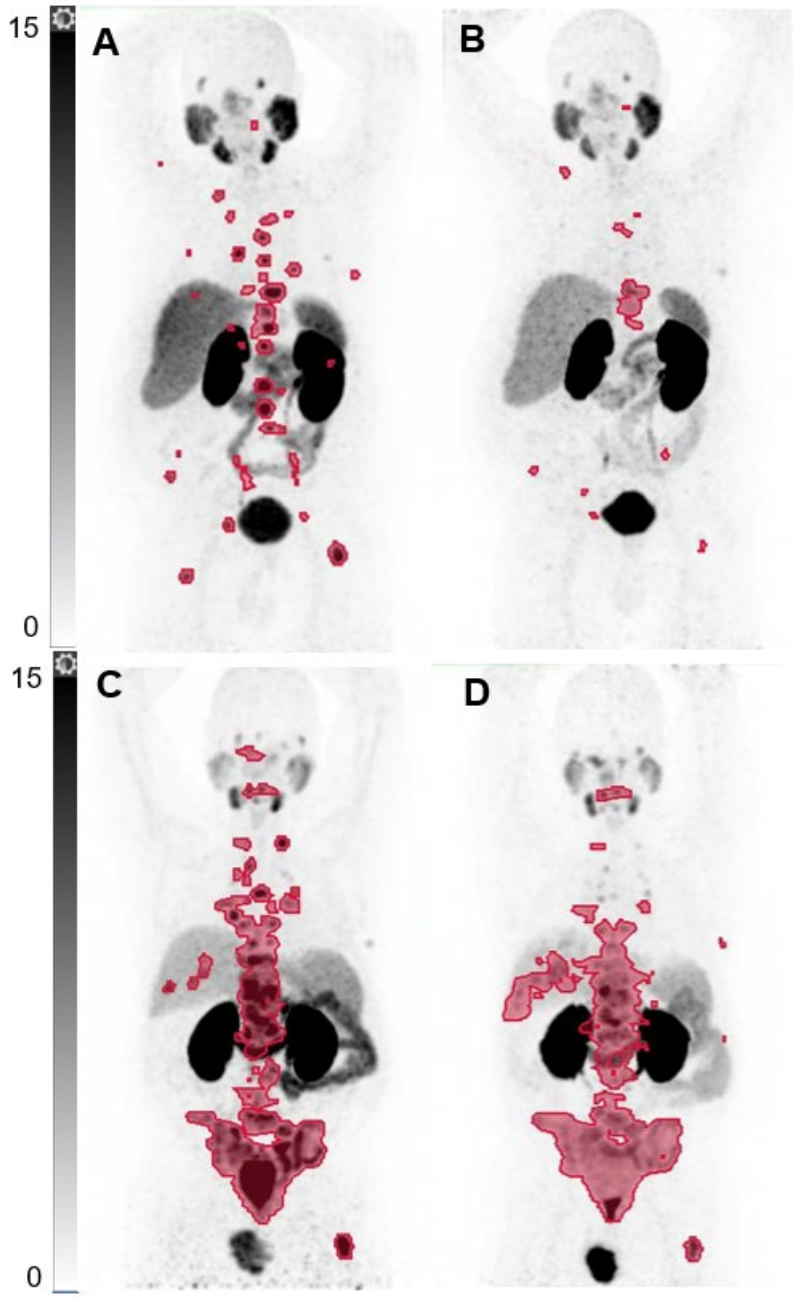

Figure 5-1. (A and B) Quantitative analysis for patient with reduced PSMA TTV between baseline (A) and after treatment (B). (C and D) Quantitative analysis for progressing patient at baseline (C) and after treatment (D). In patients with high-volume disease, it can be difficult to visually identify extent of volume change. In this second case, volume increase is 25%.

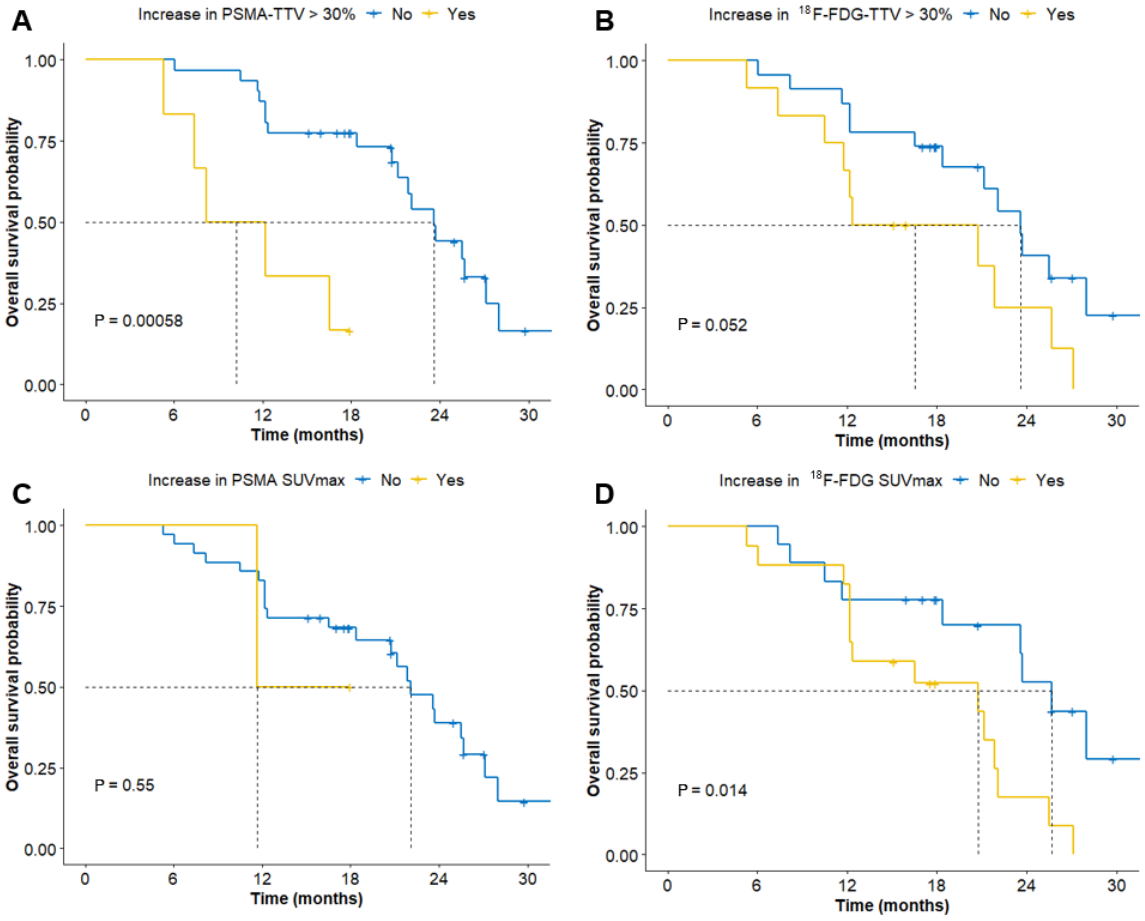

Figure 5-2. Kaplan–Meier curves for OS stratified by increase in PSMA TTV ≥ 30% from baseline (A), increase in 18F-FDG TTV ≥ 30% from baseline (B), increase in PSMA SUVmax from baseline (C), and increase in 18F-FDG SUVmax from baseline (D).

18F-FDG PET Quantitation

Analysis of screening and posttreatment 18F-FDG PET demonstrated that 51% (18/35) had a reduced 18F-FDG SUVmax (median absolute change, 0.1; interquartile range, 24 to 11) and that 66% (23/35) had a reduced 18F-FDG

SUVmean (median absolute change, 20.4; interquartile range, 21 to 10.4) in response to treatment. An increase in 18F-FDG SUVmax was associated with worse PSA PFS (HR, 3.0 [95% CI, 1.4–6.4]; P 5 0.01) and OS (HR, 3.0 [95% CI, 1.2–7.3]; P = 0.02). The median change in 18F-FDG TTV was -0.01 liters (interquartile range, -0.05 to +0.02). 18F-FDG TTV was increased in 37% (13/35) at trial exit. Any increase in 18F-FDG TTV was associated with worse PSA PFS (HR, 3.1 [95% CI, 1.4–6.8]; P = 0.005) and OS (median, 16.2 vs. 23.1 mo; HR, 2.7 [95% CI, 1.1–6.2]; P = 0.02). An increase in 18F-FDG TTV by at least 30% was significantly associated with worse PSA PFS (HR, 2.7 [95% CI, 1.2–6.0]; P = 0.01) but was not significantly associated with OS (Fig. 2).

No 18F-FDG–positive/68Ga-PSMA PET–negative progressive sites were identified in this cohort at the time of exit imaging. One patient had a significant increase in 18F-FDG–avid volume at 1 site, whereas PSMA tumor volume and SUVmax were reduced (Fig. 3).

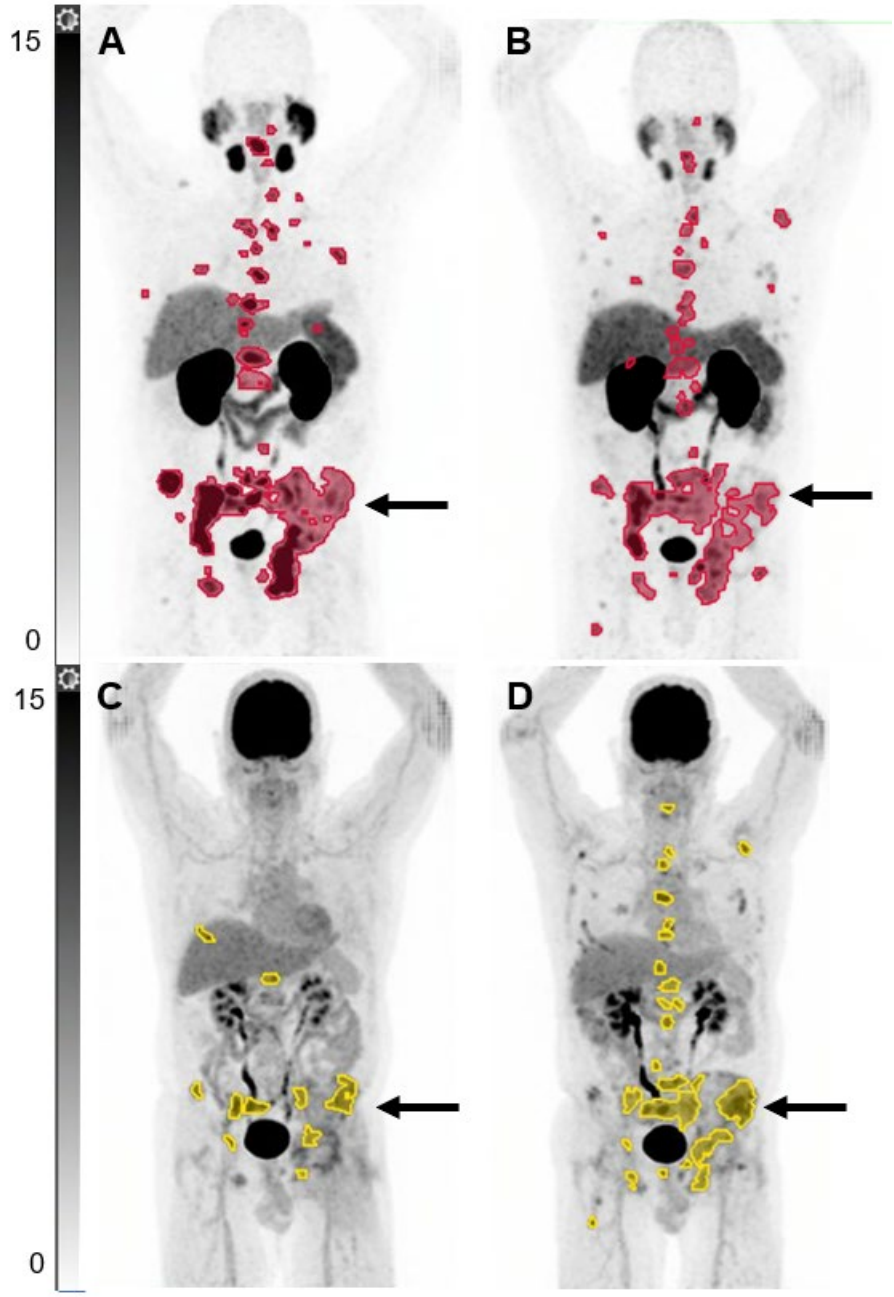

Figure 5-3. Quantitative analysis of baseline and posttreatment PSMA and 18F-FDG PET images from same patient: baseline 68Ga-PSMA PET (A), posttreatment 68Ga-PSMA PET (B), baseline 18F-FDG PET (C), and posttreatment 18F-FDG PET (D). Arrows indicate lesion in iliac bone that reduced in volume and intensity on 68Ga-PSMA PET but increased in volume and intensity of 18F-FDG PET.

Molecular Response Patterns and Patient Outcomes

Multivariable analysis including change in PSMA TTV, 18F-FDG TTV, and 18F-FDG SUVmax, as well as PSA progression and radiographic progression, found that only PSMA TTV and PSA progression remained independently prognostic of OS (HR, 5.1 [95% CI, 1.5–17.1; P = 0.008] and HR 3.5 [95% CI, 1.1–10.9; P = 0.03], respectively) (Table 3).

Variable	**Hazard ratio**
Increase in PSMA-TTV †	5.1 (1.5-17.1) [0.008]
Increase in FDG-TTV †	1.04 (0.4-2.9) [0.93]
Increase in FDG SUVmax †	1.3 (0.4-4.5) [0.67]
PSA progression†	3.5 (1.1-10.9) [0.03]
Radiographic progression†	1.8 (0.5-6.0) [0.36]

Hazard ratios are presented as HR (95%CI) [p value]. † at trial exit

Table 5-3. Multivariable Cox regression analysis for association of clinical and imaging parameters with OS. TTV = total tumour volume

DISCUSSION

This study has found that increasing TTV on posttreatment 68Ga-PSMA PET identifies early disease progression and shorter OS independently of PSA, raising its

potential for use as a prognostic biomarker. Metastatic castration-resistant prostate cancer is characterized by phenotypic and molecular heterogeneity, with marked PSMA heterogeneity previously demonstrated at both an imaging and a cellular level (*9,10*). Although the VISION and TheraP trials have found high treatment responses and improved quality-of-life parameters, the duration of treatment responses with 177Lu-PSMA-617 remains limited. Identifying effective predictive and prognostic biomarkers is critical to deepening and prolonging responses to PSMA-targeted treatments with appropriate combinations and judicious treatment sequencing.

A second key finding in this study is that in contrast to 18F-FDG PET, reduced PSMA SUVmax or SUVmean occurred in almost all patients in response to 177Lu-PSMA-617 therapy and was not predictive of either treatment response or OS. This lack of correlation between change in PSMA SUVmax or SUVmean and treatment outcomes has previously been shown. Kurth et al. found that PSMA intensity decreased in both clinically responding and progressing patients (*7,16*). Grubmuller et al. also found no correlation between change in whole-body PSMA SUVmean and OS in an analysis of posttreatment 68Ga-PSMA PET after 177Lu-PSMA-617 therapy. This lack of prognostic value of change in PSMA SUVmean and SUVmax with PSMA-targeted therapy is not unexpected. 177Lu-PSMA-617 preferentially targets highly PSMA-expressing cells, leading to persistent populations of low PSMA-expression disease that may be less responsive to treatment. The lack of predictive or prognostic value for change in PSMA SUVmean and SUVmax is important to highlight, as we intuitively use reduction in intensity (18F-FDG PET) to denote treatment response with systemic therapy (*17*).

We need to think differently when developing 68Ga-PSMA PET response criteria for PSMA-targeted therapy. Increasing PSMA TTV was an independent predictor of PSA

PFS and OS in this study. Similar to Gafita et al. and Grubmuller et al., we confirmed that an increase in quantitative PSMA TTV is a poor prognostic factor for OS (*7,18*). An increase in PSMA TTV by at least 30% was associated with poor OS, supporting the inclusion of this metric in the 68Ga-PSMA PET progression criteria (*19*).

However, accurate assessment of change in TTV visually can be difficult, especially in high-volume disease, and quantitative PET analysis may become an important tool in PSMA-targeted therapy. We found that patients with an increase in PSMA TTV at trial exit had a PSMA SUVmax of more than 15, significantly higher than in patients without progressive disease, and above a range at which PSMA-targeted therapy is expected to be effective. This finding may indicate that radiation resistance is an important mechanism of treatment failure. Further evaluation in conjunction with genetic analysis may help identify optimal treatment combinations in patients who currently have a limited treatment response to 177Lu-PSMA-617 alone.

18F-FDG PET was undertaken both at screening and at trial exit, with 18F-FDG PET screening parameters previously shown to be predictive of OS in this study cohort and other 177Lu-PSMA-617 trials (*1,12,20*). Although we found that an increase in 18F-FDG SUVmax and 18F-FDG TTV was associated with poor OS in univariable analysis, they did not remain significant on multivariate analysis. Further, the incidence of discordant progressive lesions (18F-FDG PET–positive/68Ga-PSMA PET–negative) was low, with only 1 patient having a significant increase in 18F-FDG–avid volume at 1 site, whereas PSMA TTV was reduced.

RECIST progression is the standard of care for identifying progressive disease on imaging and has a strong correlation with OS in prostate cancer (*21*). However, RECIST progression was less prognostic than change in either PSMA TTV or PSA

progression at study exit. The promising prognostic value for molecular imaging parameters suggests that more work needs to be done validating PET for treatment response in metastatic castration-resistant prostate cancer, potentially as an alternative to CT and bone scanning currently used in Prostate Cancer Working Group 3 criteria.

This study had several limitations. Its small sample size made it purely exploratory, and the findings will need to be validated in larger trials. The sample size also did not allow for a multivariable analysis incorporating other known prognostic factors. Additionally, selection of patients for this analysis was biased toward those well enough to complete posttreatment imaging, explaining the higher rate of at least 50% PSA decline and longer OS in this subset of patients than previously published for the trial. The interval between screening and posttreatment imaging was variable, with 32% exiting the trial early because of clinical progression.

Finally, PET quantitation software remains of limited availability and time-intensive to achieve accurate results. Further automation in quantitation is required to minimize the time required to derive reproducible results, in addition to harmonization and validation of quantitative methods. Nevertheless, the prognostic value of quantified PSMA TTV in this study suggests that investment in PET quantitation will yield significant clinical benefit.

CONCLUSION

Change in quantitative PSMA TTV has strong potential as a prognostic biomarker with 177Lu-PSMA-617 therapy, independently of 18F-FDG parameters, PSA, or

radiographic progression. Further research into the value of posttreatment PET as an imaging biomarker is warranted.

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6 Quantifying patterns of response and progression on PSMA PET/CT using a novel Traffic Light Workflow analysis method in men with metastatic castration-resistant prostate cancer within a phase 1/2 clinical trial of 177Lu-PSMA-617 and NOX66 (LuPIN).

6.1 Overview

This chapter is a manuscript aimed at nuclear medicine and oncology clinicians and researchers. The study used clinical and PET imaging data collected as part of a phase I/II trial. The study used a novel imaging analysis workflow to track and quantify lesional changes after 177Lu-PSMA-617 treatment. The overall response pattern was then divided into three response groups. The association between overall survival and the response pattern was assessment.

This manuscript has been submitted to the Journal of Nuclear Medicine.

Manuscript details

Pathmanandavel, S., Crumbaker, M., Nguyen, A., Yam, A. O. W., Wilson, P., Niman, R., Ayers, M., Sharma, S., Eu, P., Martin, A. J., Stockler, M. R., Joshua, A. M., & Emmett, L. Quantifying patterns of response and progression on PSMA PET/CT using a novel Traffic Light Workflow analysis method in men with metastatic castration-resistant prostate cancer within a prospective phase 1/2 clinical trial of 177Lu-PSMA-617 and NOX66 (LuPIN).

Contributions of the authors

SP: research proposal and concept development, data acquisition, data analysis, interpretation of results, drafted and revised the manuscript.

MC: contributed to the data acquisition, data analysis, interpretation of results, helped revise the manuscript.

AN, AY, PW, RN, MA, SS, PE, AMJ: contributed to the data acquisition, and helped revise the manuscript.

AJM, MS: data analysis, interpretation of results, and helped revise the manuscript.

LE: research proposal and concept development, interpretation of results, and helped revise the manuscript.

6.2 Manuscript

ABSTRACT

Purpose: 177Lutetium PSMA-617 (177Lu-PSMA-617) improves survival in metastatic, castration-resistant prostate cancer (mCRPC). Better tools are needed to measure response to 177Lu-PSMA-617, and to identify treatment resistance earlier, so that alternative treatment strategies can be sought. This study evaluated a quantitative workflow to track lesional changes on PSMA PET/CT that might be prognostic for overall survival following treatment with 177Lu-PSMA-617.

Methods: We performed quantitative analyses of baseline and post-treatment 68Ga-PSMA-11 PET images using a novel Traffic Light Workflow (TLW) to track lesional changes in tumour volume and SUVmax in men with mCRPC treated with up to six doses of 177Lu-PSMA-617 and a radiation sensitiser (NOX66) in the LuPIN trial. Individual lesions were classified as ‘reducing’ (≥30% decrease in volume), ‘stable’ (<30% change in volume/SUVmax), or ‘increasing’ (≥30% increase in volume/SUVmax, or new lesions). Overall patient responses were categorised as ‘responder’ (no ‘increasing’/new lesions), ‘low-volume progressor’ (≤50% of total tumour volume (TTV) classified as ‘increasing’), or ‘high-volume progressor’ (>50% of TTV classified as ‘increasing’). TLW response category, PSA response, and radiographic progression were correlated with OS. Stratification by TLW response category and Response Evaluation Criteria in PSMA PET (RECIP 1.0) were compared.

Results: Thirty-seven men had baseline and post-treatment PET imaging, 68% (25/37) after completing six cycles of 177Lu-PSMA-617 + NOX66, and 32% (12/37)

after completing 2-5 cycles of treatment. PSA decline > 50% was achieved in 70% (26/37) of participants. The median PSA-PFS was 8.6 months, and the median OS was 22 months. 54% (20/37) had disease progression at time of post-treatment imaging. On TLW analysis, 24% (9/37) were classified ‘responders’, and 41% (15/37) were ‘low-volume progressors’ and 35% (13/37) ‘high-volume progressors’. ‘Responders’ had longer OS than ‘low-volume progressors’ (median 17.7 vs 12 months, HR 1.4, 95%CI 0.4-4.6) or ‘high-volume progressors’ (median 7.5 months, HR 4.8, 95%CI 1.4-15.8, p=0.005). When classified by RECIP 1.0, 24% (9/37) demonstrated RECIP-PR, 51% (19/37) RECIP-SD, and 24% (9/37) RECIP-PD.

Conclusion: This study demonstrates the feasibility of classifying complex patterns of response with a novel, quantitative TLW. Further work is needed to validate the TLW and to optimise classification of responses that could be used to guide decisions about treatment.

INTRODUCTION

177Lutetium-PSMA-617 (177Lu-PSMA-617) is an active treatment in advanced prostate cancer and had a higher PSA response rate than chemotherapy with cabazitaxel in metastatic castration-resistant prostate cancer (mCRPC) (*1,2*). 68Ga-PSMA-11 (PSMA) PET/CT and 18F-fluorodeoxyglucose (FDG) have been used as screening tools for trials to help select participants most likely to respond to 177Lu-PSMA-617 (*2-5*). Less work has been done to evaluate molecular imaging as a method for monitoring responses to treatment with 177Lu-PSMA-617, and to characterize patterns of failure and response (*6,7*). Preclinical studies have confirmed that there is considerable inter-patient and intra-patient heterogeneity of PSMA expression in prostate carcinoma (*8,9*). Serial imaging with PSMA PET has the potential to track changes in PSMA expression and tumour volume, allowing a more nuanced characterization of response than with conventional imaging (computed tomography and whole body bone scintigraphy).

We report here the development and evaluation of a novel, quantitative imaging workflow for tracking changes in tumour volume and PSMA SUVmax at an individual lesional level, correlating patterns of response and progression to clinical outcomes in a clinical trial of 177Lu-PSMA-617 (*10*). We refer to this approach as the Traffic Light Workflow (TLW). We compared our TLW with the Response Evaluation Criteria in PSMA PET (RECIP) 1.0, and discuss future directions for PSMA PET response assessment criteria.

MATERIALS AND METHODS

The LuPIN trial is a single-centre, phase 1/2, trial with dose-escalation and expansion stages, evaluating the combination 177Lu-PSMA-617 and NOX66 in mCRPC previously treated with at least one line of taxane chemotherapy, and an androgen signalling inhibitor. The clinical results have been previously published (*10,11*). St Vincent's Hospital institutional review board approved the study protocol (HREC/17/SVH/19 ACTRN12618001073291) and all participants provided signed, written, informed consent.

Screening

Men with mCRPC that was progressing according to either conventional imaging or a rising PSA (based on Prostate Cancer Working Group 3 (PCWG3) criteria were eligible for screening (*12*). Prior treatment with at least one line of taxane chemotherapy (docetaxel and/or cabazitaxel) and an androgen signalling inhibitor (abiraterone and/or enzalutamide) were required for inclusion. Men underwent screening with FDG and PSMA PET/CT and conventional imaging. Eligibility required a Standardized Uptake Value (SUV) maximum > 15 on PSMA PET at ≥ 1 site, an SUVmax >10 at all measurable sites, and no FDG avidity without corresponding PSMA uptake.

Study Treatment

177Lu-PSMA-617 was administered via intravenous injection on the first day of a six week cycle for a maximum of six cycles. NOX66 was administered as a suppository

on day 1-10 of each cycle. All cohorts received 7.5 GBq of 177Lu-PSMA-617 via slow intravenous (IV) injection and NOX66 suppositories were administered at 400mg, 800mg and 1200mg doses as per a dose escalation protocol (*10*).

Imaging procedures and analysis

PSMA PET/CT scans were performed at baseline and post-treatment (after completing all six cycles or when treatment was ceased). Patients were injected with 2.0 MBq/kg 68Ga-PSMA with matched imaging parameters (dose, time post injection and imaging protocols) for each patient. All PET/CT imaging was undertaken using a Phillips Ingenuity TOF-PET/64 slice CT scanner. A non-contrast low dose CT scan was performed 60 minutes post tracer injection. Immediately after CT scanning, a whole-body PET scan was acquired for 2 minutes per bed position. The emission data were corrected for randoms, scatter, and decay.

Diagnostic contrast enhanced CT of the chest, abdomen, and pelvis, and whole-body bone scintigraphy were also performed at baseline and post-treatment.

Quantitative analysis

PET/CT scans were analysed semi-quantitatively using MIM EncoreTM, version 7.1 (MIM Software, Inc., Cleveland, USA). A standardised, semiautomated workflow delineated regions of interest with a minimum SUV cut-off of 3 for PSMA. After the semiautomated segmentation, a nuclear medicine physician removed areas of uptake that did not represent disease. All lesions were aggregated into a Total

Tumour Burden contour, and the total tumour volume (TTV), SUVmax, SUVmean, and total lesional activity were derived (*13*).

A second MIM Workflow, the Traffic Light Workflow (TLW) analysis, was developed to match lesions on the baseline and post-treatment PSMA PET/CT scans to measure changes in volume and PSMA SUVmax at a lesional level. Semiautomated matching of lesions was performed by the workflow and then reviewed and adjusted by the user to ensure accurate lesion matching. The TLW grouped lesions into three categories: ‘reducing’ lesions shown in green (defined as a lesion with a ≥30% reduction in volume, or a lesion which had resolved), ‘stable’ lesions shown in yellow (<30% change in volume or PSMA SUVmax), ‘increasing’ lesions (≥30% increase in volume or PSMA SUVmax), and new lesions shown in red (Figure 1). We chose, a priori, to use a 30% threshold to define a significant change, based on EORTC criteria for FDG PET and PSMA PET consensus guidelines (*14-17*).

Based on the TLW, each patient’s overall tumour response was classified into one of four categories: ‘responder’ when no lesions were categorized as ‘increasing’; ‘stable disease’ when no ‘reducing’, ‘increasing’ or new lesions were identified; ‘low-volume progressor’ when the proportion of ‘increasing’/new disease was less than 50% of the total tumour volume on post-treatment imaging (TTV); or ‘high-volume progressor’, when the proportion of ‘increasing’/new disease was more than 50% of the TTV on post-treatment imaging (Figure 1).

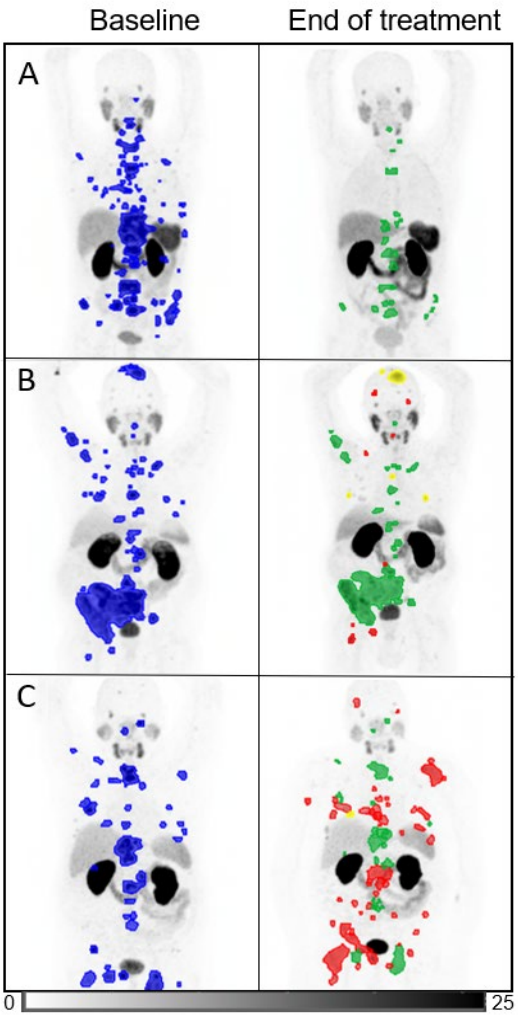


Figure 6-1. Traffic Light Workflow analysis identifying reducing (green), stable (yellow) and increasing or new (red) lesions. Based on TLW, overall response was categorised into (A) 'responder', (B) 'low-volume progressor', (C) 'high-volume progressor'.

Reproducibility of the TLW was assessed by repeating the TLW on each of the paired baseline and post-treatment scans. Analyses of test-retest differences and repeatability coefficients were done for each lesion, i.e. at a lesional level. The repeatability of the TLW overall response categories was also calculated.

Participants were also classified according to RECIP 1.0, based on changes in quantitative PSMA TTV and identification of new lesions (*6*).

Statistical Analyses

Only participants with PSMA PET/CT at both baseline and post-treatment were included in the analysis. We measured PSA decline from baseline (reduction at any point), ≥50% reduction from baseline (PSA50) at any time-point, PSA progression-free survival (PSA-PFS) as defined by PCWG3 criteria, radiographic progression defined by RECIST 1.1 and PCWG3 criteria, and overall survival (OS) (*12,18*). Time-to-event endpoints (PSA-PFS and OS) were defined as the interval from the date of post-treatment imaging to the event date, or the date last known to be event-free (at which point the observation was censored). Multistate modelling was used to account for potential bias related to time on treatment (*19*).

Two-sided, exact binomial 95% confidence interval (CI) were calculated for PSA response rates. The Kaplan-Meier method was used to characterise time-to-event endpoints and estimate medians (presented with 95% CIs). We correlated overall response patterns, changes in tumour volume, and changes in PSMA SUVmax with time-to-event outcomes, using univariable Cox proportional hazards regression. P-values below 5% were considered significant, without control for multiplicity. Models based on TLW and RECIP 1.0 were compared using the concordance index (C-index).

We assessed reproducibility of the TLW workflow using repeatability statistics that were calculated from a hierarchical, linear, mixed model that accounted for variance in score at the lesion-within-patient level (one level of clustering), and at the patient

level (a higher level of clustering) (*20*). The model also included fixed effect terms for lesion type (increased, reduced, resolved, and stable) and time point (baseline and post-treatment). 95% confidence intervals for the repeatability statistics were derived by bootstrapping. Analyses were performed using R (v4.0.5) (*21*).

RESULTS

Patient Characteristics

Baseline characteristics are summarized in Table 1. Thirty-seven LuPIN participants were evaluable (with scans before and after treatment): 68% (25/37) of whom had post-treatment imaging after completing all six cycles of 177Lu-PSMA-617 in combination with NOX66, and 32% (12/37) of whom had post-treatment imaging after 2-5 cycles. Seventy percent (26/37) of participants had a PSA50 response. The median reduction in PSA was 77% (IQR 34-92%). After a median follow-up of 26 months, the median PSA-PFS was 8.6 months (95% CI 5.6 – 11.6) and median OS was 22 months (95% CI 18.6-25.6).

Characteristic	**N=37**
Age (years)	68 (65-74)
ECOG	
0 or 1	32 (86)
2	5 (14)
PSA at C1 (ug/L)	91 (41.3-380)
Haemoglobin (Normal Range 130-180 g/L)	122 (112-131)
Alkaline Phosphatase (NR 30-100 U/L)	124 (83-359)

Albumin (NR 36-52 g/L)	40 (36-42)
De novo metastatic disease	19 (51)
Gleason Score	
≤ 7	7 (19)
8-10	21 (57)
Unknown/Not Available	9 (24)
Prior Systemic treatments	
LHRH agonist/antagonist	37 (100)
Chemotherapy	37 (100)
Docetaxel	37 (100)
Cabazitaxel	34 (92)
Androgen Signalling Inhibitor (ASI)	37 (100)
Time between baseline 68Ga-PSMA PET and cycle 1 of therapy	14 (8-20)
Time between last cycle of therapy and post-treatment 68Ga-PSMA PET	42 (29-43)

Numbers are presented as absolute counts (percentage) or median (interquartile range).

Table 6-1. Baseline patient characteristics.

Disease progression (based on PSA and/or clinical progression) had occurred in 54% (20/37) at the time of post-treatment imaging. Ninety-two percent (34/37) of participants had conventional post-treatment imaging (CT and bone scan), with radiographic progression identified in 18% (6/34).

Analysis of PSMA-PET using the Traffic Light Workflow

Changes in TTV, SUVmax, and SUVmean using whole-body quantitation of both PSMA PET and FDG PET in LuPIN have been previously reported (23). The distributions of resolved, reducing, stable, increasing, and new lesions identified by the TLW for each participant are illustrated in Figure 2.

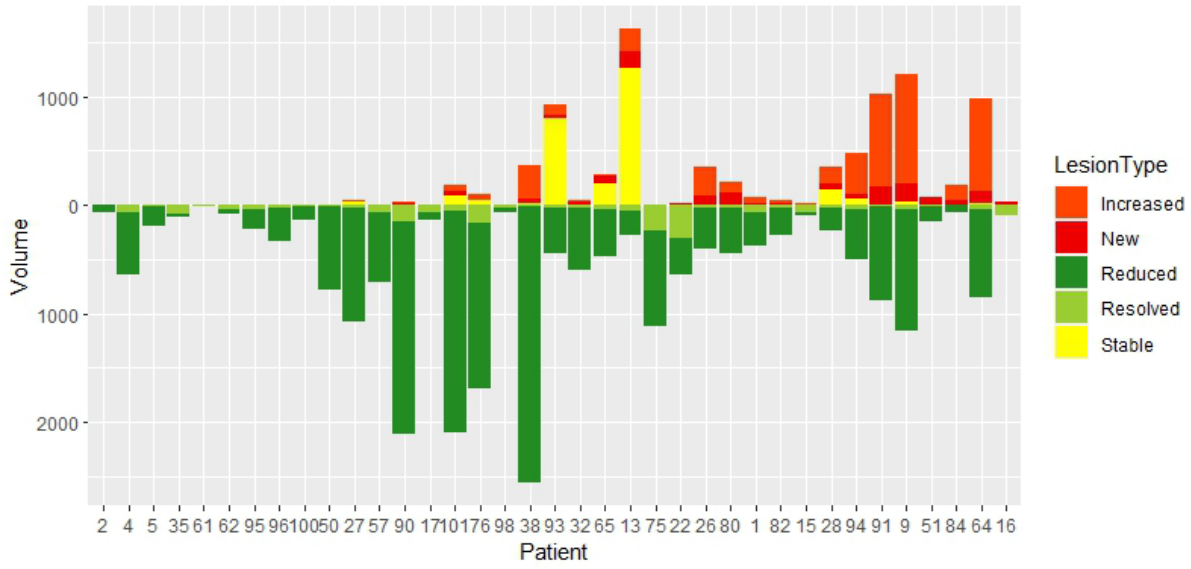


Figure 6-2. The distributions of resolved, reducing, stable, increasing, and new lesions identified for each participant. Most participants had a heterogenous tumour response.

The TLW categorised the overall response pattern for each participant. 24% (9/37) of participants were categorised as ‘responders’, none as having stable disease, 41% (15/37) as ‘low-volume progressors’, and 35% (13/37) as ‘high-volume progressors’.

Radiographic progression occurred in 18% (6/34). The TLW identified all six participants with radiographic disease progression, plus an additional 19/34 who had radiographically stable disease on conventional imaging were identified as 'progressors' using the TLW (Table 2).

Characteristic	**TLW responders (n=9)**	**TLW progressors (n=28)**
Baseline PSMA PET/CT		
TTV	125 (0.6-567)	48.7 (0.9-1002.7)
SUVmax	37.8 (5-122)	20.4 (5.6-122.3)
SUVmean	8.5 (3.9-12)	6.6 (3.7-22.6)
Post-treatment PSMA PET/CT		
TTV	39 (0.6-116)	103 (2.4-2308)
SUVmax	7.8 (1.6-12.5)	16 (6.2-73.1)
SUVmean	3.7 (0.9-5.4)	4.8 (3.6-10.4)
PSA progression*	1/9 (11)	19/28 (68)
Radiographic progression*	0/9 (0)	6/28 (21)

Numbers are presented as absolute counts (percentage) or median (interquartile range). *at time of post-treatment imaging

Table 6-2. Comparison of baseline and post-treatment PSMA PET/CT, PSA and radiographic characteristics for TLW 'responders' and 'progressors'.

Thirty-two percent (9/28) of participants identified as 'progressors' using the TLW had no evidence of PSA progression, while 68% (19/28) had both PSA progression and

progression based on the TLW. Among TLW ‘responders’, only 11% (1/9) had PSA progression (Table 2).

When compared with the change in PSMA TTV measured by whole body quantitation, 100% (9/9) ‘responders’ demonstrated any reduction in PSMA TTV. In contrast, only 27% (4/15) ‘low-volume progressors’ and 62% (8/13) ‘high-volume progressors’ demonstrated any increase in PSMA TTV. In the remaining 16 participants, although PSMA TTV decreased, the TLW also identified lesions that were either ‘increasing’ or new.

TLW Response Patterns and survival outcomes

TLW ‘responders’ had a median OS from time of post-treatment imaging of 17.7 months (95%CI 12.7-22.8), “low-volume progressors” had a median OS of 12 months (95%CI 9.1-14.9) and “high-volume progressors” a median OS of 7.5 months (95%CI 3-12). ‘Responders’ had longer OS than ‘low-volume progressors’ (HR 1.4 (95%CI 0.4-4.6) and ‘high-volume progressors’ (HR 4.8, 95%CI 1.4-15.8, overall p=0.005) (Table 3, Figure 3).

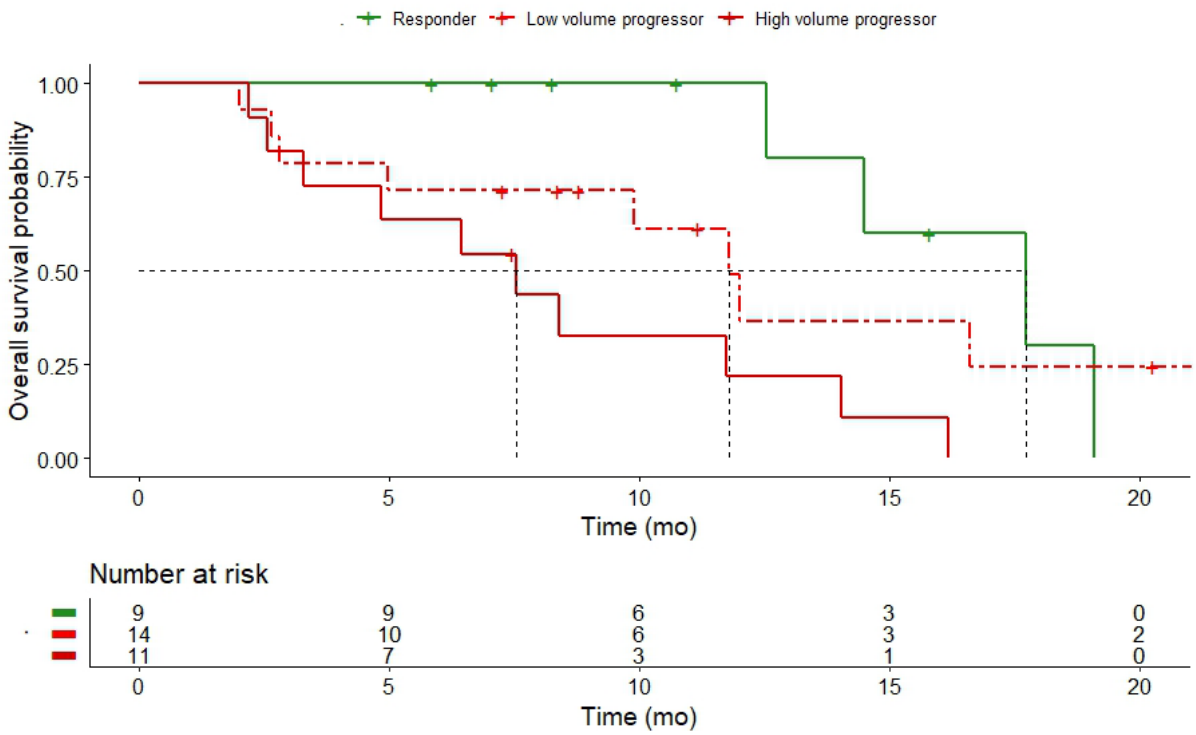


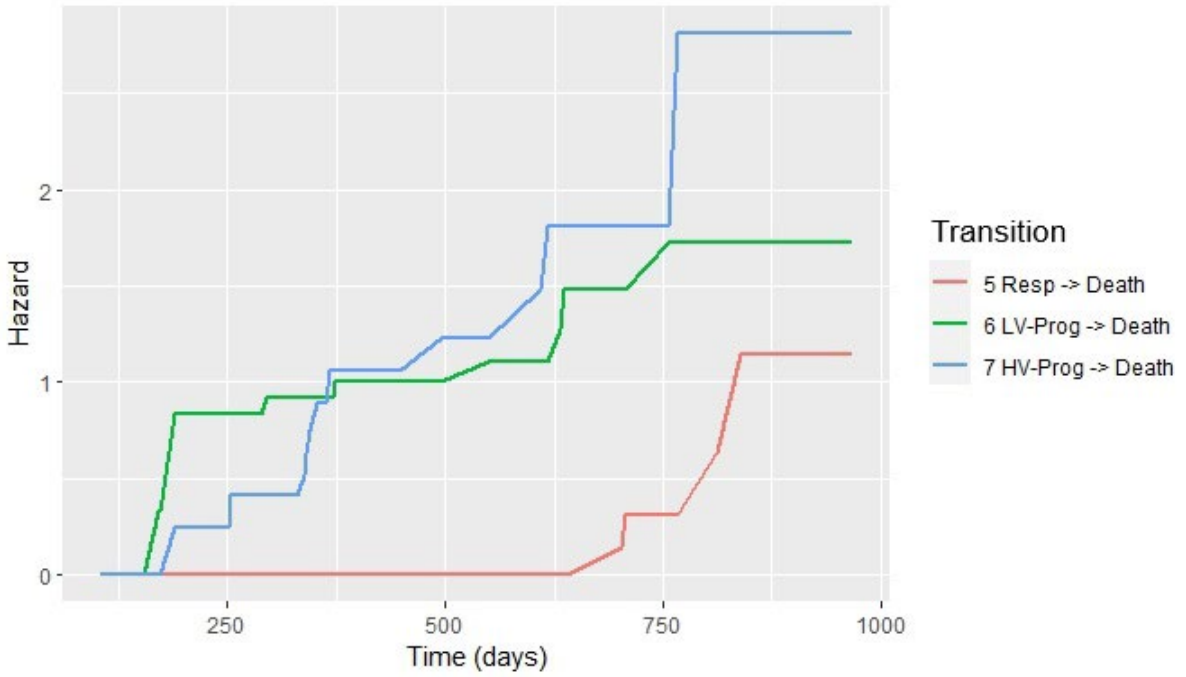
Figure 6-3. Kaplan-Meier curves for overall survival according to TLW response category.

Univariable analysis	**Overall survival** **HR (95% CI)**
TLW response pattern	
Responder	1.00 [ref]
Low-volume progressor	1.4 (0.4-4.5)
High-volume progressor	4.8 (1.4-15.8) p=0.01
PSA progression*	4.8 (1.8-13.4) p=0.002
Radiographic progression *	6.7 (2.2-20) p=<0.001

Hazard ratios are presented as HR (95%CI) [reference group]. * at time of post-treatment imaging

Table 6-3. Univariable Cox regression analysis for association with overall survival from time of post-treatment PSMA PET.

Multistate modelling confirmed that the hazard for death was consistently lower in ‘responders’ than in ‘low-volume progressors’ or in ‘high-volume progressors’ from the time of study entry (Supplementary Figure 1). PSA progression and radiographic progression at time of post-treatment imaging were also associated with worse OS (HR 6.2 (95%CI 2.2-17), p=0.002 and HR 6.6, 95%CI 2.2-20, p<0.001 respectively).



Supplementary Figure 1. A multi-state model was constructed to examine the hazard for death from the date of enrolment, incorporating the effect of TLW response category as a covariable. The model partitions each patient's survival into transitions between the following states: Event Free, Responder, LV Progressor, HV Progressor, Death. The transitions hazard plot shows the hazard over time of transitioning from either Responder-> Death, LV Progressor-> Death, or HV Progressor->Death. The Responder->Death group have a consistently lower hazard for death at any time point from the date of enrolment, compared with LV Progressor-> Death, or HV Progressor->Death.

Comparison with RECIP 1.0 response

In this cohort, 24% (9/37) were classified as RECIP-PR, 51% (19/37) RECIP-SD, and 24% (9/37) RECIP-PD. Participants with a RECIP-PR had a median survival of 17.7 months (95%CI 12.7-22.8), RECIP-SD 14 months (95% CI 10.3-17.7), RECIP-

PD 3.3 months (95%CI 1.3-5.2). RECIP-PR was associated with longer survival than RECIP-SD (HR 1.5 (95%CI 0.5-4.8) and RECIP-PD (HR 15 (95%CI 3.4-67), p<0.001). The C-indices were similar for TLW and RECIP 1.0 (0.70 and 0.75 respectively).

Patterns of progression

The predominant phenotype in TLW 'progressors' was a persistently high PSMA SUVmax (median 16, range 6-73) with a stable SUVmean (median 4.8, range 4-10), compared to median SUVmax 20.4 (range 5.6-122) and median SUVmean 6.6 (range 3.7-22.6) at baseline. By contrast, 'responders' had a significant reduction in both SUVmax (median 7.8, range 1.6-12.5) and SUVmean (median 3.7 (range 0.9-5.4) on post-treatment imaging in comparison with their baseline levels (median SUVmax 37.8 (range 5-122) and median SUVmean 8.5 (3.9-12) (both p<0.001) (Table 2).

The mean difference between SUVmax and SUVmean narrowed in 'responders' (difference of 28 down to 4 post-treatment) compared with 'progressors' (difference of 14, rising to 20 post-treatment) suggesting a higher degree of heterogeneity in 'progressors' (Figure 4).

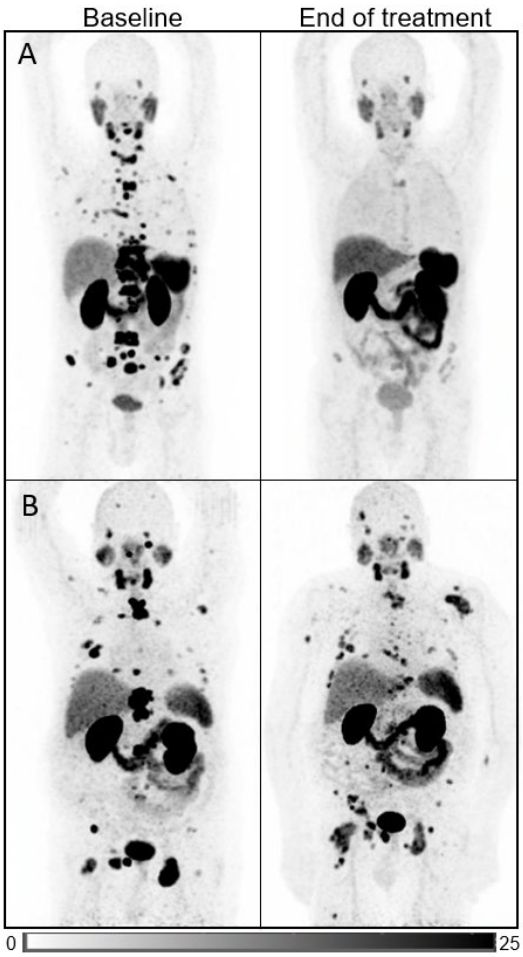


Figure 6-4. Patient A is a 'responder', showing a homogeneous reduction in PSMA intensity. Patient B is a 'progressor', with persistently high and heterogeneous PSMA expression.

Sixty-one percent (17/28) of 'progressors' as compared with none (0/9) of 'responders' had an SUVmax > 15 on post-treatment imaging (above trial entry criteria), suggesting that sites of treatment failure were more commonly persistently PSMA avid, rather than failing to respond to treatment due to low PSMA avidity (Table 2). TLW analysis also enabled identification of patients with oligo-progression (<5 new/increasing lesions) and disease progression with low PSMA expression (Figure 5).

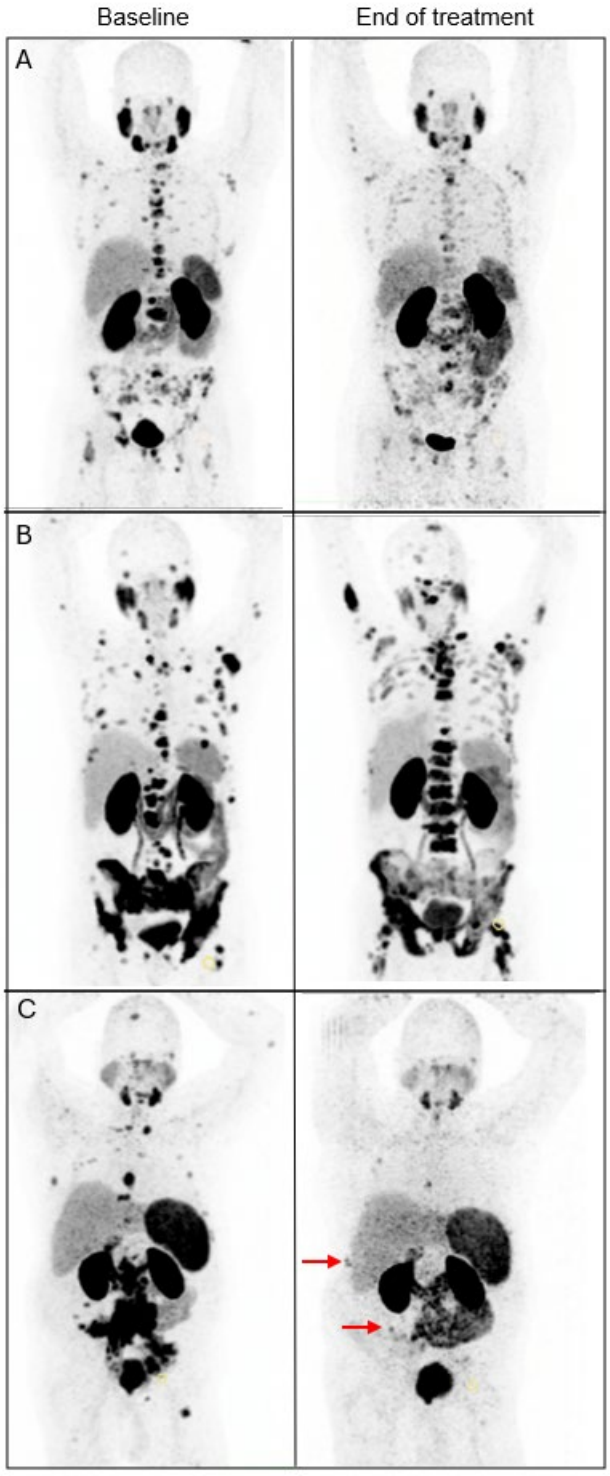


Figure 6-5. Patterns of progression: Patient A, low PSMA expression progressive disease. Patient B, persistently high PSMA expression disease progression. Patient C, oligoprogression with two new bony metastases (arrows).

TLW Analysis Reproducibility

Repeatability of the TLW analysis was assessed in 37 participants with a total of 228 lesions. Results from the hierarchical, linear, mixed model showed no evidence of a systematic difference between test and retest for SUVmax, SUVmean or lesional tumour volume. As expected, there was evidence of an effect of lesion type (reducing, stable, or increasing) on SUVmax, SUVmean, and tumour volume ($p<0.001$ for all). Repeatability estimate for SUVmax was 0.96 (95%CI 0.94-0.97), SUVmean was 0.97 (95%CI 0.96-0.98), lesion volume was 0.96 (95%CI 0.94-0.97), and TLW response category was 0.93 (95% CI 0.87-0.97) (Table 4).

	Absolute test-retest difference *	**Repeatability estimate**
SUVmax	0.67 (-0.47 to 1.82) [0.25]	0.96 (0.94-0.97)
SUVmean	0.08 (-0.07 to 0.22) [0.31]	0.97 (0.96-0.98)
Tumour volume (mL)	10.33 (11.02 to 31.69) [0.34]	0.96 (0.94-0.97)
TLW response pattern	NA	0.93 (0.87-0.97)

Results presented as Estimate (95%CI) [p value]. * Adjusted for lesion type

Table 6-4. .Repeatability of TLW response pattern and SUVmax, SUVmean and tumour volume.

DISCUSSION

We developed the TLW to assess the response of individual lesions using integrated measurements of tumour volume and SUV intensity. We hypothesized that TLW would be useful for characterising heterogenous responses to treatment with 177Lu-PSMA-617. This is the first study to track tumour responses in individual lesions, and in aggregate, to classify an overall response pattern for each participant. The TLW allows identification and characterisation of treatment-resistant sites of disease, and could be used to indicate the need for a change in treatment. The TLW demonstrated good repeatability, and future iterations have the potential for automation, requiring less time and manual adjustment by a nuclear medicine physician.

In our previously published data from the LuPIN trial, PSMA intensity reduced in 95% of patients after treatment with 177Lu-PSMA-617 (*22*). Hence, we required a reduction in lesion volume (with or without a reduction in SUVmax) to identify a responding lesion. To identify a progressive lesion, we required a ≥30% increase in volume or in PSMA SUVmax, while lesions that were within the 30% range were classified as stable disease.

Previous studies have evaluated whole body quantitation of serial PSMA PET scans to monitor PSMA-targeted therapy, but this global measure may mislabel patients with discordant or mixed tumour responses (*7,22-24*). In this study, 43% (16/37) of participants had a reduction in total tumour volume despite having sites of progressive disease. TLW response category was independently associated with overall survival, and we identified a trend towards worse overall survival even among those with low-volume progression in the presence of predominant tumour response.

Prognostic models for overall survival based on TLW and RECIP 1.0 performed similarly (C-index 0.70 and 0.75 respectively). However, when we applied the RECIP 1.0 criteria to our cohort, 51% (19/37) patients were categorised as RECIP-SD. Thus, more than half the cohort fall into an undifferentiated middle group, without further information to guide therapeutic decisions. Within the participants classified as RECIP-SD, all had new lesions, and 16/19 had new lesions in the presence of a reduction in total tumour volume.

Unlike RECIP 1.0, TLW also characterises the extent and phenotype of both new and progressive lesions on serial imaging with PET. Only 24% of participants demonstrated a response in all metastatic lesions (categorised as ‘responder’ by TLW and RECIP-PR), with the remainder having a heterogeneous response with at least one site of progression. The TLW allowed for recognition and characterisation of those with a mixed response and demonstrated shorter survival in ‘high-volume progressors’ than ‘low-volume progressors’. Additionally, the lesional information provided by TLW distinguishes different phenotypes of disease progression including progression with high PSMA expression, versus low PSMA expression, and oligo-progressive disease.

Identification of early sites of progression despite a good initial PSA response and reduction in total tumour volume may allow changes in treatment, either by adding a radiation sensitiser, changing the radionuclide or ligand, considering local treatment for oligo-progressive sites, or changing to an alternative systemic therapy. For example, heterogenous PSMA expression has been identified as a potential mechanism of treatment resistance to 177Lu-PSMA-617 (*8,9,25*). Many participants with progressive sites of disease in this cohort had persistently high PSMA SUVmax, with 61% of ‘progressors’ above the LuPIN trial entry criteria. Persistently high PSMA

expression may indicate radiation resistance, which might be overcome by alpha-emitting radioligands (*26-28*). Although this study applied the TLW to post-treatment imaging, further evaluation of the TLW is warranted earlier in the treatment course to determine its utility as an early marker of response that might be used to guide decisions about treatment.

The prognostic utility of the TLW needs investigation in independent and larger datasets. Our sample size limited our ability to comment on important subsets, such as those with visceral metastases. Additionally, our study only included participants able to complete imaging after treatment, potentially biasing spectrum of responses we observed.

CONCLUSION

This study demonstrated the feasibility of tracking complex patterns of response to PSMA-targeted treatment using a novel, quantitative TLW. The TLW provides a more detailed characterisation of the phenotype and burden of progressive disease, and the overall response category according to the TLW was prognostic for overall survival. Further research is warranted to improve automation of the workflow and evaluate the TLW in larger cohorts.

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7 Evaluation of 177Lu-PSMA-617 SPECT/CT Quantitation as a Response Biomarker Within a Prospective 177Lu-PSMA-617 and NOX66 Combination Trial (LuPIN).

7.1 Overview

This chapter is a published manuscript aimed at nuclear medicine and oncology clinicians and researchers. The study used 177Lu-PSMA-617 SPECT (177Lu SPECT) imaging data collected as part of a phase I/II trial. The study used a novel imaging analysis workflow to measure quantitative imaging indices on 177Lu SPECT imaging at cycle 1 and cycle 3. The association between PSA progression free and overall survival and a change in quantitative imaging indices was examined.

This research was originally published in the Journal of Nuclear Medicine.

Publication details

Pathmanandavel, S., Crumbaker, M., Ho, B., Yam, A. O., Wilson, P., Niman, R., Ayers, M., Sharma, S., Hickey, A., Eu, P., Stockler, M., Martin, A. J., Joshua, A. M., Nguyen, A., & Emmett, L. (2023). Evaluation of 177Lu-PSMA-617 SPECT/CT Quantitation as a Response Biomarker Within a Prospective 177Lu-PSMA-617 and NOX66 Combination Trial (LuPIN). Journal of Nuclear Medicine, 64(2), 221-226. https://doi.org/https://dx.doi.org/10.2967/jnumed.122.264398

Contributions of the authors

SP: research proposal and concept development, data acquisition, data analysis, interpretation of results, drafted and revised the manuscript.

MC, BH, AY, PW, RN, MA, SS, AH, PE, AMJ: contributed to the data acquisition, and helped revise the manuscript.

AN, AJM, MS: data analysis, interpretation of results, and helped revise the manuscript.

LE: research proposal and concept development, interpretation of results, and helped revise the manuscript.

7.2 Manuscript

ABSTRACT

177Lu-PSMA-617 is an effective and novel treatment in metastatic castration-resistant prostate cancer (mCRPC). Our ability to assess response rates and therefore efficacy may be improved using predictive tools. This study investigated the predictive value of serial 177Lu-PSMA-617 SPECT/CT (177Lu SPECT) imaging in monitoring treatment response.

Methods: Fifty-six men with progressive mCRPC previously treated with chemotherapy and novel androgen signaling inhibitor were enrolled into the LuPIN trial and received up to 6 doses of 177Lu-PSMA-617 and a radiation sensitizer (3-(4-hydroxyphenyl)-2H-1-benzopyran-7-ol [NOX66]). 68Ga-PSMA-11 and 18F-FDG PET/CT were performed at study entry and exit, and 177Lu SPECT from vertex to mid thighs was performed 24 h after each treatment. SPECT quantitative analysis was undertaken at cycles 1 (baseline) and 3 (week 12) of treatment.

Results: Thirty-two of the 56 men had analyzable serial 177Lu SPECT imaging at both cycle 1 and cycle 3. In this subgroup, median prostate-specific antigen (PSA) progression-free survival (PFS) was 6.3 mo (95% CI, 5–10 mo) and median overall survival was 12.3 mo (95% CI, 12–24 mo). The PSA 50% response rate was 63% (20/32). 177Lu SPECT total tumor volume (SPECT TTV) was reduced in 68% (22/32; median, 20.20 m3 [95% CI, 21.4 - 20.001]) and increased in 31% (10/32; median, 0.36 [95% CI, 0.1–1.4]). Any increase in SPECT TTV was associated with shorter PSA PFS (hazard ratio, 4.1 [95% CI, 1.5–11.2]; P 5 0.006). An increase of 30% or more in SPECT TTV was also associated with a shorter PSA PFS

(hazard ratio, 3.3 [95% CI, 1.3–8.6]; P 50.02). Tumoral SUVmax was reduced in 91% (29/32) and SUVmean in 84% (27/32); neither was associated with PSA PFS or overall survival outcomes. PSA progression by week 12 was also associated with a shorter PSA PFS (hazard ratio, 26.5 [95% CI, 5.4–131]). In the patients with SPECT TTV progression at week 12, 50% (5/10) had no concurrent PSA progression (median PSA PFS, 4.5 mo [95% CI, 2.8–5.6 mo]), and 5 of 10 men had both PSA and SPECT TTV progression at week 12 (median PSA PFS, 2.8 mo [95% CI, 1.8–3.7 mo]).

Conclusion: Increasing SPECT TTV on quantitative 177Lu SPECT predicts a short PFS and may play a future role as an imaging response biomarker.

BACKGROUND

Although treatment resistance and short response duration remain common, 177Lu-PSMA-617 is an effective therapy in metastatic castration-resistant prostate cancer (mCRPC) (*1-4*). Accurate monitoring of response to 177Lu-PSMA-617 may improve patient outcomes by enabling treatment escalation, change in treatment, or a treatment holiday, dependent on imaging results. Interim and serial prostate specific membrane antigen (PSMA) PET has recently been shown to be predictive of progression-free survival (PFS) with PSMA targeted radionuclide therapy (*5*). Quantitative 177Lu-PSMA-617 SPECT/CT (177Lu SPECT) imaging after each 177Lu-PSMA-617 dose may also be valuable in response monitoring in addition to providing dosimetric information. This LuPIN trial substudy aimed to determine whether quantitative parameters on serial 177Lu SPECT imaging 24 h after 177Lu-PSMA-617 therapy were predictive of treatment response and PFS.

MATERIALS AND METHODS

The LuPIN trial is a prospective single-center phase I/II dose escalation and expansion trial of combination 177Lu-PSMA-617 and 3-(4-hydroxyphenyl)-2H-1-benzopyran-7-ol (NOX66) for men with mCRPC previously treated with at least 1 line of taxane chemotherapy and androgen signaling inhibitor. The clinical results have been previously published (*6,7*). The St. Vincent's Hospital institutional review board approved the study protocol (HREC/17/SVH/19 and ACTRN12618001073291), and all patients provided informed written consent.

Screening

Men with progressive mCRPC, based on either conventional imaging (CT and bone scanning) or a rising prostate-specific antigen (PSA) level according to Prostate Cancer Working Group 3 criteria (*8*), were eligible for screening. Prior treatment with at least 1 line of taxane chemotherapy (docetaxel or cabazitaxel) and an androgen signaling inhibitor (abiraterone or enzalutamide) was required for inclusion.
Men underwent screening with 18F-FDG and 68Ga-HBEDD-PSMA-11 PET/CT, bone scanning, and CT of the chest, abdomen, and pelvis. Molecular screening criteria were based on SUVmax rather than physiologic activity (liver or parotid). Men were eligible if they had an SUVmax of more than 15 on PSMA PET at 1 or more sites, an SUVmax of more than 10 at all measurable sites, and no 18F-FDG avidity without corresponding PSMA uptake.

Study Treatment

Men received up to 6 doses of 177Lu-PSMA-617 at 6-wk intervals, with 3 dose-escalated cohorts of NOX66 (400, 800, 1,200 mg). NOX66 suppositories were administered as a radiosensitizer on days 1–10 after each 177Lu-PSMA-617 injection. All cohorts were administered 7.5 GBq of 177Lu-PSMA-617 on day 1 via slow intravenous injection. The PSMA-617 precursor (AAA Novartis) was radiolabeled to no-carrier added 177Lu-chloride according to the manufacturer’s instructions. Quality control tests for radionuclide and radiochemical purity were performed using high-pressure liquid chromatography and thin-layer chromatography. Blood was prospectively collected before assessment of adverse events and biochemical responses. The patients were treated on trial until they were no longer clinically benefiting from treatment.

Imaging Procedures and Analysis

68Ga-PSMA and 18F-FDG PET/CT scans were obtained at baseline and trial exit (after completing 6 cycles or when treatment was ceased), using the imaging acquisition and analysis parameters previously published (*7*). 177Lu SPECT (vertex to mid thighs) was performed 24 h after 177Lu-PSMA-617 injection using a Discovery 670 system (GE Healthcare) with the following parameters: medium-energy collimators, 3 bed positions, 60 projections over 360° with an acquisition time of 10 s per frame, 128 x 128 matrix, and 4.42 x 4.42 mm pixel size. An energy window centered on 208 keV ± 10% with a 165 keV ± 6.5% scatter window was used. An unenhanced low-dose CT scan was obtained immediately afterward using the following parameters: pitch of 1, tube voltage of 120 kV, automatic mAs control (reference mAs, 90), slice thickness of 3.7 mm, matrix of 512 x 512, and field of view of 40 cm. The SPECT projection images were reconstructed with an iterative ordered-subset estimation-maximum algorithm that used 4 iterations and 10 subsets using SPECTRA Quant (MIM Software, Inc.). No pre- or postreconstruction filters were applied. CT-based attenuation correction, dual-energy-window scatter correction, collimator-based resolution recovery, and quantitative conversion to SUV were performed during the reconstruction. The conversion from counts to units of activity was based on a cylinder phantom with known activity.

Quantitative Analysis

177Lu SPECT and 68Ga-PSMA PET/CT were analyzed semiquantitatively by a nuclear medicine physician using MIM (LesionID; MIM Software Inc.) software and a standardized semiautomated workflow to delineate regions of interest with a

minimum SUV cutoff of 3. All lesions identified quantitatively were manually reviewed and physiologic activity removed. Whole-body quantitation was used to derive total tumor volume (TTV), SUVmax, and SUVmean for both 68Ga-PSMA PET and 177Lu SPECT (*9*).

Statistical Analysis

We measured PSA decline from baseline (>50%) at any time point, PSA PFS as defined by Prostate Cancer Working Group 3 criteria, and overall survival (*8,10*). The Kaplan–Meier method was used to characterize time-to-event endpoints and estimate medians (presented with 95% CIs). We correlated changes in TTV, PSMA intensity, clinical parameters, and biochemical parameters with time-to-event outcomes, using univariate Cox proportional-hazards regression models (*11,12*). Continuous variables included increase in TTV, SUVmax, and SUVmean. P values below 5% were considered significant. We compared 68Ga-PSMA PET TTV with cycle 1 SPECT TTV using scatterplots and Pearson correlation coefficients. Reproducibility testing of SPECT TTV and PSMA SUVmax was undertaken using repeatability statistics calculated from a hierarchic linear mixed model that accounted for variance in score at the patient level (*13*). A 95% CI for the repeatability statistics was derived via bootstrapping. Analyses were performed using R (version 4.0.5).

RESULTS

Baseline Patient Characteristics

Of the men enrolled in LuPIN, 57% (32/56) had 177Lu SPECT imaging suitable for analysis, 30% (17/56) had incomplete SPECT data precluding analysis, and 13% (7/56) did not reach cycle 3 of treatment. Baseline characteristics are summarized in Table 1. In this LuPIN substudy, 53% (17/32) completed 6 cycles of treatment whereas 47% (15/32) completed between 3 and 5 cycles, and 63% (20/32) achieved at least a 50% PSA decline from baseline at any time point. At the time of analysis, 84% (27/32) were deceased.

There was no difference in either PSA PFS or overall survival based on NOX66 dose. Overall, median overall survival was 12.3 mo (95% CI, 11.7–23.6 mo). Median PSA PFS was 6.3 mo (95% CI, 5.1–9.8 mo).

Characteristic	N=32
Age (years)	69 (66-73)
ECOG	
0 or 1	25 (78)
2	7 (22)
Prior Systemic treatments	
LHRH agonist/antagonist	32 (100)
Chemotherapy	32 (100)
Docetaxel	32 (100)
Cabazitaxel	29 (91)
Androgen Signalling Inhibitor (ASI)	32 (100)
Sites of Disease	
Lymph nodes	18 (56)
Bone	30 (94)
Viscera	6 (19)
Median PSMA tumour volume at screening (mL)	670 (275-1736)
Number of cycles of ^{177}LuPSMA-617 received	6 (3-6)

Numbers are presented as absolute counts (percentage) or median (interquartile range).

Table 7-1.Baseline patient characteristics.

177Lu SPECT Quantitation

SPECT quantitation measures at baseline and week 12, including SPECT TTV, SUVmax, and SUVmean, are summarized in Table 2. SPECT TTV was reduced between baseline and week 12 in 68% (22/32; median, -0.20 m^3 [95% CI, -1.4 to -0.001]) and increased in 31% (10/32; median, 0.36 m^3 [95% CI, 0.1–1.4]). A 30% increase in SPECT TTV by week 12 was identified in 19% (6/32). SUVmax was reduced between baseline and week 12 in 91% (29/32; median, -28.9 [95% CI, -195 to +42]), and SUVmean was reduced in 84% (27/32; median, -2.6 [95% CI, -12 to +10]).

	Cycle 1 SPECT	**Cycle 3 SPECT**
TTV (mL)	787 (282-1298)	492 (191-1190)
SUVmax	70 (57-100)	36 (27-60)
SUVmean	10.1 (8-12)	8 (6-9)

Results presented as median (IQR).

Table 7-2. Summary of ^{177}Lu-SPECT/CT quantitation at C1 and C3.

Correlation with Patient Outcomes

An increase in SPECT TTV between baseline and week 12 was associated with a significantly worse PSA PFS (hazard ratio, 4.1 [95% CI, 1.5–11.2]; P = 0.006). Median PSA PFS in those with an increase in SPECT TTV was 4.5 mo (95% CI, 2.8–5.6 mo), compared with 7.1 mo (95% CI, 6.3–10.7 mo) for those with no increase in SPECT TTV. A SPECT TTV increase of at least 30% was also associated with a shorter PSA PFS (hazard ratio, 3.3 [95% CI, 1.3–8.6], P = 0.02) (Fig. 1). Increased

SUVmax or SUVmean between baseline and week 12 was not associated with PSA PFS or overall survival (Table 3).

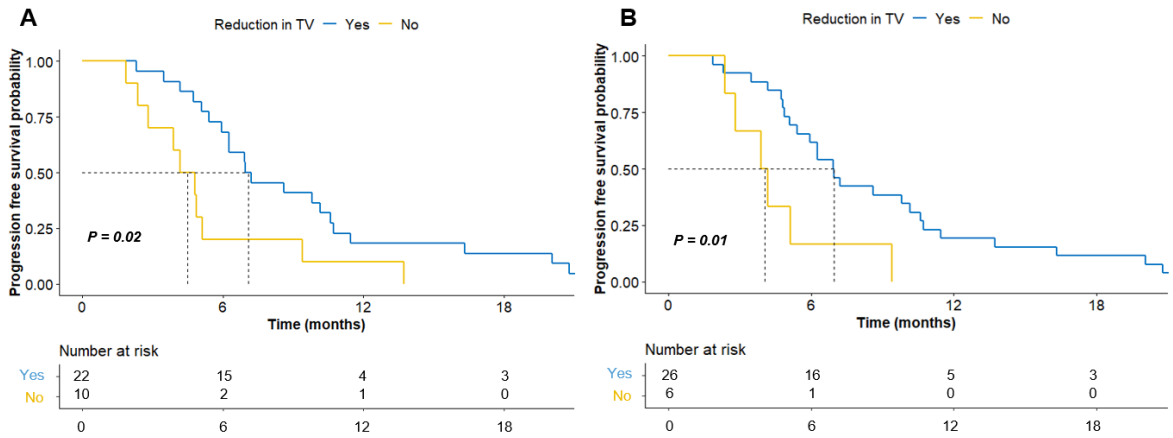

Figure 7-1. Kaplan-Meier curve for PSA-PFS stratified by (A) any increase in SPECT-TTV at cycle 3 (B) >30% increase in SPECT-TTV at cycle 3.

Univariable analysis	Overall survival	PSA progression free survival
Increase in SPECT TTV (litres) †	1.5 (0.6-4.1) [0.40]	4.1 (1.5-11.2) [0.006]
Increase in SPECT SUVmax †	1.002 (0.99-1.01) [0.75]	1.004 (0.99-1.02) [0.51]
Increase in SPECT SUVmean †	1.11 (0.98-1.24) [0.09]	1.11 (0.99-1.23) [0.06]
PSA progression †	5.6 (2.1-14.8) [<0.001]	26.5 (5.4-131) [<0.001]
PSA decline ≥ 50% †	0.31 (0.1-0.8) [0.02]	0.40 (0.2-0.9) [0.02]
Time since diagnosis	0.95 (0.88-1.03) [0.18]	0.93 (0.87-1.01) [0.09]

Hazard ratios are presented as HR (95%CI) [p value]. † at week 12

Table 7-3. Univariable analysis of clinical and imaging markers and association with PSA-PFS and OS

By week 12, 25% (8/32) of patients demonstrated PSA progression. PSA progression at week 12 was associated with significantly worse PSA PFS (hazard ratio, 26.5 [95% CI, 5.4–131]; P < 0.001). Patients with PSA progression at week 12 had a median PSA PFS of 3.5 mo (95% CI, 1.1–4.5 mo), versus 7.9 mo (95% CI, 6.3–10.7 mo) in those without PSA progression. In the 10 patients with SPECT TTV progression at week 12, 50% (5/10) had no concurrent PSA progression (median PSA PFS, 4.5 mo [95% CI, 2.8–5.6 mo]), and 5 of 10 men had both PSA and SPECT

TTV progression at week 12 (median PSA PFS, 2.8 mo [95% CI, 1.8–3.7 mo]) (Fig. 2).

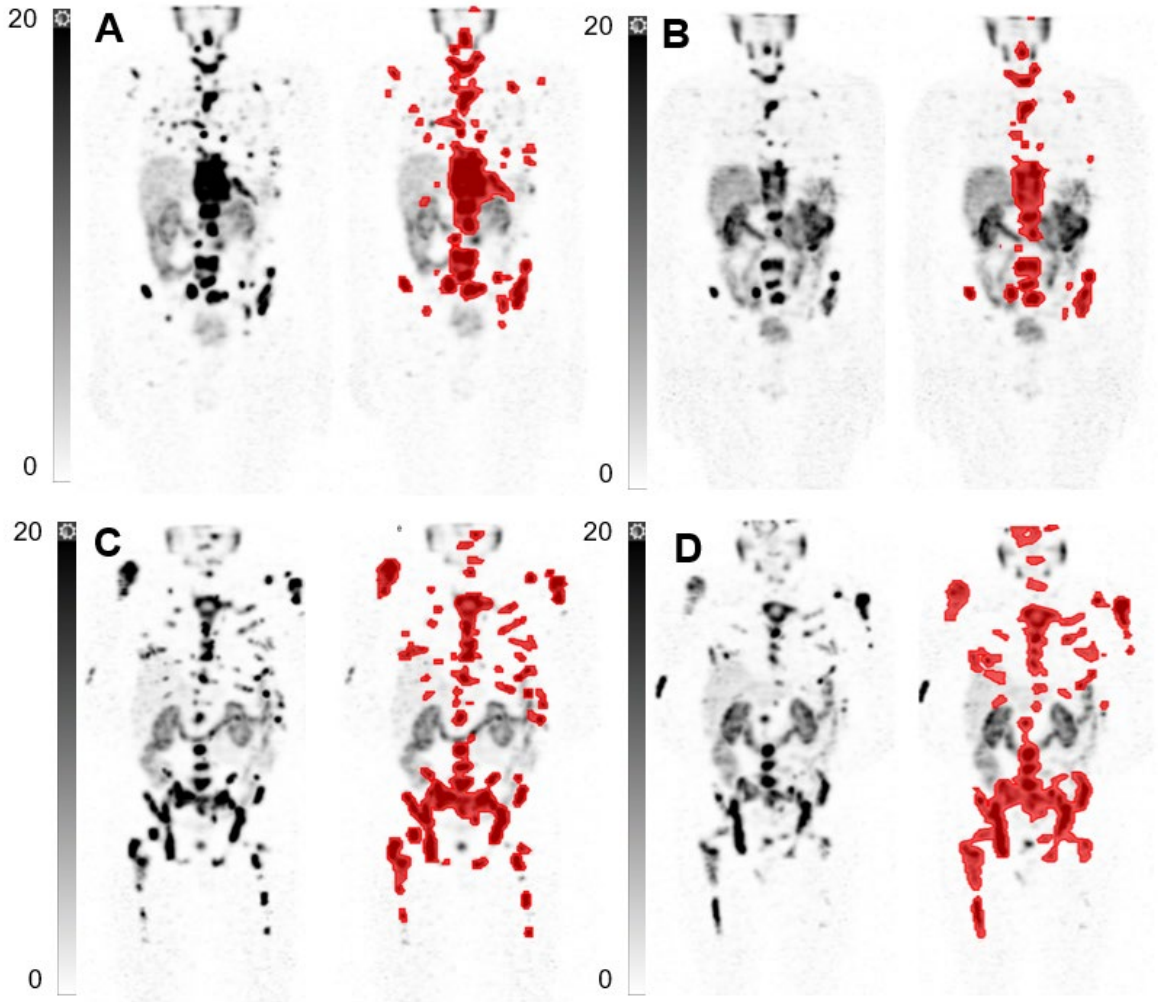

Figure 7-2. Maximum intensity projection (MIP) and quantitation of 177Lu-SPECT at cycle 1 (A) and cycle 3 (B) for a patient with reduction in SPECT-TTV and PSA (PSA-PFS 22 months). MIP and quantitation of 177Lu-SPECT at cycle 1 (C) and cycle 3 (D) for a patient with increase in SPECT-TTV > 30% but no increase in PSA (PSA-PFS 5 months.)

Reproducibility

TTV was compared between 68Ga PSMA-11 PET/CT at screening (PSMA PET TTV) and the baseline 177Lu SPECT (median time between scans, 15 d [range, 6–56 d]). There was a strong correlation between PSMA PET TTV and cycle 1 SPECT TTV (R = 0.87 [95% CI, 0.74–0.93], P < 0.001) (Fig. 3). Mean TTV was similar between PSMA PET and SPECT (PET TTV, 925 ± 856 cm^3; SPECT TTV, 949 ± 852 cm^3). The repeatability of 177Lu SPECT quantitative analysis was assessed in all 32 patients. There was no evidence of a systematic difference between test and retest for SUVmax, SUVmean, or TTV. The repeatability estimate was 0.99 for SUVmax (95% CI, 0.97–0.99), 0.90 for SUVmean (95% CI, 0.81–0.95), and 0.99 for TTV (95% CI, 0.98–0.99) (Table 4).

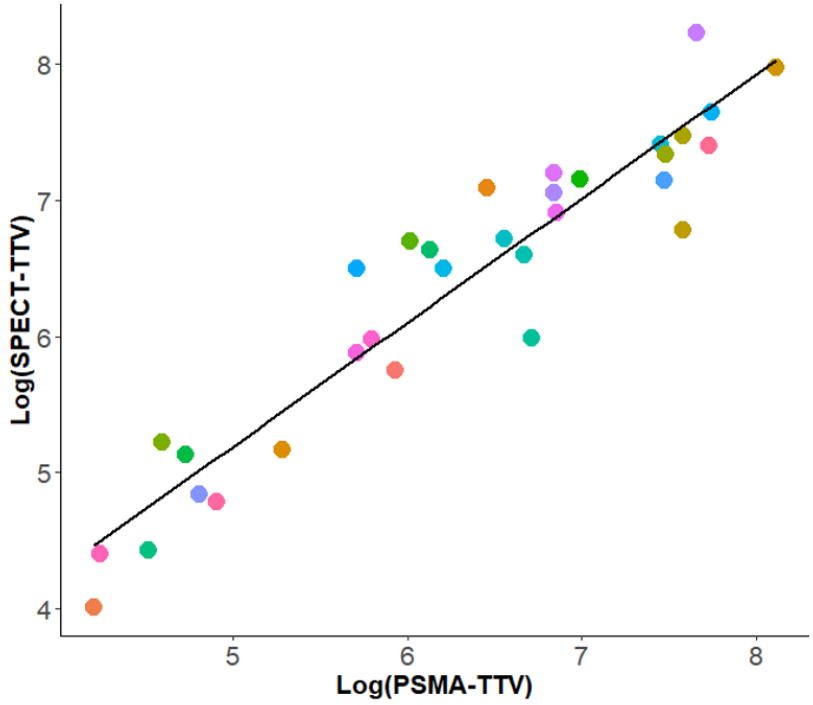

Figure 7-3. Scatter plot of PET and SPECT C1 volume quantitation.

	Absolute test-retest difference	**Repeatability estimate**
SUVmax	1.47 (-1.46 to 4.39)	0.99 (0.97-0.99) [<0.001]
SUVmean	-0.29 (-1.06 to 0.48)	0.90 (0.81-0.95) [<0.001]
Tumour volume (mL)	34.21 (-14.62 to 83.03)	0.99 (0.98-0.99) [<0.001]

Results presented as Estimate (95%CI) [p value].

Table 7-4. Repeatability of 177Lu-SPECT quantitation measures.

DISCUSSION

This study found that quantified changes in SPECT TTV between baseline and 12-week 177Lu-PSMA-617 predict PFS in men treated on a prospective PSMA-targeted therapy trial. To our knowledge, this was the first study to evaluate SPECT parameters for response biomarker capability, a potentially valuable development that uses a readily available tool to potentially enhance personalized treatment by directly assessing treatment response. 177Lu-PSMA-617 has proven an effective therapy for mCRPC, with randomized trials demonstrating both improved overall survival and improved radiographic PFS compared with the standard of care (*3*), as well as increased PSA 50% response rates and improved patient-reported outcomes compared with cabazitaxel (*2*). However, responses can be heterogeneous, and PFS remains relatively short (*2,3*). Combination trials with 177Lu-PSMA-617 are under way to investigate whether combining 177Lu-PSMA-617 with other agents may deepen and prolong responses (NCT04419402, NCT03658447, and NCT03874884) (*14*).

Predictive and interim response biomarkers, both imaging and genomic, will be critical in personalizing treatments to optimize longer-term responses to PSMA-targeted radionuclide therapy (*5,7*). Although there are limitations in spatial resolution with 177Lu SPECT, its elegant potential as a response biomarker warrants further evaluation. Molecular imaging as an interim response biomarker has been particularly successful in optimizing treatment responses with 12-wk 18F-FDG PET in lymphoma (*15-19*). More recently, Gafita et al. proposed a 12-wk interim PSMA PET scan on the basis of its predictive value for early disease progression in a multicenter 177Lu-PSMA-617 therapy trial (RECIP 1.0) (*5*). Being able to identify treatment-resistant phenotypes early could allow either intensification with the addition of synergistic drugs or a change in treatment, thereby maximizing opportunities for treatment response in individuals and avoiding the clinical and financial costs of continuing futile treatment. Generally, this has been done in mCRPC by monitoring serum PSA response (*20*). Similarly to PSMA, there is heterogeneity of PSA expression, meaning it is not an accurate measure of disease volume in a significant proportion of men with mCRPC (*21*).

In this study, both SPECT TTV and PSA progression were predictive of PFS. However, 21% of those in this study with no PSA progression had SPECT TTV progression. Gafita et al. had a similar finding, with 14% demonstrating PSMA PET progression before PSA progression (*5*). Larger numbers are required to determine whether use of SPECT TTV in combination with PSA can more effectively identify disease progression, but this study provides strong preliminary evidence, with 177Lu SPECT identifying progression in a subset of patients who had not yet experienced a PSA rise. These results raise the question of whether 177Lu SPECT or PSMA PET

should be the preferred interim imaging response biomarker for 177Lu-PSMA-617 therapy.

Posttherapy imaging both with planar imaging and with 177Lu SPECT after radionuclide therapy has traditionally been used for dosimetric calculations to determine the dose to nontarget organs and tumor (*22-25*). Because of its significantly lower spatial resolution and inability to detect small lesions relative to PET imaging, 177Lu SPECT has not been considered for treatment response. In our direct comparison of quantitative findings between 68Ga-PSMA PET and 177Lu SPECT within 2 wk, TTV was very similar between 177Lu SPECT and PET, with a high correlation between the 2 modalities, although theoretically 177Lu SPECT will underestimate small-volume disease (*26*). However, evaluating disease progression requiring treatment change or intensification should not depend on identifying small-volume disease. A lesion that is below the spatial resolution for detection on 177Lu SPECT will become visible as its size increases. Although the findings from this trial confirm that 177Lu SPECT has potential for identifying clinically significant disease progression, the opposite may be a more difficult issue. 177Lu SPECT may struggle to confirm complete resolution of all sites of disease in men with exceptional responses to 177Lu-PSMA-617 therapy. Confirmation of an exceptional response may indeed require the spatial resolution of PSMA PET, and further research is required to more precisely define the limitations of 177Lu SPECT and appropriate minimal volume changes required to identify progression.

This study relied on quantitation of 177Lu SPECT data, rather than visual assessment, to determine an increase in TTV. It is becoming clear that assessment of treatment response using PSMA-based imaging for PSMA-targeted therapy must focus on changes in volume rather than measures of intensity (*27,28*). Accurate

visual assessment of changes in tumor volume can be difficult, especially in the presence of large-volume metastatic bone disease. We found that the repeatability of tumor volume on 177Lu SPECT was high and comparable to PSMA PET/CT (*29*). However, quantitation remains outside routine reporting guidelines and is time-intensive. Further work is needed both to evaluate the accuracy of quantitation over visual assessment and to streamline quantitation to be more user friendly for integration into routine clinical practice (*9*).

Changes in SPECT TTV were predictive of progression-free but not overall survival in this study. Although this finding may be due to the small patient cohort, it may also be due to the many patients with progressive disease on 12-wk posttherapy SPECT who were taken off trial and changed to an agent that proved effective. Further work is required to evaluate the benefit of 177Lu SPECT as a prognostic biomarker.

There were several limitations to this study. First, the patient numbers were small, and a larger cohort is needed to validate these findings. This was a single-center study, and 177Lu SPECT quantitative measures can vary significantly between centers and systems (*30*). Further evaluation is required to harmonize image acquisition and reconstruction across centers and imaging systems for results to be reproducible. Finally, appropriate volume cutoffs for a significant increase in SPECT TTV need to be defined. This will require trials with larger patient numbers and outcome data. However, this study provided a strong foundation on which to build further work.

CONCLUSION

Increasing SPECT TTV on quantitative 177Lu SPECT predicted a short PFS and identified progression in some men who had yet to demonstrate PSA progression. This tool shows promise as an imaging response biomarker.

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8 Discussion

8.1 Synthesis of key findings

The work reported in this thesis examined the use of 68Ga-PSMA-11 and 18F-FDG PET imaging in men with metastatic, castration-resistant prostate cancer (mCRPC) treated with 177Lu-PSMA-617. The chapters address baseline and post-treatment imaging assessment including baseline PET imaging (chapter 4), post-treatment PET imaging (chapter 5 and 6), and interim 177Lu SPECT response assessment (chapter 7). In this discussion chapter, the key findings of each of the 'results' chapters will first be summarised, followed by a review of the key themes arising from the research, and the strengths and limitations of the work. Finally, the significance of the work in the research and clinical contexts will be discussed.

Chapter 4 examined the baseline 68Ga-PSMA and 18F-FDG PET imaging and clinical characteristics associated with prostate specific antigen (PSA) response and overall survival (OS) in men being treated with 177Lu-PSMA-617. In multivariable analysis, higher baseline PSMA SUVmean and lower baseline PSMA total tumour volume (TTV) were associated with increased odds of response (defined as a PSA reduction ≥ 50%), while higher baseline PSMA TTV and shorter duration of treatment with androgen signalling inhibitor (ASI) (for less than <12 months) were associated with shorter OS. FDG total tumour volume (TTV) and presence of visceral metastatic disease did not remain prognostic in the multivariable model.

In chapter 5, 68Ga-PSMA and 18F-FDG PET imaging performed at baseline and post-treatment were evaluated for changes in intensity of PSMA/FDG expression and change in total tumour volume. We found that any increase in PSMA TTV and

PSA progression were independently associated with shorter overall survival. Although an increase in FDG TTV was associated with shorter PSA progression free survival (PSA PFS) and OS, it did not remain independently prognostic in multivariable analyses.

In chapter 6, a novel quantitative PET imaging analysis workflow (Traffic Light Workflow; TLW) was developed to classify the response of individual lesions and then categorise the overall disease response pattern in each participant. The workflow was feasible and demonstrated good repeatability. Overall responses were stratified into ‘responder’, ‘low volume progressor’ and ‘high volume progressor’. We found that heterogenous treatment responses are common, and even low volume disease progression in the presence of predominant response elsewhere was associated with shorter overall survival. Qualitative comparison with an existing set of criteria assessing response with PET imaging (RECIP 1.0) showed similar prognostic stratification by TLW response category.

Chapter 7 described a novel method for measuring total tumour volume on 177Lu SPECT imaging acquired 24 hours after treatment. 177Lu SPECT imaging analysis was feasible and showed high repeatability. A reduction in SPECT TTV at cycle 3 was associated with improved PSA PFS.

Theme 1: Change in total tumour volume on PSMA PET or 177Lu SPECT is important for assessing response to 177Lu-PSMA-617, but cannot be used as a single, definitive measure of response

The studies in chapter 5 and 7 demonstrated that a change in TTV (measured on PSMA PET or 177Lu SPECT respectively) is critical for assessing response, and that an increase in TTV following treatment is associated with poorer clinical outcomes.

In contrast, 95% of study participants had a reduction in measures of PSMA intensity (SUVmean and SUVmax), therefore these indices were not useful for assessing tumour response. These principles were applied in the novel TLW workflow studied in chapter 6, where a reduction in tumour volume defined a responding lesion, while progressing lesions were defined by an increase in volume and/or PSMA intensity.

However, the study in chapter 6 demonstrated that heterogeneous tumour responses were common (28/37 participants). Additionally, there was often discordance between the response assessment based on whole body PSMA TTV and the presence of new/increasing lesions. In this cohort, 16/37 participants had a reduction in PSMA TTV in the presence of new/increasing lesions. Therefore, criteria for assessment of response should incorporate both an assessment of PSMA TTV and an evaluation for new/increasing lesions. Furthermore, TLW enables identification of patterns of progression (such as progressive disease with persistently high PSMA expression, or oligoprogression) which may help individualise subsequent treatment selection. For example, we hypothesise that imaging showing disease progression with persistently high PSMA expression should be considered an indication for treatment with an alpha emitter.

Theme 2: 18F-FDG PET imaging indices were not prognostic of progression free or overall survival in this cohort

Chapters 3 and 4 identified that baseline FDG TTV was associated with shorter OS, and that an increase in FDG TTV on post-treatment imaging was associated with shorter PSA PFS and OS. However, neither of these 18F-FDG PET indices remained prognostic on multivariable analysis. This may be because there were a lower volumes and lower intensities of FDG-avid disease in this cohort than in

previously published studies (*1,2*). Additionally, no participants in our cohort had FDG-positive, PSMA-negative sites of disease progression, so we could not assess the significance of this phenotype.

Theme 3: Interim response assessment with 177Lu SPECT is feasible and prognostic for clinical outcomes

The study in chapter 6 demonstrated the feasibility of interim response assessment using imaging with 177Lu SPECT, and that an increase in SPECT TTV at cycle 3 was prognostic for PSA PFS. This suggests that patients who were not responding to treatment with 177Lu-PSMA-617 might be identified earlier in their treatment course, allowing for potential changes or escalation of their treatment. Interim response assessment with 68Ga-PSMA PET might be similarly useful. Further work is needed to validate the use of interim imaging (with either 68Ga-PSMA PET or 177Lu SPECT) for response assessment.

8.2 Strengths

The strengths of each study have been discussed in each chapter. These will be reviewed and considered collectively here.

The studies in this thesis used data collected from a prospective, single arm, single centre study that included pre-specified eligibility criteria, adherence to a treatment protocol, and follow up for pre-defined clinical endpoints. This is superior to previous work in the field which used retrospective designs, data collection, and analysis plans associated with a high risk of confounding and bias.

All our studies used semiautomated imaging analysis workflows to extract imaging parameters. Use of semiautomated workflows should reduce inter- and intra-observer variability, compared with manual lesion contouring. Additionally, the use of semiautomated workflows allows imaging parameters to be extracted more quickly, increasing the feasibility, efficiency and clinical applicability of these techniques.

The studies in chapters 5 and 6 developed novel imaging analysis workflows to assess response to treatment with 177Lu-PSMA-617. Both studies included work to define the repeatability of the new imaging assessment methods. Further work is planned to assess the inter-observer reliability of these methods (discussed in section 8.5).

8.3 Limitations

The data for studies above was drawn from a single centre study (LuPIN) including 56 participants. Participants in LuPIN may differ systematically from the wider general population of patients being treated with 177Lu-PSMA-617 outside of clinical trials.

Clinical trial participants may differ systematically from those in the general patient population. For example, patients suitable for trial may have fewer comorbidities and better functional status, and thus may have better clinical outcomes. The study was neither randomised nor comparative, limiting conclusions about treatment effects.

Furthermore, eligible participants in LuPIN trial were treated with at least two prior lines of therapy including chemotherapy with a taxane, and an ASI. Studies published after the commencement of LuPIN have demonstrated the activity of 177Lu-PSMA-617 used in combination with ASI, or in the second line setting

following progression on ASI (*3,4*). The findings of this thesis may not be applicable to patients treated with the combination of ASI and 177Lu-PSMA-617.

Additionally, the LuPIN trial selected individuals with high PSMA expression (participants were required to have SUVmax >15 on 68Ga-PSMA PET at 1 or more sites, an SUVmax of more than 10 at all measurable sites), which are more stringent than the eligibility criteria used in subsequent randomised trials. For example, the VISION trial required at least 1 metastatic lesion that was PSMA-positive (defined as uptake greater than that of liver parenchyma) and no PSMA-negative measurable lesions. Given that higher PSMA expression is associated with higher odds of treatment response, the rates and durations of treatment responses described in this work may also exceed those observed in routine clinical practice.

The LuPIN study utilised conventional imaging (CT and bone scan) at baseline and post-treatment, however no interim conventional imaging was undertaken. Therefore in chapter 7, interim 177Lu SPECT could not be compared with conventional imaging as a method of interim response assessment. More frequent use of conventional imaging may also have increased the detection of disease progression which was not 68Ga-PSMA or 18F-FDG avid.

Due to the modest sample size of our studies, important subgroups, such as patients with visceral disease or with significant 18F-FDG-avid disease, were not well represented. It is important that the findings of this thesis be tested in larger cohorts, to better evaluate these groups.

The sample size also limited the use of multivariable analyses. Assessment within a larger cohort would allow the prognostic markers identified in this thesis to be incorporated and tested within existing multivariable prognostic models.

The work in chapter 5 and 6 examined post-treatment PET. The imaging was performed after the completion of the planned 6 cycles of therapy, or after cessation of therapy earlier due to disease progression. The PET imaging performed in the LuPIN study was not incorporated into clinical decision-making for the study participants. Similarly, the work in chapter 7 examined interim 177Lu SPECT at cycle 3, but did not change study treatments. Therefore, the utility of PET or 177Lu SPECT in guiding treatment decisions cannot be evaluated. Future studies should aim to incorporate either PET or 177Lu SPECT imaging earlier in the treatment course.

8.4 Significance of the findings

Implications for clinical practice: Patient selection

The findings from chapter 3 may be applied clinically in the selection of patients for 177Lu-PSMA-617 treatment. The study identified that a higher PSMA SUVmean was associated with higher odds of response to 177Lu-PSMA-617. It was previously established that expression of the PSMA target is needed for Lu-PSMA to be a rational treatment. Our work builds on this observation by indicating that there is a relationship between increasing PSMA SUVmean and improved outcomes. Further work is needed to determine whether there is an optimal SUVmean cutoff point below which 177Lu-PSMA-617 treatment is unlikely to be effective.

Since the publication of these studies, a large retrospective study (*5*), and two relevant randomised clinical trials (TheraP and VISION) have been completed (*6-8*). The results of these three studies will be discussed below.

The retrospective multicentre study included 270 participants treated with 177Lu-PSMA-617. Almost all had prior treatment with an androgen signalling inhibitor (95%)

and most had prior docetaxel treatment (81%). The population was divided into a model development cohort (196 patients) and a validation cohort (74 patients). Baseline clinical and imaging markers were incorporated into a multivariable model to predict overall survival, and then validated in a separate cohort. In multivariable analysis, PSMA SUVmean (as a continuous variable) was associated with higher odds of PSA50 response (HR 2.88 95% CI 1.80 to 4.62) and longer overall survival (HR 0.94 95% CI 0.90 to 0.98). PSMA TTV was not assessed in this study. The validated model for overall survival incorporated statistically significant variables including: time since diagnosis, prior chemotherapy, plasma haemoglobin concentration, PSMA SUVmean, having more than 20 lesions, presence of bone metastases, and presence of liver metastases (*5*).

TheraP was a randomised trial that recruited men previously treated with docetaxel and an androgen signalling inhibitor, and randomly assigned them to treatment with either the standard of care chemotherapy (cabazitaxel), or 177Lu-PSMA-617. In univariable analysis, TheraP found that a PSMA SUVmean greater than 10 was associated with higher odds of PSA response (HR 12.19 95% CI 3.42 to 58.76). In a post-hoc subgroup analysis, there was a trend to favour cabazitaxel rather than 177Lu-PSMA-617 in participants with PSMA SUVmean less than 6.9 (HR 0.53 95% CI 0.15 to 1.74), but favoured 177Lu-PSMA-617 rather than cabazitaxel in those with PSMA SUVmean 6.9 or higher. Univariable analysis of PSMA TTV was not performed in this study (*2,7*).

The VISION trial recruited men previously treated with taxane chemotherapy and an androgen signalling inhibitor, with PSMA-positive disease (defined as at least one PSMA positive lesion with activity greater than physiological liver activity; and no PSMA-negative lesions). Participants were randomised to either best standard of

care or best standard of care with 177Lu-PSMA-617 (*8*). In an exploratory study of the baseline quantitative 68Ga-PSMA PET indices in 826 participants, they found that PSMA SUVmean was associated with higher odds of response (OR 1.39 95% CI 1.22 to 1.58), longer radiographic PFS (HR 0.86 95% CI 0.82 to 0.90) and longer overall survival (HR 0.88 95% CI 0.84 to 0.91) in the 177Lu-PSMA-617 arm. Although there appeared to be a linear relationship between increasing SUVmean and longer radiographic PFS and OS, an optimal SUVmean cutoff could not be determined. Tumour load (defined as PSMA TTV x SUVmean) was also associated with shorter radiographic PFS (HR 1.02 95% CI 1.01 to 1.04) and shorter OS (HR 1.04 95% CI 1.03 to 1.05), in the 177Lu-PSMA-617 arm. PSMA TTV as a standalone marker was not included in the multivariable model for the 177Lu-PSMA-617 arm (*9*).

Overall, our work and the studies described above support the use of PSMA SUVmean as a prognostic marker for response and overall survival in men with mCRPC.

The data in chapter 4 identified that a higher pre-treatment PSMA total tumour volume was prognostic for lower odds of PSA50 response (defined by PSA reduction > 50%) and shorter overall survival. Therefore, identifying and treating suitable patients earlier at a lower tumour volume may improve treatment outcomes. The data from VISION showed that a higher tumour burden, a measure which incorporates PSMA TTV (PSMA TTV x SUVmean), was associated with shorter radiographic PFS and OS, which is in line with the findings of this thesis (*9*). However, none of the other studies discussed above evaluated PSMA TTV as an independent prognostic marker for tumour response or survival.

In the TheraP study, FDG TTV > 200mL was predictive of worse overall survival, regardless of treatment arm (*2*). This may indicate that FDG avid disease has a more aggressive phenotype. This association was not identified in our study, although as previously discussed, the incidence and median volume of FDG-positive disease in our study was lower than in TheraP.

Overall, the prognostic utility of PSMA TTV remains unclear and warrants further evaluation.

Implications for clinical practice: Tumour response assessment

The work in this thesis also provides evidence supporting the use of change in PSMA TTV for assessment of tumour response to treatment with 177Lu-PSMA-617. When interpreting post-treatment PSMA PET, clinicians must be aware that a reduction in PSMA SUVmax or SUVmean does not always correlate with treatment response; this is in direct contrast to reductions in SUVmean and SUVmax on 18F-FDG PET imaging in other cancers which do consistently indicate a favourable response to treatment (*10,11*). Additionally, chapter 5 identified that heterogeneous responses to treatment may result in a reduction in PSMA total tumour volume and in PSA, in the presence of increasing or new lesions elsewhere. Clinicians need to search vigilantly for sites of progressive disease.

Recent studies support the use of PSMA TTV as a useful, early marker of response to 177Lu-PSMA-617 treatment. Response Evaluation Criteria In PSMA-PET/CT (RECIP) 1.0 propose four categories of response based on change in PSMA TTV and the presence or absence of new lesions after two cycles of treatment with 177Lu-PSMA-617 (*12*):

Response category	Definition
RECIP-CR (complete response)	Absence of any PSMA-uptake on interim PET
RECIP-PR (partial response)	Decline in PSMA TTV > 30% without appearance of new lesions
RECIP-SD (stable disease)	Insufficient decline in PSMA TTV to qualify for PR or Decline PSMA TTV > 30% with appearance of new lesions or Insufficient increase in PSMA TTV to qualify for PD or Increase in PSMA TTV > 20% without appearance of new lesions
RECIP-PD (progressive disease)	Increase in PSMA TTV > 20% with appearance of new lesions

Table 8-1. RECIP 1.0 response category definitions (Adapted from Gafita et al 2022) (12).

In this retrospective study of 124 men treated with at least 2 cycles of 177Lu-PSMA-617, baseline and post treatment PSMA PET performed at week 12 were examined using RECIP 1.0 criteria. Participants with RECIP-PD had a significantly shorter overall survival than those with RECIP-SD (HR 2.3 [95%CI 1.5 to 3.5]) or RECIP-PR (HR 3.0 [95%CI 1.8 to 5.0]). The study authors noted that 13% of participants had a reduction in PSMA TTV, but also had new lesions, and were classified as RECIP-SD.

Two other studies have applied RECIP 1.0 to assess response to treatment and demonstrated similar findings: one in a cohort treated with 177Lu-PSMA-617 and the other in a cohort with biochemical relapse (*13,14*). RECIP 1.0 appears to be effective in identifying responders and non-responders.

However, further work is needed to determine how to categorise and treat patients with mixed responses to treatment. In chapter 5, where we categorised LuPIN participants using RECIP 1.0, 51% were categorised as RECIP-SD. In other words, approximately half the participants had an indeterminate response to therapy. However, the TLW enabled identification of different patterns of disease progression within this group, including individuals with progressive disease with low PSMA expression, progressive disease with persistently high PSMA expression, or oligoprogression. Assessment of response to 177Lu-PSMA-617 with the TLW provided clinically relevant information that could be used to guide subsequent treatment.

In Chapter 6, we reported that quantitative analysis of 177Lu SPECT was feasible, and that a reduction in SPECT TTV between cycle 1 and cycle 3 was associated with improved PSA PFS. Two subsequent studies examining reductions in SPECT TTV between cycle 1 and cycle 2 also demonstrated an association with longer OS

(*15,16*). Another study using same-day 177Lu SPECT (1-2 hours after administration of 177Lu-PSMA-617) demonstrated an association between a reduction in SPECT TTV of >30% after 2 cycles and longer PSA PFS and longer OS (*17*). These studies support the use of 177Lu SPECT imaging as an early, interim marker of response, which may provide additional useful information above and beyond assessment of clinical and PSA indices during treatment with 177Lu-PSMA-617.

Most Australian nuclear medicine departments do not currently have access to the resources needed for quantitative analysis of tumour volume and PSMA intensity indices. Typical workstations only provide measures of SUVmax in a region of interest. This is an important barrier to implementing imaging biomarkers into clinical practice, patient selection, and response assessment.

Implications for the conduct of research

This thesis has implications for the design and conduct of future trials of 177Lu-PSMA-617. It is clear that quantitative analysis is important for patient selection and response assessment. Harmonisation of 68Ga-PSMA PET acquisition parameters is needed for centres participating in 177Lu-PSMA-617 clinical trials, so that 68Ga-PSMA PET imaging acquired from multiple sites is comparable (*18-20*). Collaboration from multiple centres is also integral to achieving the adequate power and sample size to validate response assessment criteria. Routine, quantitative analysis and assessment of response using 68Ga-PSMA PET should be integrated into future multicentre clinical trials.

Incorporation of quantitative analysis of 68Ga-PSMA PET and 177Lu SPECT would provide useful information in future studies of 177Lu-PSMA-617. However, it is

relatively costly, time consuming, and labour-intensive, and these are likely to be barriers to its wider, routine implementation and adoption. The development of accessible, semiautomated, workflows for imaging analysis should be a priority for future research, to make routine quantitative analysis more feasible in a research and routine clinical practice.

8.5 Future research

The findings in this thesis raise a number of important questions for future research.

The work in chapter 4 identified that a higher PSMA avid TTV was associated with lower odds of response to treatment and shorter OS. This may be overcome by tailoring the 177Lu-PSMA-617 dose to tumour volume. There is existing data demonstrating that patients with higher PSMA avid TTV demonstrate lower PSMA uptake in normal organs including salivary glands, liver and kidneys, and therefore a higher dose of 177Lu-PSMA-617 may be delivered without excess toxicity to non-target organs (*21*). Additionally, a study demonstrated that up to 22GBq 177Lu-PSMA-617 could be administered without increased toxicity in a dose fractionated single cycle (*22*). Future work could employ dosimetry to calculate a suitable 177Lu-PSMA-617 dose based on PSMA avid TTV.

There is still a paucity of data regarding patterns of response to treatment with 177Lu-PSMA-617, and future, larger studies should evaluate the characteristics and outcomes of subgroups, such as those with visceral disease or with FDG-positive disease. The recently reported ENZAp trial of 177Lu-PSMA-617 in addition to enzalutamide in mCRPC being treated with an androgen signalling inhibitor (ASI) for the first time, did not exclude patients with sites of disease that were FDG-positive

and PSMA-negative; evaluation of this subgroup in ENZA-p may provide useful information about changes in FDG-PET indices after treatment with 177Lu-PSMA-617 (*23*).

As discussed in section 8.4, optimal criteria for assessing response to 177Lu-PSMA-617 with 68Ga-PSMA PET have not yet been determined. As a starting point, future clinical trials of treatment with 177Lu-PSMA-617 should incorporate progress imaging with 68Ga-PSMA PET after 2 cycles of treatment to evaluate proposed criteria for assessment of response. This should include assessment of interobserver reliability and validation of TLW analysis in larger cohorts. Concurrent assessment and validation of 177Lu SPECT, particularly its interobserver reliability and use assessing response would also be valuable.

Once response assessment criteria have been validated, clinical trials implementing early response assessment after 2 cycles of treatment may be developed. Following early response assessment, participants with an excellent response might be warrant de-intensification or cessation of treatment with 177Lu-PSMA-617.

A retrospective analysis of a clinical cohort of 125 individuals treated with 177Lu-PSMA-617 outside of clinical trials, demonstrated how interim 177Lu SPECT imaging could be integrated into routine clinical practice. The analysis stratified patients into three response groups based on changes in SPECT TTV and PSA between cycle 1 and cycle 2. Treatment was adjusted according to the interim stratification of response shown in Table 8-2 below. The study authors reported that patients in group with the best response (group 1), had excellent subsequent outcomes (median OS 19 months, 95%CI: 17–21) and a median ‘treatment holiday’ of 6 months (*24*).

Response group	Definition	Treatment adjustment after 2 cycles
Response group 1	>50% reduction in PSA OR >30% reduction in total tumour volume	a break in treatment until subsequent PSA rise, then re-treatment
Response group 2	Stable or < 50% reduction in PSA OR change in total tumour volume < 30%	6-weekly treatments until six doses, or no longer clinically benefitting
Response group 3	Any rise in PSA OR >30% increase in total tumour volume	Recommended for an alternative treatment

Table 8-2. Response group definitions (Adapted from Emmett et al 2023) (24).

Further prospective studies are needed to corroborate these promising findings and approach, which might avoid the costs of serial imaging with 68Ga-PSMA PET, and might improve clinical outcomes by promptly identifying those who might benefit from treatment escalation and de-escalation.

An important finding of the work in this thesis was that participants with progressive disease often maintained high expression of PSMA. This may represent radiation resistant clones of disease, however further work is needed to understand the

radiobiology of prostate cancer treated with 177Lu-PSMA-617. More research is needed to identify suitable subsequent treatments for those who are not responding to 177Lu-PSMA-617 alone. For example, the use of alpha-emitting radioisotopes with higher linear energy transfer, such as Actinium-225, might overcome resistance to treatment with 177Lu-PSMA-617 (*25,26*). Alternatively, escalation to combination therapy may be of use in the population not responding to 177Lu-PSMA-617 alone. A single arm dose escalation study has shown activity of the combination of 177Lu-PSMA-617 with the PARP inhibitor olaparib, which increases the susceptibility of tumour cells to DNA damage by inhibiting DNA repair mechanisms (*27*). Another approach combining 177Lu-PSMA-617 with the immune checkpoint inhibitor pembrolizumab, which uses 177Lu-PSMA-617 to cause antigenic cell death, thus enhancing the activation of the body's immune system, has also shown promise (*28,29*).

8.6 Conclusions

We propose that imaging biomarkers are useful to better select patients, and to better assess response to treatment with 177Lu-PSMA-617. The work in this thesis examined candidate imaging biomarkers and demonstrated associations with improved clinical outcomes after treatment with 177Lu-PSMA-617.

Our analyses of baseline PET imaging revealed that PSMA SUVmean and PSMA TTV were prognostic of response to 177Lu-PSMA-617. An optimal cutoff for either PSMA SUVmean or PSMA TTV has not yet been established. However, clinicians should consider PSMA SUVmean when selecting patients for treatment with 177Lu-PSMA-617, and when discussing the probability of a response to treatment.

Following 177Lu-PSMA-617 treatment, a reduction in PSMA TTV was associated with longer overall survival, and might be used to identify responders and non-responders to 177Lu-PSMA-617. However, the Traffic Light workflow analysis demonstrated that heterogeneous treatment responses were common, and patients with a mixed response tended to have worse clinical outcomes. Further research is needed to develop response assessment criteria that capture the complexity of treatment response, and facilitate optimal adjustment of therapy.

Quantitative analysis of interim imaging with 177Lu SPECT was feasible, and changes in 177Lu SPECT TTV were associated with progression free survival. This would allow earlier assessment of response without the burden of additional PET imaging. Prospective studies are needed to test and validate this approach, and to evaluate response-adapted approaches to treatment with 177Lu-PSMA-617.

8.7 References

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9 Appendices: Publications arising from this thesis

9.1 177Lu-PSMA-617 and Idronoxil in Men with End-Stage Metastatic Castration-Resistant Prostate Cancer (LuPIN): Patient Outcomes and Predictors of Treatment Response in a Phase I/II Trial

^{177}Lu-PSMA-617 and Idronoxil in Men with End-Stage Metastatic Castration-Resistant Prostate Cancer (LuPIN): Patient Outcomes and Predictors of Treatment Response in a Phase I/II Trial

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^{177}Lu-PSMA-617 is an effective therapy for metastatic castration-resistant prostate cancer (mCRPC). However, treatment resistance occurs frequently, and combination therapies may improve outcomes. We report the final safety and efficacy results of a phase I/II study combining ^{177}Lu-PSMA-617 with idronoxil (NOX66), a radiosensitizer, and examine potential clinical, blood-based, and imaging biomarkers. **Methods:** Fifty-six men with progressive mCRPC previously treated with taxane chemotherapy and novel androgen signaling inhibitor (ASI) were enrolled. Patients received up to 6 doses of ^{177}Lu-PSMA-617 (7.5 GBq) on day 1 in combination with a NOX66 suppository on days 1–10 of each 6-wk cycle. Cohort 1 ($n = 8$) received 400 mg of NOX66, cohort 2 ($n = 24$) received 800 mg, and cohort 3 ($n = 24$) received 1,200 mg. ^{68}Ga-PSMA and ^{18}F-FDG PET/CT were performed at study entry, and semiquantitative imaging analysis was undertaken. Blood samples were collected for analysis of blood-based biomarkers, including androgen receptor splice variant 7 expression. The primary outcomes were safety and tolerability; secondary outcomes included efficacy, pain scores, and xerostomia. Regression analyses were performed to explore the prognostic value of baseline clinical, blood-based, and imaging parameters. **Results:** Fifty-six of the 100 men screened were enrolled (56%), with a screening failure rate of 26% (26/100) for PET imaging criteria. All men had received prior treatment with ASI and docetaxel, and 95% (53/56) had received cabazitaxel. Ninety-six percent (54/56) of patients received at least 2 cycles of combination NOX66 and ^{177}Lu-PSMA-617, and 46% (26/56) completed 6 cycles. Common adverse events were anemia, fatigue, and xerostomia. Anal irritation attributable to NOX66 occurred in 38%. Forty-eight of 56 had a reduction in prostate-specific antigen (PSA) level (86%; 95% CI, 74%–94%); 34 of 56 (61%; 95% CI, 47%–74%) had a PSA reduction of at least 50%. Median PSA progression-free survival was 7.5 mo (95% CI, 5.9–9 mo), and median overall survival was 19.7 mo (95% CI, 9.5–30 mo). A higher PSMA SUV_{mean} correlated with treatment response, whereas a higher PSMA tumor volume and prior treatment with ASI for less than 12 mo were associated with worse overall survival. **Conclusion:** NOX66 with ^{177}Lu-PSMA-617 is a safe and feasible strategy in men being treated with third-line therapy and beyond for mCRPC. PSMA SUV_{mean}, PSMA-avid tumor volume, and duration of treatment with ASI were independently associated with outcome.

Key Words: metastatic prostate cancer; theranostics; lutetium-PSMA

J Nucl Med 2022; 63:560–566
DOI: 10.2967/jnumed.121.262552

Metastatic castration-resistant prostate cancer (mCRPC) is a lethal disease, and treatment options remain limited. ^{177}Lu-prostate-specific membrane antigen-617 (^{177}Lu-PSMA-617) is a radioligand therapy that targets prostate-specific membrane antigen (PSMA), a receptor highly expressed on prostate cancer cells (*1*). ^{177}Lu-PSMA-617 has shown promising results in prospective single-center studies, the phase II TheraP trial, and the phase III VISION trial (*2–5*). However, secondary treatment resistance hinders longer-term outcomes for many men (*2*,*3*,*6*).

Combination therapies may overcome resistance mechanisms and improve clinical outcomes. Idronoxil (NOX66) is a derivative of the flavonoid genistein that binds to external NADH oxidase 2, a tumor-specific enzyme that induces apoptosis and inhibits topoisomerase II. It has shown potential as a radiation sensitizer in prostate cancer (*7–9*). We hypothesized that combining NOX66 with ^{177}Lu-PSMA-617 may improve treatment responses, with a minimal increase in toxicity.

Received May 10, 2021; revision accepted Jun. 29, 2021.
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Published online Jul. 29, 2021.

Improving treatment response with targeted radionuclide therapy involves not only optimizing treatment responses through effective combinations but also improving patient selection. Quantitative parameters on ^{68}Ga-HBEDD-PSMA-11 and ^{18}F-FDG PET/CT have shown potential as predictive and prognostic biomarkers for ^{177}Lu-PSMA-617 therapy (*6*,*10*–*13*). The duration of prior treatments and other markers of treatment resistance, such as androgen receptor splice variant 7, may also have prognostic utility (*11*,*14*,*15*). We report the results of a trial of combination NOX66 and ^{177}Lu-PSMA-617. Additionally, we evaluate the predictive and prognostic potential of blood-based markers, clinical factors, and molecular imaging.

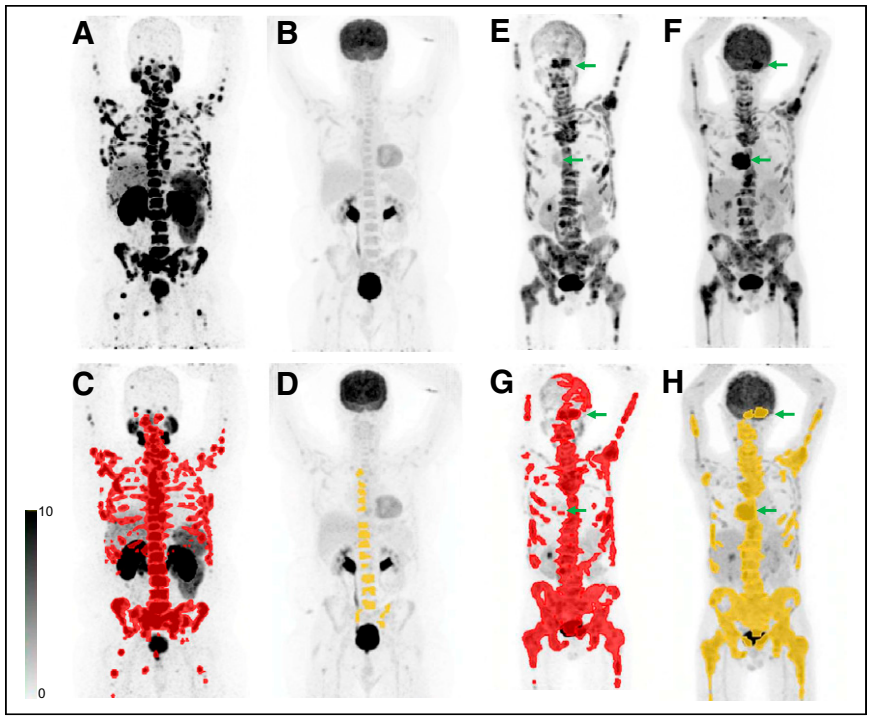

FIGURE 1. (A–D) Patient who was eligible on the basis of imaging with PSMA-avid disease (A) and no sites of discordant ^{18}F-FDG (B). Quantitative PSMA tumor volume (C) and ^{18}F-FDG tumor volume (D) are also shown. (E–H) Patient who was ineligible on the basis of imaging showing 2 sites (arrows) with higher ^{18}F-FDG avidity (F) than PSMA avidity (E). Quantitative PSMA tumor volume (G) and ^{18}F-FDG tumor volume (H) are also shown.

MATERIALS AND METHODS

Study Design

This was a prospective single-center phase I/II dose escalation/expansion trial of combination ^{177}Lu-PSMA-617 and NOX66. The St. Vincent's Hospital institutional review board approved the study protocol (HREC/17/SVH/19 and ACTRN12618001073291), and all patients provided written informed consent.

Screening

Men with mCRPC experiencing progression on conventional imaging (CT and bone scanning) or a rising level of prostate-specific antigen (PSA) based on Prostate Cancer Working group 3 criteria (*16*), and previously treated with at least 1 line of taxane chemotherapy (docetaxel or cabazitaxel) and at least 1 androgen signaling inhibitor (ASI) (abiraterone or enzalutamide), were screened. All patients had adequate organ function (baseline hemoglobin $\geq$ 100 g/L, platelet count $\geq$ 100 $\times$ 10^9/L, and estimated glomerular filtration rate $\geq$ 40 mL/min), an estimated life expectancy of more than 12 wk, and a World Health Organization Eastern Cooperative Oncology Group performance status of no more than 2.

Men underwent screening with ^{18}F-FDG and PSMA PET/CT, bone scanning, and CT and were eligible if they had an SUV_{max} of more than 15 on PSMA PET at 1 or more sites, an SUV_{max} of more than 10 at all measurable sites, and no ^{18}F-FDG avidity without corresponding PSMA uptake (Fig. 1).

Study Treatment

All men received up to 6 cycles of ^{177}Lu-PSMA-617 at 6-wk intervals in combination with 1 of 3 doses of NOX66 (400, 800, and 1,200 mg). NOX66 was administered via suppository on days 1–10 after each ^{177}Lu-PSMA-617 injection. All cohorts were administered 7.5 GBq of ^{177}Lu-PSMA-617 on day 1 via a slow intravenous injection. In addition, participants in cohort 1 (n = 8) received 400 mg of NOX66. After interim safety data reviews, the dose of NOX66 was escalated to 800 mg for cohort 2 (n = 24) and 1,200 mg for cohort 3 (n = 24).

The PSMA-617 precursor (AAA, Novartis) was radiolabeled to no-carrier-added ^{177}Lu-chloride according to the manufacturer's instructions by a qualified radiopharmacist or radiochemist. Quality control tests for radionuclidic and radiochemical purity were performed using high-pressure liquid chromatography and thin-layer chromatography. NOX66 (Noxopharm Ltd.) was commercially produced.

Imaging Procedures and Analysis

^{68}Ga-HBEDD-CC PSMA-11 was produced on-site in compliance with good laboratory practices using a Trasis automated radiopharmacy cassette. ^{18}F-FDG was produced off-site commercially. Radiopharmacy quality control testing used a high-pressure liquid chromatography method. Patients were injected with a 2.0 MBq/kg dose of PSMA and a 3.5 MBq/kg dose of ^{18}F-FDG, with identical imaging parameters (dose, time after injection, and imaging protocols) for each patient. All PET/CT imaging was undertaken using a Phillips Ingenuity TOF-PET/64-slice CT scanner. An unenhanced low-dose CT scan was performed 60 min after tracer injection. Immediately after CT, a whole-body PET scan was acquired for 2 min per bed position.

PET/CT scans were analyzed semiquantitatively using MIM software and a standardized semiautomated workflow to delineate regions of interest with a minimum SUV_{max} cutoff of 3 for PSMA and blood pool intensity, plus 1.5 SDs for ^{18}F-FDG (*17*). Quantitation derived total metabolic tumor volume, SUV_{max}, SUV_{mean}, and total lesional activity for both ^{18}F-FDG and PSMA (MIM Software).

Study Endpoints

Safety and tolerability were assessed using the National Cancer Institute Common Terminology Criteria for Adverse Events (version 5.0) every 2 wk during each 6-wk cycle until 6 wk after the final study treatment. To assess efficacy, we measured the PSA decline from baseline (absolute and $\geq$50% [PSA50]) at any time point, PSA progression-free survival (PFS), and overall survival (OS). Time-to-event endpoints (PSA PFS and OS) were defined as the interval from the date of enrollment to the event date, or the date last known to be event-free (at which point the observation was censored). Patient-reported outcomes were measured on day 1 of each cycle and during follow-up using the University of Michigan xerostomia-related quality-of-life scale (XeQoLS) (*18*) and the short form of the brief pain

inventory (*19*). Pain palliation was defined as a reduction by at least 30% in the worst pain intensity score over the last 24 h observed at 2 consecutive evaluations (*20*).

Clinical, Blood-Based, and Molecular Analysis

Clinical information regarding initial diagnosis, Gleason score, previous lines of therapy, and prior treatment responses was collected. Blood was prospectively collected for biomarker measurement, including hemoglobin, platelets, alkaline phosphatase, albumin, and PSA. Whole-blood samples were collected at baseline, before cycle 3, and before cycle 6 for analysis of potential molecular biomarkers, including androgen receptor splice variant 7. Androgen receptor splice variant 7 analysis was performed using the method described by To et al. (*21*).

Statistical Analyses

The study sample size was calculated to characterize the toxicity profile of the combination, based on the expectation that an adverse event with a true 5% incidence would be detected with 70% probability in a sample of 24 and detected with over 90% probability in a sample of 56. All patients who received at least 1 cycle of study treatment were included in the safety and efficacy analyses. *P* values below 5% were considered significant but interpreted cautiously. A 2-sided exact binomial 95% CI was calculated for PSA response rates. The Kaplan–Meier method was used to characterize time-to-event endpoints (PSA PFS, and OS) and to estimate medians (presented with 95% CIs). The 3 NOX66 dose levels were compared in terms of adverse events, PSA50, and OS.

Cox proportional-hazards regression, and logistic regression, were used to identify prognostic factors for time-to-event (PSA PFS, and OS) and binary endpoints (PSA50), respectively. The covariates investigated included baseline clinical, blood-based, and imaging parameters, including tumor volume and intensity scores (SUV_{max} and SUV_{mean}). In the absence of compelling evidence of a dose–response effect on PSA50, the cohorts were grouped, and prognostic analyses were performed on the grouped cohort.

We used the relaxed LASSO (least absolute shrinkage and selection operator) regression method to identify covariates for inclusion in a multivariable model (*22*). These were fitted in a standard multivariable Cox regression model to obtain conventional hazard ratios (HRs), 95% CIs, and *P* values.

All patients with a worst pain score of at least 4 were included in the analysis, and changes in score between baseline, precycle 3, and end of treatment were compared. Scores from the XeQoLS questionnaire were compared between baseline, precycle 3, and end of treatment. A 2-tailed paired *t* test was used to assess for a change in scores. Analyses were performed using R (version 4.0.5) and SPSS (version 25).

RESULTS

Baseline Patient Characteristics

One hundred men were screened, of whom 56 (56%) were enrolled between November 2017 and February 2020. Twenty-six percent were ineligible on the basis of the PET imaging criteria (13% because of low-PSMA-intensity disease and 13% because of sites with ^{18}F-FDG/PSMA mismatch). Remaining screening fails (18%) were from clinical deterioration (6%), concurrent illness (3%), low hemoglobin (7%), or personal reasons (2%). Baseline characteristics are summarized in Table 1. All patients had prior treatment with at least 1 ASI and taxane chemotherapy, with 95% (53/56) having 2 lines of taxane chemotherapy; 66% (37/56) had at least 20 PSMA-avid metastases, 88% (49/56) had metastases in bone, 55% (31/56) had metastases in lymph nodes, and 19% (11/56) had visceral metastases.

TABLE 1
Patient Characteristics (n = 56)

Characteristic	Data
Age (y)	69 (64–74)
Eastern Cooperative Oncology Group status	
0 or 1	49 (88)
2	7 (12)
PSA at C1 (μg/L)	115 (46–476)
Hemoglobin (reference range [RR], 130–180 g/L)	122 (110–131)
Alkaline phosphatase (RR, 30–100 U/L)	113 (86–231)
Albumin (RR, 36–52 g/L)	38 (34–41)
De novo metastatic disease	29 (52)
Gleason score	
≤7	9 (16)
8–10	35 (63)
Unknown/not available	12 (21)
Prior systemic treatments	
Luteinizing hormone–releasing hormone agonist or antagonist	56 (100)
Chemotherapy	56 (100)
Docetaxel	56 (100)
Cabazitaxel	53 (91)
Other chemotherapy	5 (9)
ASI	56 (100)
Enzalutamide only	27 (48)
Abiraterone only	13 (23)
Abiraterone + enzalutamide	16 (29)
Clinical trial medication	4 (7)
PSMA PET	
SUV_{mean}	8 (7–10)
SUV_{max}	39 (29–61)
Volume (L)	0.64 (0.19–1.21)
^{18}F-FDG PET	
SUV_{mean}	4 (3–5)
SUV_{max}	8 (5–10)
Volume (L)	0.07 (0.02–0.31)
Disease volume	
<20 metastases	19 (33)
≥ 0 metastases	37 (66)
Sites of disease on PSMA PET	
Bone	49 (88)
Lymph node	31 (55)
Viscera	12 (21)

Qualitative data are absolute counts and percentage; continuous data are median and interquartile range.

Because of the small numbers in each NOX66 dose cohort, with ^{177}Lu-PSMA-617 as the key treatment, we combined the 3 patient cohorts for reporting of outcomes and for exploratory analysis of

TABLE 2
Summary of Common and Therapeutically Relevant Adverse Events (n = 56)

Adverse event	Grade 1	Grade 2	Grade 3	All grades
Anemia	31 (55)	16 (29)	4 (7)	51 (91)
Xerostomia	30 (54)	3 (5)	0 (0)	33 (59)
Fatigue	27 (48)	8 (14)	0 (0)	35 (63)
Anal inflammation	18 (32)	3 (5)	0 (0)	21 (38)
Nausea	15 (27)	0 (0)	0 (0)	15 (27)
Thrombocytopenia	12 (21)	3 (5)	0 (0)	15 (27)
Constipation	11 (20)	1 (2)	0 (0)	12 (21)
Neutropenia	5 (9)	0 (0)	0 (0)	5 (9)
Pneumonitis*	0 (0)	1 (3)	0 (0)	1 (3)

*Attributed to radiation therapy prior to enrollment.
Data are number followed by percentage.

biomarkers of response and survival. Analysis of the 3 dose escalation cohorts of NOX66 did not reveal any statistical differences in adverse events, PSA response rate, PSA PFS, or OS.

Safety and Tolerability

Adverse events were predominantly grade 1 (149/188; 79%). The most common toxicities were anemia (50/56; 89%), fatigue (36/56; 64%), and xerostomia (33/56; 59%) (Table 2). Anal inflammation due to the NOX66 suppository occurred in 38% (21/56), with 27% (15/56) requiring topical treatment for anal inflammation. The rate of grade 1 anal inflammation was higher in cohort 3 (46%) than in cohort 1 or 2 (25% and 21%, respectively). Two men in cohort 2 and 1 man in cohort 3 required dose reduction or omission of NOX66. Four cases of grade 3 anemia were reported. There were no other significant differences in toxicities across the 3 cohorts and no grade 4–5 adverse events or treatment-related deaths.

Treatment Duration

Participants received a median of 5 (interquartile range, 3–6) cycles of ^{177}Lu-PSMA-617 and NOX66; 96% (54/56) received at least 2 cycles, and 46% (26/56) completed all 6 cycles. Of the 30 participants who ceased treatment before completing 6 cycles, 2 participants ceased because of exceptional responses, and the other patients ceased because of progressive disease (46%, n = 26), withdrawal of consent (2%, n = 1), or inability to continue the study because of COVID-19 travel restrictions (2%, n = 1). One participant ceased NOX66 because of grade 2 anal inflammation but continued ^{177}Lu-PSMA-617. No participants ceased LuPSMA-617 because of toxicity.

Treatment Response

At a median follow-up of 21.8 mo, PSA50 occurred in 61% (34/56; 95% CI, 47%–74%), whereas any decline in PSA occurred in 86% (48/56; 95% CI, 74%–94%). The waterfall plot of best PSA responses at any time point is shown in Figure 2. At the time of this analysis, 91% (51/56) of participants have had PSA progression and 66% (37/56) have died. The median PSA PFS was 7.5 mo (95% CI, 5.9–9.0 mo) (Fig. 3A), and the median OS was 19.7 mo (95% CI, 9.5–30.0 mo) (Fig. 3B).

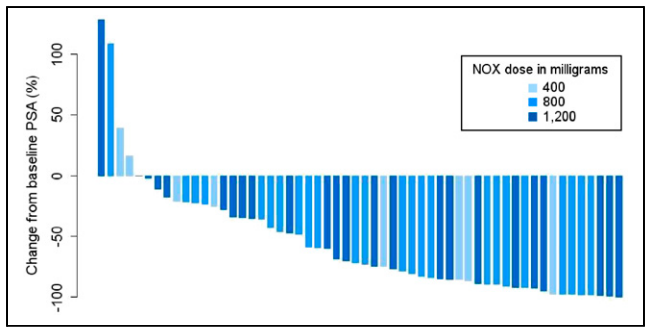

FIGURE 2. Waterfall plot of best PSA responses as indicated by maximum percentage change from baseline at any time point. NOX = NOX66.

Quality of Life

The baseline brief pain inventory assessment (short form) was completed by 95% (53/56) of the men. The mean worst pain score at baseline was 4.21 (range, 0–10; SD, 2.99). Fifty-six percent (29/52) of the men recorded a worst pain score of at least 4, and of these, 41% (12/29) experienced pain palliation at any time point.

The baseline XeQoLS assessment was completed by 48 (86%) of 56 men at baseline, with serial results at cycle 3 (48/56) and cycle 6 (26/48). There was no significant difference in XeQoLS scores between baseline and cycle 3, but a statistically significant difference was found between baseline and cycle 6 (P = 0.04). There were no differences in XeQoLS scores among the 3 dose levels.

Potential Prognostic Factors

We performed exploratory univariable analysis to identify potential markers of PSA50 and OS.

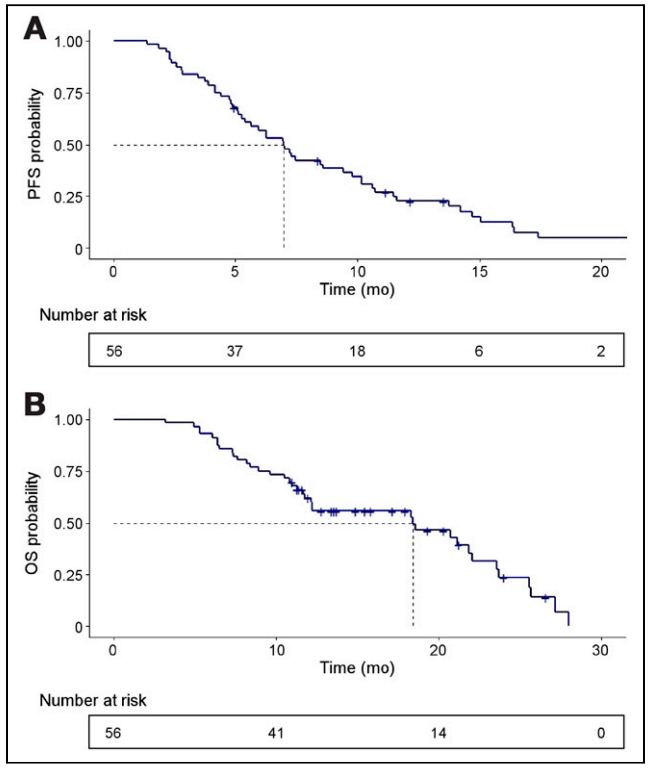

FIGURE 3. Kaplan–Meier curves for PSA progression-free survival (A) and OS (B).

Quantitative PET Imaging Markers. Comparative screening imaging characteristics are detailed in Table 1. A higher tumor volume on PSMA PET was associated with a lower likelihood of achieving PSA50 (odds ratio [OR], 0.41 [95% CI, 0.19–0.87]; $P = 0.02$) and shorter OS (HR, 2.18 [95% CI, 1.36–3.51]; $P = 0.001$). PSMA SUV_{mean} was associated with an increased likelihood of achieving PSA50 (OR, 1.57 [95% CI, 1.12–2.19]; $P = 0.008$). A higher ^{18}F-FDG–avid tumor volume at baseline was associated with a worse OS (HR, 3.02 [95% CI, 1.04–8.79]; $P = 0.04$). The presence of visceral metastases was also associated with a worse OS (HR, 2.35 [95% CI, 1.06–5.20]; $P = 0.04$).

Impact of Prior Treatments on Outcome. The most common treatment immediately before enrollment in the trial was cabazitaxel (70%, 39/56). Receiving either chemotherapy or ASI immediately before the trial did not predict treatment response or survival. Similarly, the duration of chemotherapy did not predict a response to therapy. However, an ASI treatment duration of more than 12 mo was significantly associated with improved OS (HR, 0.45 [95% CI, 0.22–0.91]; $P = 0.03$).

Blood-Based Markers. A higher baseline level of hemoglobin was associated with higher odds of PSA50 (OR, 1.05 [95% CI, 1.01–1.10]; $P = 0.03$) and improved OS (HR, 0.96 [95% CI, 0.93–0.99]; $P = 0.004$). Other known prognostic markers, including baseline alkaline phosphatase, PSA, and use of opioid analgesia, did not correlate with outcome.

Thirty-five patients had androgen receptor splice variant 7 (ARV7) assessed before cycle 1; of these, 9 (26%) were ARV7-positive. Two patients remained positive at cycle 3, and 2 patients became positive while on treatment. A total of 11 patients had ARV7 detected at any time point. The presence of ARV7 at any time point was not significantly associated with treatment response or survival.

Multivariable Analysis of Potential Prognostic Factors. A higher PSMA mean intensity score (SUV_{mean}) (OR, 1.61 [95% CI, 1.12–3.32]; $P = 0.01$) and a lower PSMA tumor volume (OR, 0.42 [95% CI, 0.18–0.94]; $P = 0.04$) remained predictive of PSA50, whereas PSMA tumor volume (HR, 2.19 [95% CI, 1.38–3.46]; $P = 0.001$) predicted a worse OS (Fig. 4). The only clinical parameter predictive of survival was treatment with ASI for more than 12 mo (HR, 0.42 [95% CI, 0.20–0.87]; $P = 0.02$). Baseline ^{18}F-FDG tumor volume, presence of visceral disease, and hemoglobin did not remain independently predictive of outcome (Tables 3 and 4).

DISCUSSION

PSMA-targeted radionuclide therapy is emerging as a new treatment paradigm in men with mCRPC. The randomized TheraP trial demonstrated a significantly improved treatment response (PSA50), PFS, and quality-of-life parameters for ^{177}Lu-PSMA-617, compared with cabazitaxel chemotherapy, in mCRPC. However, whereas results from TheraP are encouraging, PFS remains short, with a median of 5.1 mo (95% CI, 3.4–5.7) (*4*). We postulate that deepening and prolonging responses to ^{177}Lu-PSMA-617 therapy for men with mCRPC may be possible by targeting intracellular resistance mechanisms to maximize treatment effect. This article reports the results of a dose escalation trial of ^{177}Lu-PSMA-617 with a radiation sensitizer (NOX66) and evaluates potential predictive markers of response to PSMA-targeted therapy.

Treatment response rates to the ^{177}Lu-PSMA-617 and NOX66 combination were high with a 61% PSA50, even though most men in this trial had high-volume disease, baseline anemia, high baseline opiate requirements, and 95% had undergone 2 lines of taxane therapy. Despite these high-risk features, the treatment response rate is in line with previous prospective single-center trials and the TheraP study (range, 36%–66%) (*2–4*). Further, PFS and OS were longer than those reported in previous studies with ^{177}Lu-PSMA-617 and longer than those reported using alternative treatments after taxane chemotherapy (*3*,*23*). These results are encouraging for men with ASI and taxane-refractory mCRPC, but a randomized trial—possibly with less stringent imaging enrollment criteria—will be required to determine whether these results are due to the novel treatment combination or to patient selection.

NOX66 was included in the trial as a potential tumor-specific radiation sensitizer that binds to external NADH oxidase 2, a tumor-specific enzyme inducing apoptosis and inhibiting topoisomerase II and demonstrating radiation sensitization in preclinical models (*7*). We did not find an association between an increasing dose of NOX66 and PSA50 or OS. However, the role of NOX66 in the study was as a radiation sensitizer, rather than having a direct effect on therapy, and it may be that either the lower dose of NOX66 was sufficient to induce radiation sensitivity or the impact of NOX66 was limited. The safety of combination therapy with ^{177}Lu-PSMA-617 and NOX66 has been previously reported for the first 2 cohorts of the LuPIN trial and was confirmed in this study (*24*).

Predictors of treatment response are important to further improve PSMA-targeted therapy. Men were screened for this study with molecular imaging, with a requirement for an SUV_{max} of at least 15 on PSMA PET and no sites of ^{18}F-FDG/PSMA PET mismatch. An SUV_{max} of at least 15 has been previously reported to stratify men into responders and nonresponders for ^{177}Lu-PSMA-617 therapy (*2*). Hence it is not surprising that PSMA SUV_{max} was not predictive of a treatment response in this study. However, PSMA SUV_{mean} was an independent predictor of treatment response in this

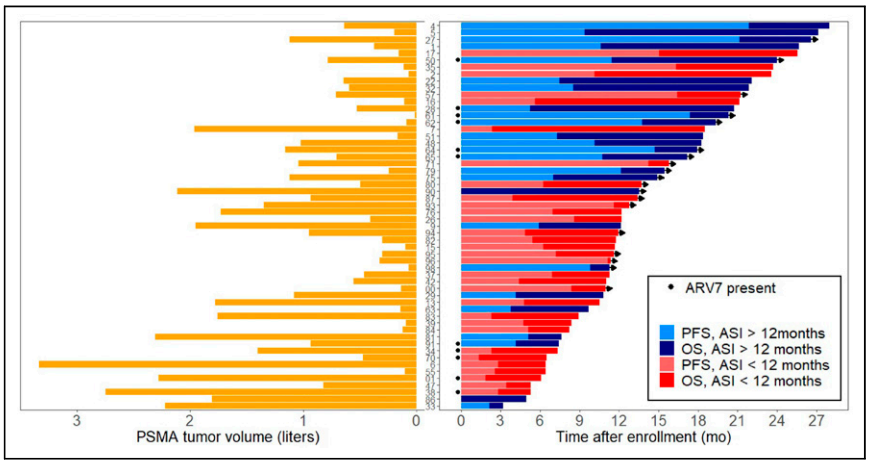

FIGURE 4 Graphical representation of important markers of OS. (Right) Swimmer plot showing individual-patient PFS and OS. (Left) Graph showing corresponding baseline tumor volume for each patient.

TABLE 3
Final Multivariable Model for Association of Baseline Markers with PSA50

Variable	LASSO OR	Multivariable logistic regression			Backward elimination model		
		OR	95% CI	*P*	OR	95% CI	*P*
PSMA TV	0.73	0.47	0.20–1.09	0.08	0.42	0.18–0.94	0.04
PSMA SUV_{mean}	1.20	1.61	1.10–2.34	0.01	1.61	1.12–2.32	
Hemoglobin	1.02	1.04	0.99–1.10	0.12	NA	NA	NA

TV = tumor volume; NA = not applicable.

TABLE 4
Final Multivariable Model for Association of Baseline Markers with OS

Variable	LASSO OR	Multivariable Cox regression			Backward elimination model		
		HR	95% CI	*P*	HR	95% CI	*P*
PSMA TV	1.67	2.05	1.19–3.53	0.009	2.19	1.381–3.463	0.001
ASI > 12 mo	0.70	0.56	0.24–1.31	0.56	0.42	0.202–0.869	0.02
^{18}F-FDG tumor volume (L)	NA	0.99	0.25–3.98	0.99	NA	NA	NA
Hemoglobin	0.99	0.98	0.95–1.02	0.30	NA	NA	NA
Presence of visceral disease	1.499	2.01	0.89–4.53	0.09	NA	NA	NA

TV = tumor volume; NA = not applicable.

study and previously (*10*). A relationship between a higher SUV_{mean} and improved clinical outcomes is biologically plausible. Intra- and interlesional heterogeneity of PSMA is common in mCRPC, and high heterogeneity of expression is likely to impact treatment response (*25*). SUV_{mean} is likely a better indicator of lesional heterogeneity than is SUV_{max}. Further, dosimetry studies have shown that SUV_{mean} correlates with the mean absorbed radiation dose and treatment response (*13*). Although SUV_{mean} requires quantitative analysis, its repeated association with treatment response suggests that it may have a future role as a predictive biomarker for PSMA-targeted radionuclide therapy.

PSMA tumor volume at baseline was the strongest independent predictor of treatment response and was also prognostic for OS. ^{18}F-FDG tumor volume was also prognostic, but not independently of other variables. Essentially, men with higher tumor volumes responded poorly to treatment. This finding agrees with retrospective analyses of men undergoing ^{177}Lu-PSMA-617 therapy (*12*,*26*) and raises questions about the timing of PSMA-targeted therapy in men with mCRPC, suggesting that earlier referral for treatment after prior treatment failure may both improve treatment responses and prolong survival.

The duration of response to prior therapies may help predict the treatment response to PSMA-targeted agents and OS. We found that men with a shorter duration of response to ASI (<12 mo) had a worse OS, though the depth of the treatment response was not affected. By contrast, the duration of the response to chemotherapy, or whether the patient received chemotherapy or ASI immediately before the trial, was not predictive of either survival or response.

This study enrolled a population of heavily pretreated men with mCRPC; therefore, the identified prognostic markers may not be generalizable to other stages of prostate cancer. Larger studies are needed to validate the prognostic markers identified in this study.

CONCLUSION

^{177}Lu-PSMA-617 in combination with NOX66 is a safe treatment for heavily pretreated men with mCRPC, with encouraging results that warrant further evaluation. PSMA SUV_{mean} and tumor volume merit further investigation as imaging markers of treatment response and survival.

DISCLOSURE

This investigator-initiated study was sponsored by St. Vincent's Hospital Sydney and supported by a Cancer Institute NSW prostate translational research grant. Noxopharm Limited provided funding for drug and PET scans, and AAA/Novartis provided PSMA-617 ligand. Edmond Kwan receives honoraria from Janssen, Ipsen, and Astellas Pharma; has a research review, consulting, or advisory role with Astellas Pharma, Janssen and Ipsen; and receives research funding from Astellas Pharma and AstraZeneca. Anthony Joshua has an advisory role with Noxopharm Limited and receives institutional funding from Novartis. Louise Emmett has an advisory role with Noxopharm Limited and receives trial support from Novartis and Astellas. No other potential conflict of interest relevant to this article was reported.

ACKNOWLEDGMENTS

We thank the patients, as well as the clinical trials teams at the Department of Theranostics and Nuclear Medicine and the Kinghorn Cancer Centre, St. Vincent's Hospital, for their support.

KEY POINTS

QUESTION: Is combination therapy with ^{177}Lu-PSMA-617 and NOX66 feasible and safe?

PERTINENT FINDINGS: This phase I/II dose escalation and expansion study found that the combination is feasible and potentially efficacious. Evaluation of clinical, blood-based, and quantitative imaging markers identified PSMA SUV_{mean}, tumor volume, and duration of prior treatment with ASI as potential prognostic markers.

IMPLICATIONS FOR PATIENT CARE: Further randomized studies combining ^{177}Lu-PSMA-617 and NOX66 are needed. Quantitative imaging markers correlate with treatment response and survival and should be explored further.

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9.2 The Prognostic Value of Posttreatment 68Ga-PSMA-11 PET/CT and 18F-FDG PET/CT in Metastatic Castration-Resistant Prostate Cancer Treated with 177Lu-PSMA-617 and NOX66 in a Phase I/II Trial (LuPIN).

The Prognostic Value of Posttreatment ^{68}Ga-PSMA-11 PET/CT and ^{18}F-FDG PET/CT in Metastatic Castration-Resistant Prostate Cancer Treated with ^{177}Lu-PSMA-617 and NOX66 in a Phase I/II Trial (LuPIN)

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^{177}Lu-PSMA-617 therapy has shown high prostate-specific antigen (PSA) response rates in men with metastatic castration-resistant prostate cancer. However, early treatment resistance is common. This LuPIN substudy aimed to determine the prognostic value of posttreatment quantitative PET for PSA progression-free survival (PFS) and overall survival (OS) with ^{177}Lu-PSMA-617 therapy. **Methods:** Fifty-six men with progressive metastatic castration-resistant prostate cancer were enrolled in the LuPIN trial and received up to 6 doses of ^{177}Lu-PSMA-617 and a radiation sensitizer (NOX66). ^{68}Ga-PSMA-11 and ^{18}F-FDG PET/CT, diagnostic CT, and bone scanning were performed at study entry and exit. Quantitative analysis tracked change in total tumor volume (TTV) and SUV. Univariable and multivariable analyses were conducted to examine the association of change in TTV (continuous and >30%), SUV_{max}, PSA, and radiographic progression with PSA PFS and OS. **Results:** All men (37/56) who underwent both screening and posttreatment molecular imaging were analyzed; 70% (26/37) had a PSA response of more than 50%. Median PSA PFS was 8.6 mo, and median OS was 22 mo. Clinical progression had occurred at trial exit in 54% (20/37). In response to treatment, a reduced PSMA SUV_{max} was demonstrated in 95% (35/37) and a reduced PSMA TTV in 68% (25/37). An increase in PSMA TTV by at least 30% was associated with worse OS (median, 10.2 vs. 23.6 mo; $P = 0.002$). Change in PSMA SUV_{max} was not associated with PSA PFS or OS. ^{18}F-FDG SUV_{max} was reduced in 51% (18/35) and ^{18}F-FDG TTV in 67% (22/35). An increased ^{18}F-FDG SUV_{max} was associated with worse OS (median, 20.7 vs. 25.7 mo; $P < 0.01$). An ^{18}F-FDG TTV increase by more than 30% was associated with a short PSA PFS (median, 3.5 vs. 8.6 mo; $P < 0.001$) but not OS. Both PSA and radiographic progression were associated with shorter OS (median, 14.5 vs. 25.7 mo [$P < 0.001$] and 12.2 vs. 23.6 mo [$P = 0.002$]). On multivariable analysis, only increased PSMA TTV and PSA progression remained independently prognostic of OS (hazard ratio, 5.1 [95% CI, 1.5–17.1; $P = 0.008$] and 3.5 [95% CI, 1.1–10.9; $P = 0.03$], respectively). **Conclusion:** Change in quantitative PSMA TTV has strong potential as a prognostic biomarker with ^{177}Lu-PSMA-617 therapy, independent of ^{18}F-FDG PET parameters, PSA, or radiographic progression. Further research into the value of posttreatment PET as an imaging biomarker is warranted.

Key Words: metastatic prostate cancer; theranostics; lutetium-PSMA; prognosis; therapy response

J Nucl Med 2023; 64:69–74
DOI: 10.2967/jnumed.122.264104

In the VISION trial, ^{177}Lu-PSMA-617–targeted therapy improved overall survival (OS) and progression-free survival (PFS) in metastatic castration-resistant prostate cancer when compared with the standard of care, and in the TheraP trial it yielded a higher prostate-specific antigen (PSA) response rate and PSA PFS than second-line chemotherapy with cabazitaxel (*1,2*). However, further work is needed to deepen treatment response and prolong survival. ^{68}Ga-PSMA-11 PET/CT (^{68}Ga-PSMA PET) and ^{18}F-FDG PET/CT (^{18}F-FDG PET) have been used as screening tools in prospective trials to select patients most likely to respond to ^{177}Lu-PSMA-617–targeted treatments (*1–4*). Less work has been done using molecular imaging to monitor treatment response to ^{177}Lu-PSMA-617 therapy (*5–8*). Preclinical studies have confirmed that there is considerable interpatient and intrapatient heterogeneity of PSMA expression (*9,10*). We hypothesized that an increase in uptake or tumor volume on ^{68}Ga-PSMA PET or ^{18}F-FDG PET might have potential as a prognostic biomarker in men being treated with ^{177}Lu-PSMA-617.

In this study, we aimed to determine whether changes in total tumor volume (TTV) and SUV on both ^{68}Ga-PSMA PET and ^{18}F-FDG PET correlate with clinical outcomes in a prospective trial of treatment with ^{177}Lu-PSMA-617.

MATERIALS AND METHODS

This was an imaging substudy of the LuPIN trial. The LuPIN trial is a prospective single center, phase I/II dose escalation and expansion trial of combining ^{177}Lu-PSMA-617 with NOX66. The study enrolled men with

Received Mar. 5, 2022; revision accepted Jun. 13, 2022.
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Published online Jun. 23, 2022.

metastatic castration-resistant prostate cancer previously treated with both at least 1 line of taxane chemotherapy and an androgen signaling inhibitor. The clinical results have been previously published (*11,12*). St. Vincent's Hospital institutional review board approved the study protocol (HREC/17/SVH/19 ACTRN12618001073291), and all participants provided written informed consent.

Screening

Men with progressive metastatic castration-resistant prostate cancer, based on either conventional imaging (CT and bone scanning) or a rising serum concentration of PSA based on Prostate Cancer Working Group 3 criteria (*13*), were eligible for screening. The men underwent screening with ^{18}F-FDG PET and ^{68}Ga-PSMA PET, bone scanning, and CT of the chest, abdomen, and pelvis. Patients were eligible if they had an SUV_{max} of more than 15 on ^{68}Ga-PSMA PET at 1 or more sites, an SUV_{max} of more than 10 at all measurable sites, and no ^{18}F-FDG PET avidity without corresponding PSMA uptake. All men with ^{68}Ga-PSMA PET and ^{18}F-FDG PET at both baseline and after treatment were included in this substudy.

Study Treatment

All men received up to 6 doses of ^{177}Lu-PSMA-617 at 6-wk intervals, with 3 dose-escalated cohorts of NOX66. NOX66 was provided as a dose-appropriate suppository taken from days 1 to 10 after each ^{177}Lu-PSMA-617 injection. All cohorts were administered 7.5 GBq of ^{177}Lu-PSMA-617 on day 1 via a slow intravenous injection. The PSMA-617 precursor (AAA Novartis) was radiolabeled to no-carrier-added ^{177}Lu-chloride according to the manufacturer's instructions. Quality control tests for radionuclide and radiochemical purity were performed using high-pressure liquid chromatography and thin-layer chromatography. NOX66 suppositories were administered at 400-, 800-, and 1,200-mg doses per a dose escalation protocol (*11*).

Imaging Procedures and Acquisition

^{68}Ga-PSMA PET and ^{18}F-FDG PET were performed at baseline (screening) and after treatment (6 wk after completing all 6 cycles or when treatment ceased earlier because of clinical progression). ^{68}Ga-HBEDD-CC PSMA-11 was produced on-site in a manner compliant with good-laboratory-practice procedures using a TRASIS automated radiopharmacy cassette. ^{18}F-FDG was produced off-site commercially under good-manufacturing-practice–compliant conditions. Radiopharmacy quality control was undertaken using a high-pressure liquid chromatography method. Patients were injected with a 2.0 MBq/kg dose of ^{68}GaPSMA-11 and a 3.5 MBq/kg dose of ^{18}F-FDG, with matched imaging parameters (dose, time after injection, and imaging protocols) for each patient. All PET/CT imaging was undertaken using a Phillips Ingenuity time-of-flight PET/64-slice CT scanner. An unenhanced low-dose CT scan was performed 60 min after tracer injection. Immediately after CT scanning, a whole-body PET scan was acquired for 2 min per bed position. The emission data were corrected for randoms, scatter, and decay.

Diagnostic contrast-enhanced CT of the chest, abdomen, and pelvis, and a whole-body bone scan, were performed at baseline and after treatment.

Imaging Analysis

All ^{68}Ga-PSMA and ^{18}F-FDG PET scans (screening and after treatment) were analyzed semiquantitatively by a nuclear medicine physician using MIM Software and a standardized semiautomated workflow to delineate regions of interest with a minimum SUV cutoff of 3 for ^{68}Ga-PSMA PET and an SUV cutoff equal to the blood pool mean intensity plus 1.5 SDs for ^{18}F-FDG PET. All lesions identified quantitatively were manually reviewed and physiologic uptake or scatter removed. Whole-body quantitation derived total metabolic tumor volume, SUV_{max}, and SUV_{mean} for both ^{18}F-FDG PET and ^{68}Ga-PSMA PET (MIM Software) (*14*). A nuclear medicine physician visually assessed both the quantified and the nonquantified PET images to identify potential sites of ^{18}F-FDG PET–positive/^{68}Ga-PSMA PET–negative progressive disease between the screening and posttreatment scans.

Statistical Analyses

We measured PSA declines from baseline (absolute and ≥50%) at any time point, PSA PFS as defined by Prostate Cancer Working Group 3 criteria, radiographic progression defined by RECIST 1.1 and Prostate Cancer Working Group 3 criteria, and OS (*13,15*). Time-to-event endpoints (PSA PFS and OS) were defined as the interval from the date of enrolment to the event date, or the date last known to be event-free (at which point the observation was censored).

A 2-sided exact binomial 95% CI was calculated for PSA response rates. The Kaplan–Meier method was used to characterize time-to-event endpoints and estimate medians (presented with 95% CIs). We correlated changes in TTV, SUV_{max}, and SUV_{mean} for ^{68}Ga-PSMA PET and ^{18}F-FDG PET with time-to-event outcomes, using univariable and multivariable Cox proportional-hazards regression models. *P* values below 5% were considered significant. Analyses were performed using R (version 4.0.5) and SPSS (version 25).

RESULTS

Patient Characteristics

Patient characteristics are summarized in Table 1. Thirty-seven of 56 (66%) men on the LuPIN trial had both baseline screening and posttreatment imaging (6 wk after completion of 6 cycles of treatment, or earlier if the trial was exited because of clinical progression). Of these, 68% (25/37) had posttreatment imaging after completing all 6 cycles of ^{177}Lu-PSMA-617 plus NOX66, 3% (1/37) after 5 cycles, 14% (5/37) after 4 cycles, 14% (5/37) after 3 cycles, and 3% (1/37)

TABLE 1
Patient Characteristics

Characteristic	Substudy data
Age (y)	68 (65–74)
Eastern Cooperative Oncology Group performance status	
0 or 1	32 (86%)
2	5 (14%)
PSA at screening (μg/L)	91 (41.3–380)
Hemoglobin (reference range, 130–180 g/L)	122 (112–131)
Alkaline phosphatase (reference range, 30–100 U/L)	124 (83–359)
Prior systemic treatments	
Luteinizing hormone-releasing hormone agonist/antagonist	37 (100%)
Chemotherapy	37 (100%)
Docetaxel	37 (100%)
Cabazitaxel	34 (92%)
Androgen signaling inhibitor	37 (100%)
Cycles of ^{177}Lu-PSMA-617 administered	6 (4–6)
Exit diagnostic CT and bone scan	34 (92%)

Qualitative data are absolute counts and percentage; continuous data are median and interquartile range.

TABLE 2
Univariable Cox Regression Analysis for Association with PSA PFS and OS

	OS			PSA PFS		
Variable	HR	95% CI	*P*	HR	95% CI	*P*
Increase in PSMA TTV (L)	6.2	2.0–19.2	0.002	2.9	1.4–6.1	0.01
Increase in PSMA SUV_{max} at trial exit	1.9	0.2–14.4	0.56	1.3	0.3–5.6	0.71
Increase in ^{18}F-FDG TTV (L)	2.7	1.1–6.2	0.02	3.1	1.4–6.8	0.005
Increase in ^{18}F-FDG SUV_{max} at trial exit	3.0	1.2–7.3	0.02	3.0	1.4–6.4	0.01
PSA progression at trial exit	5.8	2.1–16.1	<0.001	5.0	2.3–10.7	<0.001
Radiographic progression at trial exit	5.4	1.8–16	0.003	4.4	1.6–12	0.004

after 2 cycles. Nineteen of 56 (34%) did not have exit imaging because of illness (12/19), travel restrictions (3/19), or unknown reasons (3/19).

Clinical Outcomes

The median reduction in PSA was 77% (interquartile range, 34%–92%), and 70% (26/37) of patients had a PSA response of more than 50%. With a median follow-up of 26 mo, the median PSA PFS was 8.6 mo (95% CI, 5.6–11.6) and the median OS was 22 mo (95% CI, 18.6–25.6). PSA or clinical progression had occurred in 54% (20/37) at the time of exit imaging, whereas 46% (17/37) had no PSA progression after the full 6 cycles of treatment. At exit, 92% (34/37) had conventional imaging (CT and bone scanning). Radiographic progression at the time of exit imaging was identified in 18% (6/34). On univariable analysis, PSA progression and radiographic progression at trial exit were also associated with significantly worse PSA PFS and OS (Table 2).

^{68}Ga-PSMA PET Quantitation

Quantitative ^{68}Ga-PSMA PET SUV_{max} and SUV_{mean} were reduced in 95% of men independently of whether they had PSA progression at the time of exit imaging (absolute change in PSMA SUV_{max}: median, −26 [interquartile range, −40 to −13]; absolute change in PSMA SUV_{mean}: median, −3 [interquartile range, −5 to −2]). There was no correlation between an increase in PSMA SUV_{max} and either PSA PFS or OS (Table 2).

The median change in PSMA TTV was −0.64 liters (interquartile range, −0.29 to +0.07). PSMA TTV was increased in 32% (12/37). Any increase in PSMA TTV was associated with shorter PSA PFS (hazard ratio [HR], 2.9 [95% CI, 1.4–6.1]; $P = 0.01$) and OS (median, 12.2 vs. 25.5 mo; HR, 6.2 [95% CI, 2.0–19.2]; $P < 0.01$) (Fig. 1). An increase in PSMA TTV by at least 30% was significantly associated with worse OS (HR, 6.0 [95% CI, 1.9–19.2]; $P = 0.002$), whereas association with PSA PFS was not significant (Fig. 2).

All 12 patients with an increasing PSMA TTV had an SUV_{max} of more than 15 at trial exit (above trial entry criteria), compared with 52% (13/25) of those with a reduced PSMA TTV.

^{18}F-FDG PET Quantitation

Analysis of screening and posttreatment ^{18}F-FDG PET demonstrated that 51% (18/35) had a reduced ^{18}F-FDG SUV_{max} (median absolute change, 0.1; interquartile range, −4 to +1) and that 66% (23/35) had a reduced ^{18}F-FDG SUV_{mean} (median absolute change, −0.4; interquartile range, −1 to +0.4) in response to treatment. An increase in ^{18}F-FDG SUV_{max} was associated with worse PSA PFS (HR, 3.0 [95% CI, 1.4–6.4]; $P = 0.01$) and OS (HR, 3.0 [95% CI, 1.2–7.3]; $P = 0.02$).

The median change in ^{18}F-FDG TTV was −0.01 liters (interquartile range, −0.05 to +0.02). ^{18}F-FDG TTV was increased in

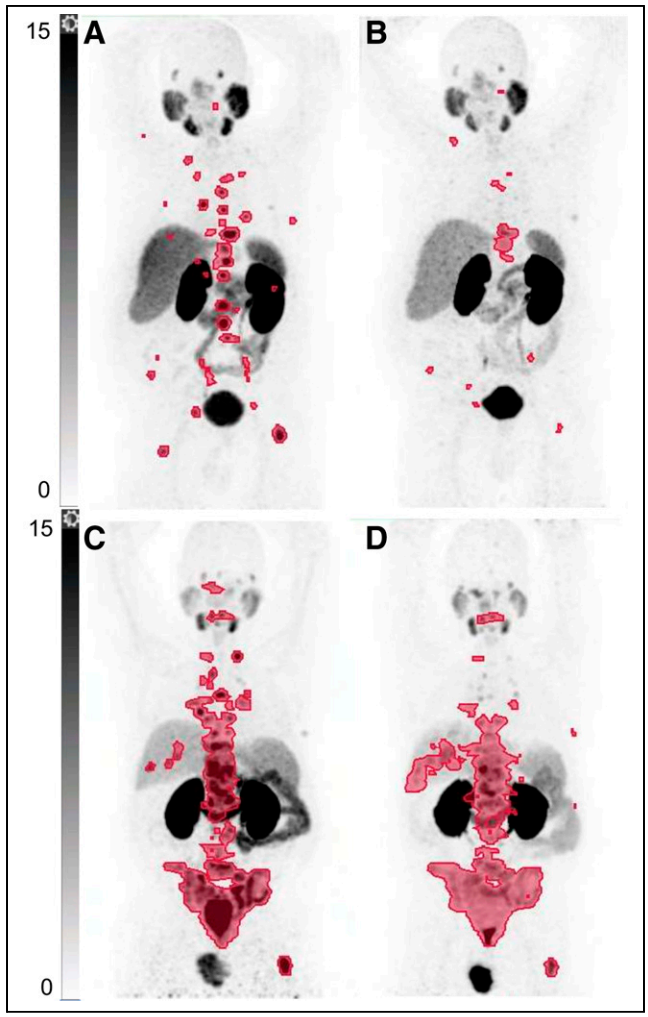

FIGURE 1. (A and B) Quantitative analysis for patient with reduced PSMA TTV between baseline (A) and after treatment (B). (C and D) Quantitative analysis for progressing patient at baseline (C) and after treatment (D). In patients with high-volume disease, it can be difficult to visually identify extent of volume change. In this second case, volume increase is 25%.

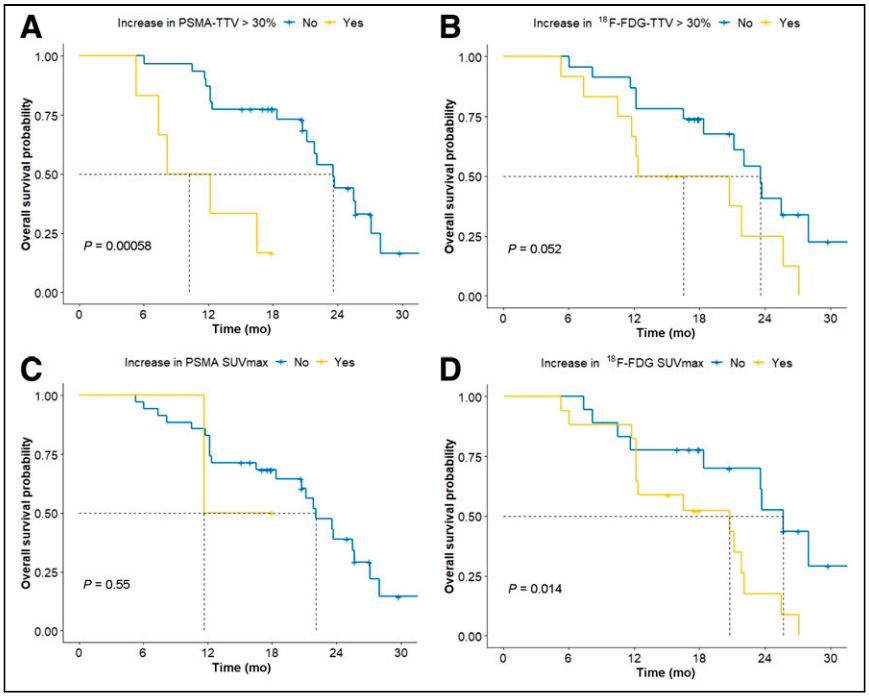

FIGURE 2. Kaplan–Meier curves for OS stratified by increase in PSMA TTV ≥ 30% from baseline (A), increase in ^{18}F-FDG TTV ≥ 30% from baseline (B), increase in PSMA SUV_{max} from baseline (C), and increase in ^{18}F-FDG SUV_{max} from baseline (D).

37% (13/35) at trial exit. Any increase in ^{18}F-FDG TTV was associated with worse PSA PFS (HR, 3.1 [95% CI, 1.4–6.8]; $P = 0.005$) and OS (median, 16.2 vs. 23.1 mo; HR, 2.7 [95% CI, 1.1–6.2]; $P = 0.02$). An increase in ^{18}F-FDG TTV by at least 30% was significantly associated with worse PSA PFS (HR, 2.7 [95% CI, 1.2–6.0]; $P = 0.01$) but was not significantly associated with OS (Fig. 2).

No ^{18}F-FDG–positive/^{68}Ga-PSMA PET–negative progressive sites were identified in this cohort at the time of exit imaging. One patient had a significant increase in ^{18}F-FDG–avid volume at 1 site, whereas PSMA tumor volume and SUV_{max} were reduced (Fig. 3).

Molecular Response Patterns and Patient Outcomes

Multivariable analysis including change in PSMA TTV, ^{18}F-FDG TTV, and ^{18}F-FDG SUV_{max}, as well as PSA progression and radiographic progression, found that only PSMA TTV and PSA progression remained independently prognostic of OS (HR, 5.1 [95% CI, 1.5–17.1; $P = 0.008$] and 3.5 [95% CI, 1.1–10.9; $P = 0.03$], respectively) (Table 3).

DISCUSSION

This study has found that increasing TTV on posttreatment ^{68}Ga-PSMA PET identifies early disease progression and shorter OS independently of PSA, raising its potential for use as a prognostic biomarker. Metastatic castration-resistant prostate cancer is characterized by phenotypic and molecular heterogeneity, with marked PSMA heterogeneity previously demonstrated at both an imaging and a cellular level (*9,10*). Although the VISION and TheraP trials have found high treatment responses and improved quality-of-life parameters, the duration of treatment responses with ^{177}Lu-PSMA-617 remains limited. Identifying effective predictive and prognostic biomarkers is critical to deepening and prolonging responses to PSMA-targeted treatments with appropriate combinations and judicious treatment sequencing.

A second key finding in this study is that in contrast to ^{18}F-FDG PET, reduced PSMA SUV_{max} or SUV_{mean} occurred in almost all patients in response to ^{177}Lu-PSMA-617 therapy and was not predictive of either treatment response or OS. This lack of correlation between change in PSMA SUV_{max} or SUV_{mean} and treatment outcomes has previously been shown. Kurth et al. found that PSMA intensity decreased in both clinically responding and progressing patients (*7,16*). Grubmüller et al. also found no correlation between change in whole-body PSMA SUV_{mean} and OS in an analysis of posttreatment ^{68}Ga-PSMA PET after ^{177}Lu-PSMA-617 therapy. This lack of prognostic value of change in PSMA SUV_{mean} and SUV_{max} with PSMA-targeted therapy is not unexpected. ^{177}Lu-PSMA-617 preferentially targets highly PSMA-expressing cells, leading to persistent populations of low-PSMA-expression disease that may be less responsive to treatment. The lack of predictive or prognostic value for change in PSMA SUV_{mean} and SUV_{max} is important to highlight, as we intuitively use reduction in intensity (^{18}F-FDG PET) to denote treatment response with systemic therapy (*17*). We need to think differently when developing ^{68}Ga-PSMA PET response criteria for PSMA-targeted therapy.

Increasing PSMA TTV was an independent predictor of PSA PFS and OS in this study. Similar to Gafita et al. and Grubmüller et al., we confirmed that an increase in quantitative PSMA TTV is a poor prognostic factor for OS (*6,7*). An increase in PSMA TTV by at least 30% was associated with poor OS, supporting the inclusion of this metric in the ^{68}Ga-PSMA PET progression criteria (*18*). However, accurate assessment of change in TTV visually can be difficult, especially in high-volume disease, and quantitative PET analysis may become an important tool in PSMA-targeted therapy.

We found that patients with an increase in PSMA TTV at trial exit had a PSMA SUV_{max} of more than 15, significantly higher than in patients without progressive disease, and above a range at which PSMA-targeted therapy is expected to be effective. This finding may indicate that radiation resistance is an important mechanism of treatment failure. Further evaluation in conjunction with genetic analysis may help identify optimal treatment combinations in patients who currently have a limited treatment response to ^{177}Lu-PSMA-617 alone.

^{18}F-FDG PET was undertaken both at screening and at trial exit, with ^{18}F-FDG PET screening parameters previously shown to be predictive of OS in this study cohort and other ^{177}Lu-PSMA-617 trials (*1,12,19*). Although we found that an increase in ^{18}F-FDG SUV_{max} and ^{18}F-FDG TTV was associated with poor OS in univariable analysis, they did not remain significant on multivariate analysis. Further, the incidence of discordant progressive lesions (^{18}F-FDG PET–positive/^{68}Ga-PSMA PET–negative) was low, with only 1 patient having a significant increase in ^{18}F-FDG–avid volume at 1 site, whereas PSMA TTV was reduced.

RECIST progression is the standard of care for identifying progressive disease on imaging and has a strong correlation with OS in prostate cancer (*20*). However, RECIST progression was less prognostic than change in either PSMA TTV or PSA progression at

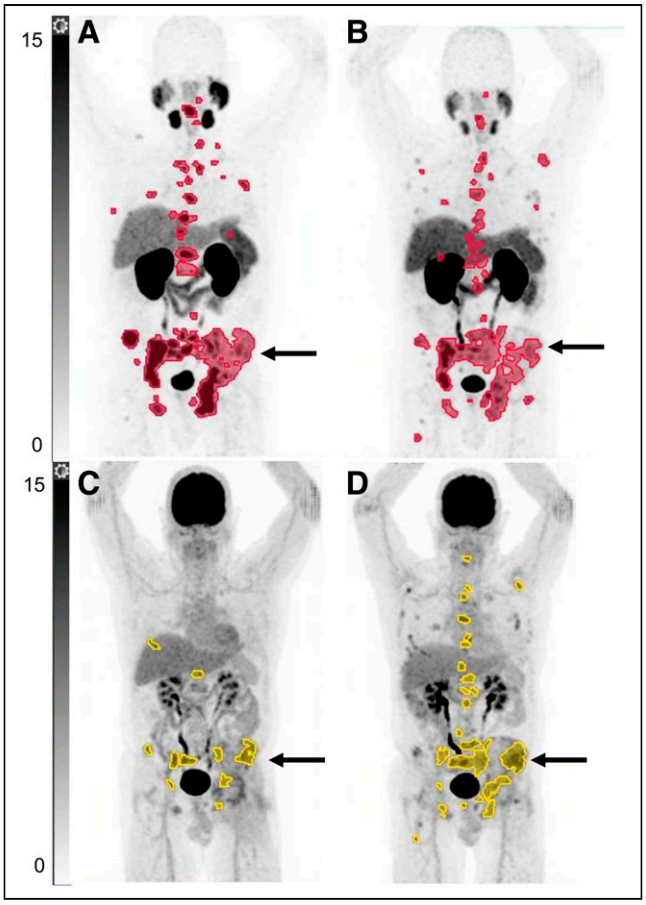

FIGURE 3. Quantitative analysis of baseline and posttreatment PSMA and ^{18}F-FDG PET images from same patient: baseline ^{68}Ga-PSMA PET (A), posttreatment ^{68}Ga-PSMA PET (B), baseline ^{18}F-FDG PET (C), and posttreatment ^{18}F-FDG PET (D). Arrows indicate lesion in iliac bone that reduced in volume and intensity on ^{68}Ga-PSMA PET but increased in volume and intensity of ^{18}F-FDG PET.

study exit. The promising prognostic value for molecular imaging parameters suggests that more work needs to be done validating PET for treatment response in metastatic castration-resistant prostate cancer, potentially as an alternative to CT and bone scanning currently used in Prostate Cancer Working Group 3 criteria.

This study had several limitations. Its small sample size made it purely exploratory, and the findings will need to be validated in larger trials. The sample size also did not allow for a multivariable analysis incorporating other known prognostic factors.

TABLE 3
Multivariable Cox Regression Analysis for Association of Clinical and Imaging Parameters with OS

Variable at trial exit	HR	95% CI	*P*
Increase in PSMA TTV	5.1	1.5–17.1	0.008
Increase in ^{18}F-FDG TTV	1.04	0.4–2.9	0.93
Increase in ^{18}F-FDG SUV_{max}	1.3	0.4–4.5	0.67
PSA progression	3.5	1.1–10.9	0.03
Radiographic progression	1.8	0.5–6.0	0.36

Additionally, selection of patients for this analysis was biased toward those well enough to complete posttreatment imaging, explaining the higher rate of at least 50% PSA decline and longer OS in this subset of patients than previously published for the trial. The interval between screening and posttreatment imaging was variable, with 32% exiting the trial early because of clinical progression.

Finally, PET quantitation software remains of limited availability and time-intensive to achieve accurate results. Further automation in quantitation is required to minimize the time required to derive reproducible results, in addition to harmonization and validation of quantitative methods. Nevertheless, the prognostic value of quantified PSMA TTV in this study suggests that investment in PET quantitation will yield significant clinical benefit.

CONCLUSION

Change in quantitative PSMA TTV has strong potential as a prognostic biomarker with ^{177}Lu-PSMA-617 therapy, independently of ^{18}F-FDG parameters, PSA, or radiographic progression. Further research into the value of posttreatment PET as an imaging biomarker is warranted.

DISCLOSURE

This investigator-initiated study was sponsored by St. Vincent's Hospital Sydney and supported by a Cancer Institute NSW prostate translational research grant. Noxopharm Limited provided funding for drug and PET scans, AAA/Novartis provided PSMA-617 ligand. Louise Emmett has an advisory role with Noxopharm, receives trial support from Novartis and Astellas, and receives grant funding support from St. Vincent's Clinic Foundation. Anthony Joshua has an advisory role with Noxopharm Limited and receives institutional funding from Novartis. Peter Wilson and Remy Niman are salaried employees of MIM Software, Inc. No other potential conflict of interest relevant to this article was reported.

ACKNOWLEDGMENTS

We thank the patients, and the clinical trials teams at the Department of Theranostics and Nuclear Medicine and the Kinghorn Cancer Centre, St. Vincent's Hospital, for their support.

KEY POINTS

QUESTION: What are the imaging findings on ^{68}Ga-PSMA PET and ^{18}F-FDG PET after ^{177}Lu-PSMA-617 therapy, and do changes in tumor volume, SUV_{max}, or SUV_{mean} correlate with clinical outcomes?

PERTINENT FINDINGS: In this LuPIN substudy, any increase in PSMA TTV and PSA progression at study exit were independently prognostic of OS.

IMPLICATIONS FOR PATIENT CARE: Change in tumor volume on ^{68}Ga-PSMA PET after ^{177}Lu-PSMA-617 therapy provides information for clinicians on patient survival and may help them make clinical decisions on timing and type of next treatments.

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9.3 Evaluation of 177Lu-PSMA-617 SPECT/CT Quantitation as a Response Biomarker Within a Prospective 177Lu-PSMA-617 and NOX66 Combination Trial (LuPIN).

Evaluation of ^{177}Lu-PSMA-617 SPECT/CT Quantitation as a Response Biomarker Within a Prospective ^{177}Lu-PSMA-617 and NOX66 Combination Trial (LuPIN)

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^{177}Lu-PSMA-617 is an effective and novel treatment in metastatic castration-resistant prostate cancer (mCRPC). Our ability to assess response rates and therefore efficacy may be improved using predictive tools. This study investigated the predictive value of serial ^{177}Lu-PSMA-617 SPECT/CT (^{177}Lu SPECT) imaging in monitoring treatment response. **Methods:** Fifty-six men with progressive mCRPC previously treated with chemotherapy and novel androgen signaling inhibitor were enrolled into the LuPIN trial and received up to 6 doses of ^{177}Lu-PSMA-617 and a radiation sensitizer (3-(4-hydroxyphenyl)-2H-1-benzopyran-7-ol [NOX66]). ^{68}Ga-PSMA-11 and ^{18}F-FDG PET/CT were performed at study entry and exit, and ^{177}Lu SPECT from vertex to mid thighs was performed 24 h after each treatment. SPECT quantitative analysis was undertaken at cycles 1 (baseline) and 3 (week 12) of treatment. **Results:** Thirty-two of the 56 men had analyzable serial ^{177}Lu SPECT imaging at both cycle 1 and cycle 3. In this subgroup, median prostate-specific antigen (PSA) progression-free survival (PFS) was 6.3 mo (95% CI, 5–10 mo) and median overall survival was 12.3 mo (95% CI, 12–24 mo). The PSA 50% response rate was 63% (20/32). ^{177}Lu SPECT total tumor volume (SPECT TTV) was reduced in 68% (22/32; median, −0.20 m^3 [95% CI, −1.4 to −0.001]) and increased in 31% (10/32; median, 0.36 [95% CI, 0.1–1.4]). Any increase in SPECT TTV was associated with shorter PSA PFS (hazard ratio, 4.1 [95% CI, 1.5–11.2]; P = 0.006). An increase of 30% or more in SPECT TTV was also associated with a shorter PSA PFS (hazard ratio, 3.3 [95% CI, 1.3–8.6]; P = 0.02). Tumoral SUV_{max} was reduced in 91% (29/32) and SUV_{mean} in 84% (27/32); neither was associated with PSA PFS or overall survival outcomes. PSA progression by week 12 was also associated with a shorter PSA PFS (hazard ratio, 26.5 [95% CI, 5.4–131]). In the patients with SPECT TTV progression at week 12, 50% (5/10) had no concurrent PSA progression (median PSA PFS, 4.5 mo [95% CI, 2.8–5.6 mo]), and 5 of 10 men had both PSA and SPECT TTV progression at week 12 (median PSA PFS, 2.8 mo [95% CI, 1.8–3.7 mo]). **Conclusion:** Increasing SPECT TTV on quantitative ^{177}Lu SPECT predicts a short PFS and may play a future role as an imaging response biomarker.

Key Words: metastatic prostate cancer; SPECT; lutetium-PSMA; response biomarker

Received May 10, 2022; revision accepted Jul. 22, 2022.

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Published online Aug. 25, 2022.

J Nucl Med 2023; 64:221–226
DOI: 10.2967/jnumed.122.264398

Although treatment resistance and short response duration remain common, ^{177}Lu-PSMA-617 is an effective therapy in metastatic castration-resistant prostate cancer (mCRPC) (*1–4*). Accurate monitoring of response to ^{177}Lu-PSMA-617 may improve patient outcomes by enabling treatment escalation, change in treatment, or a treatment holiday, dependent on imaging results. Interim and serial prostate-specific membrane antigen (PSMA) PET has recently been shown to be predictive of progression-free survival (PFS) with PSMA-targeted radionuclide therapy (*5*). Quantitative ^{177}Lu-PSMA-617 SPECT/CT (^{177}Lu SPECT) imaging after each ^{177}Lu-PSMA-617 dose may also be valuable in response monitoring in addition to providing dosimetric information. This LuPIN trial substudy aimed to determine whether quantitative parameters on serial ^{177}Lu SPECT imaging 24 h after ^{177}Lu-PSMA-617 therapy were predictive of treatment response and PFS.

MATERIALS AND METHODS

The LuPIN trial is a prospective single-center phase I/II dose escalation and expansion trial of combination ^{177}Lu-PSMA-617 and 3-(4-hydroxyphenyl)-2H-1-benzopyran-7-ol (NOX66) for men with mCRPC previously treated with at least 1 line of taxane chemotherapy and androgen signaling inhibitor. The clinical results have been previously published (*6,7*). The St. Vincent's Hospital institutional review board approved the study protocol (HREC/17/SVH/19 and ACTRN12618001073291), and all patients provided informed written consent.

Screening

Men with progressive mCRPC, based on either conventional imaging (CT and bone scanning) or a rising prostate-specific antigen (PSA) level according to Prostate Cancer Working Group 3 criteria (*8*), were eligible for screening. Prior treatment with at least 1 line of taxane chemotherapy (docetaxel or cabazitaxel) and an androgen signaling inhibitor (abiraterone or enzalutamide) was required for inclusion. Men underwent screening with ^{18}F-FDG and ^{68}Ga-HBEDD-PSMA-11 PET/CT, bone scanning, and CT of the chest, abdomen, and pelvis.

Molecular screening criteria were based on SUV_{max} rather than physiologic activity (liver or parotid). Men were eligible if they had an SUV_{max} of more than 15 on PSMA PET at 1 or more sites, an SUV_{max} of more than 10 at all measurable sites, and no ^{18}F-FDG avidity without corresponding PSMA uptake.

Study Treatment

Men received up to 6 doses of ^{177}Lu-PSMA-617 at 6-wk intervals, with 3 dose-escalated cohorts of NOX66 (400, 800, 1,200 mg). NOX66 suppositories were administered as a radiosensitizer on days 1–10 after each ^{177}Lu-PSMA-617 injection. All cohorts were administered 7.5 GBq of ^{177}Lu-PSMA-617 on day 1 via slow intravenous injection. The PSMA-617 precursor (AAA Novartis) was radiolabeled to no-carrier-added ^{177}Lu-chloride according to the manufacturer's instructions. Quality control tests for radionuclide and radiochemical purity were performed using high-pressure liquid chromatography and thin-layer chromatography. Blood was prospectively collected before assessment of adverse events and biochemical responses. The patients were treated on trial until they were no longer clinically benefiting from treatment.

Imaging Procedures and Analysis

^{68}Ga-PSMA and ^{18}F-FDG PET/CT scans were obtained at baseline and trial exit (after completing 6 cycles or when treatment was ceased), using the imaging acquisition and analysis parameters previously published (*7*). ^{177}Lu SPECT (vertex to mid thighs) was performed 24 h after ^{177}Lu-PSMA-617 injection using a Discovery 670 system (GE Healthcare) with the following parameters: medium-energy collimators, 3 bed positions, 60 projections over 360° with an acquisition time of 10 s per frame, 128 × 128 matrix, and 4.42 × 4.42 mm pixel size. An energy window centered on 208 keV ± 10% with a 165 keV ± 6.5% scatter window was used. An unenhanced low-dose CT scan was obtained immediately afterward using the following parameters: pitch of 1, tube voltage of 120 kV, automatic mAs control (reference mAs, 90), slice thickness of 3.7 mm, matrix of 512 × 512, and field of view of 40 cm. The SPECT projection images were reconstructed with an iterative ordered-subset estimation-maximum algorithm that used 4 iterations and 10 subsets using SPECTRA Quant (MIM Software, Inc.). No pre- or postreconstruction filters were applied. CT-based attenuation correction, dual-energy-window scatter correction, collimator-based resolution recovery, and quantitative conversion to SUV were performed during the reconstruction. The conversion from counts to units of activity was based on a cylinder phantom with known activity.

Quantitative Analysis

^{177}Lu SPECT and ^{68}Ga-PSMA PET/CT were analyzed semiquantitatively by a nuclear medicine physician using MIM (LesionID; MIM Software Inc.) software and a standardized semiautomated workflow to delineate regions of interest with a minimum SUV cutoff of 3. All lesions identified quantitatively were manually reviewed and physiologic activity removed. Whole-body quantitation was used to derive total tumor volume (TTV), SUV_{max}, and SUV_{mean} for both ^{68}Ga-PSMA PET and ^{177}Lu SPECT (*9*).

Statistical Analysis

We measured PSA decline from baseline (≥50%) at any time point, PSA PFS as defined by Prostate Cancer Working Group 3 criteria, and overall survival (*8,10*). The Kaplan–Meier method was used to characterize time-to-event endpoints and estimate medians (presented with 95% CIs). We correlated changes in TTV, PSMA intensity, clinical parameters, and biochemical parameters with time-to-event outcomes, using univariate Cox proportional-hazards regression models (*11,12*). Continuous variables included increase in TTV, SUV_{max}, and SUV_{mean}. *P* values below 5% were considered significant. We compared ^{68}Ga-PSMA PET TTV with cycle 1 SPECT TTV using scatterplots and Pearson correlation coefficients. Reproducibility testing of SPECT TTV and PSMA SUV_{max} was undertaken using repeatability statistics calculated from a hierarchic linear mixed model that accounted for variance in score at the patient level (*13*). A 95% CI for the repeatability statistics was derived via bootstrapping. Analyses were performed using R (version 4.0.5).

TABLE 1
Baseline Patient Characteristics

Characteristic	Data
Total patients	32
Age (y)	69 (66–73)
ECOG	
0 or 1	25 (78)
2	7 (22)
Prior systemic treatments	
LHRH agonist/antagonist	32 (100)
Chemotherapy	32 (100)
Docetaxel	32 (100)
Cabazitaxel	29 (91)
Androgen signaling inhibitor	32 (100)
Sites of disease	
Lymph nodes	18 (56)
Bone	30 (94)
Viscera	6 (19)
Median PSMA tumor volume at screening (cm^3)	670 (275–1,736)
Number of cycles of ^{177}Lu-PSMA-617 received	6 (3–6)

LHRH = luteinizing hormone-releasing hormone.
Qualitative data are absolute counts and percentage; continuous data are median and interquartile range.

RESULTS

Baseline Patient Characteristics

Of the men enrolled in LuPIN, 57% (32/56) had ^{177}Lu SPECT imaging suitable for analysis, 30% (17/56) had incomplete SPECT data precluding analysis, and 13% (7/56) did not reach cycle 3 of treatment. Baseline characteristics are summarized in Table 1. In this LuPIN substudy, 53% (17/32) completed 6 cycles of treatment whereas 47% (15/32) completed between 3 and 5 cycles, and 63% (20/32) achieved at least a 50% PSA decline from baseline at any time point. At the time of analysis, 84% (27/32) were deceased.

TABLE 2
Summary of ^{177}Lu SPECT Quantitation at Cycles 1 and 3

Parameter	Cycle 1	Cycle 3
TTV (cm^3)	787 (282–1,298)	492 (191–1,190)
SUV_{max}	70 (57–100)	36 (27–60)
SUV_{mean}	10.1 (8–12)	8 (6–9)

Data are median and interquartile range.

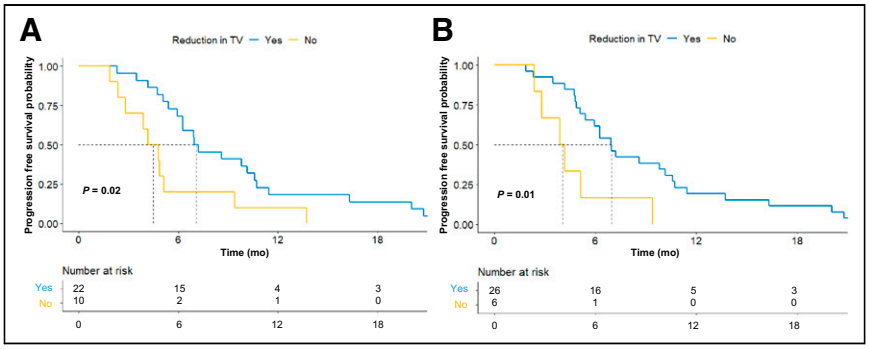

FIGURE 1. Kaplan–Meier curve for PSA PFS stratified by any increase in SPECT TTV at cycle 3 (A) or >30% increase in SPECT TTV at cycle 3 (B). TV = tumor volume.

There was no difference in either PSA PFS or overall survival based on NOX66 dose. Overall, median overall survival was 12.3 mo (95% CI, 11.7–23.6 mo). Median PSA PFS was 6.3 mo (95% CI, 5.1–9.8 mo).

^{177}Lu SPECT Quantitation

SPECT quantitation measures at baseline and week 12, including SPECT TTV, SUV_{max}, and SUV_{mean}, are summarized in Table 2. SPECT TTV was reduced between baseline and week 12 in 68% (22/32; median, −0.20 m^3 [95% CI, −1.4 to −0.001]) and increased in 31% (10/32; median, 0.36 [95% CI, 0.1–1.4]). A 30% increase in SPECT TTV by week 12 was identified in 19% (6/32). SUV_{max} was reduced between baseline and week 12 in 91% (29/32; median, −28.9 [95% CI, −195 to +42]), and SUV_{mean} was reduced in 84% (27/32; median, −2.6 [95% CI, −12 to +10]).

Correlation with Patient Outcomes

An increase in SPECT TTV between baseline and week 12 was associated with a significantly worse PSA PFS (hazard ratio, 4.1 [95% CI, 1.5–11.2]; $P = 0.006$). Median PSA PFS in those with an increase in SPECT TTV was 4.5 mo (95% CI, 2.8–5.6 mo), compared with 7.1 mo (95% CI, 6.3–10.7 mo) for those with no increase in SPECT TTV. A SPECT TTV increase of at least 30% was also associated with a shorter PSA PFS (hazard ratio, 3.3 [95% CI, 1.3–8.6], $P = 0.02$) (Fig. 1). Increased SUV_{max} or SUV_{mean} between baseline and week 12 was not associated with PSA PFS or overall survival (Table 3). By week 12, 25% (8/32) of patients demonstrated PSA progression. PSA progression at week 12 was associated with significantly worse PSA PFS (hazard ratio, 26.5 [95% CI, 5.4–131]; $P < 0.001$). Patients with PSA progression at week 12 had a median PSA PFS of 3.5 mo (95% CI, 1.1–4.5 mo), versus 7.9 mo (95% CI, 6.3–10.7 mo) in those without PSA progression. In the 10 patients with SPECT TTV progression at week 12, 50% (5/10) had no concurrent PSA progression (median PSA PFS, 4.5 mo [95% CI, 2.8–5.6 mo]), and 5 of 10 men had both PSA and SPECT TTV progression at week 12 (median PSA PFS, 2.8 mo [95% CI, 1.8–3.7 mo]) (Fig. 2).

Reproducibility

TTV was compared between ^{68}Ga PSMA-11 PET/CT at screening (PSMA PET TTV) and the baseline ^{177}Lu SPECT (median time between scans, 15 d [range, 6–56 d]). There was a strong correlation between PSMA PET TTV and cycle 1 SPECT TTV ($R = 0.87$ [95% CI, 0.74–0.93], $P < 0.001$) (Fig. 3). Mean TTV was similar between PSMA PET and SPECT (PET TTV, 925 ± 856 cm^3; SPECT TTV, 949 ± 852 cm^3).

The repeatability of ^{177}Lu SPECT quantitative analysis was assessed in all 32 patients. There was no evidence of a systematic difference between test and retest for SUV_{max}, SUV_{mean}, or TTV. The repeatability estimate was 0.99 for SUV_{max} (95% CI, 0.97–0.99), 0.90 for SUV_{mean} (95% CI, 0.81–0.95), and 0.99 for TTV (95% CI, 0.98–0.99) (Table 4).

DISCUSSION

This study found that quantified changes in SPECT TTV between baseline and 12-wk ^{177}Lu-PSMA-617 predict PFS in men treated on a prospective PSMA-targeted therapy trial. To our knowledge, this was the first study to evaluate SPECT parameters for response biomarker capability, a potentially valuable development that uses a readily available tool to potentially enhance personalized treatment by directly assessing treatment response. ^{177}Lu-PSMA-617 has proven an effective therapy for mCRPC, with randomized trials demonstrating both improved overall survival and improved radiographic PFS compared with the standard of care (*3*), as well as increased PSA 50% response rates and improved patient-reported outcomes compared with cabazitaxel (*2*). However, responses can be heterogeneous, and PFS remains relatively short (*2,3*). Combination trials with ^{177}Lu-PSMA-617 are under way to investigate whether combining ^{177}Lu-PSMA-617 with other agents may deepen and prolong responses (NCT04419402, NCT03658447, and NCT03874884) (*14*).

TABLE 3
Univariable Analysis of Clinical and Imaging Markers and Association with PSA PFS and Overall Survival

Univariable analysis	Overall survival	PSA PFS
Increase in SPECT TTV (m^3)*	1.5 (0.6–4.1); $P = 0.40$	4.1 (1.5–11.2); $P = 0.006$
Increase in SPECT SUV_{max}*	1.002 (0.99–1.01; $P = 0.75$	1.004 (0.99–1.02); $P = 0.51$
Increase in SPECT SUV_{mean}*	1.11 (0.98–1.24); $P = 0.09$	1.11 (0.99–1.23); $P = 0.06$
PSA progression*	5.6 (2.1–14.8); $P < 0.001$	26.5 (5.4–131); $P < 0.001$
PSA decline ≥ 50%*	0.31 (0.1–0.8); $P = 0.02$	0.40 (0.2–0.9); $P = 0.02$
Time since diagnosis	0.95 (0.88–1.03); $P = 0.18$	0.93 (0.87–1.01); $P = 0.09$

*At week 12.
Data are hazard ratios and 95% CIs.

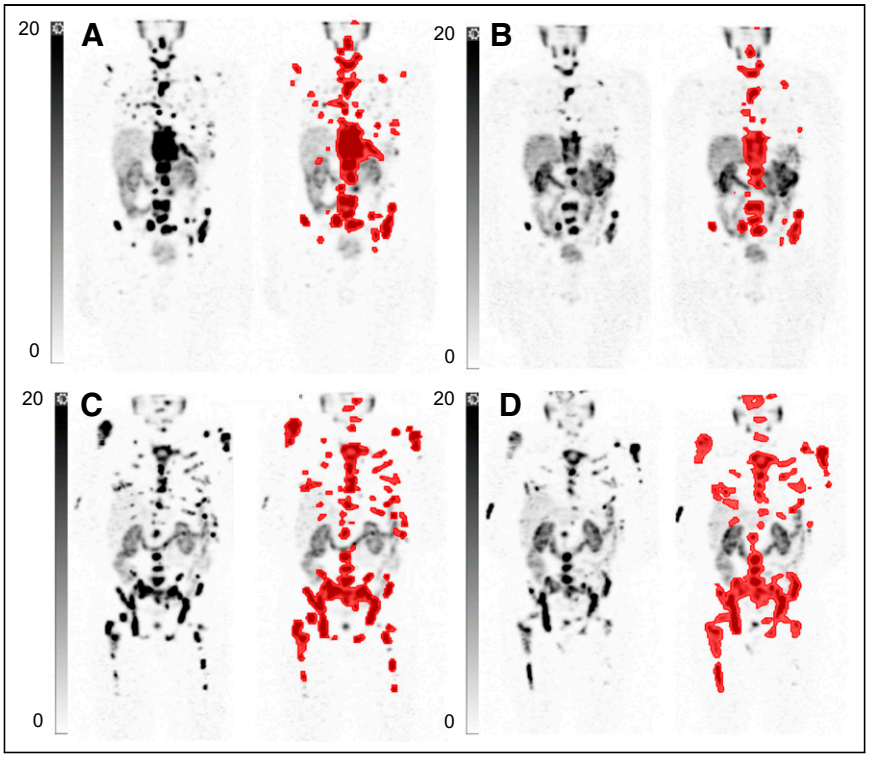

FIGURE 2. (A and B) Maximum-intensity projection and quantitation of ^{177}Lu SPECT at cycle 1 (A) and cycle 3 (B) for patient with reduction in SPECT TTV and PSA and PSA PFS of 22 mo. (C and D) Maximum-intensity projection and quantitation of ^{177}Lu SPECT at cycle 1 (C) and cycle 3 (D) for patient with increase in SPECT TTV > 30% but no increase in PSA and PSA PFS of 5 mo.

Predictive and interim response biomarkers, both imaging and genomic, will be critical in personalizing treatments to optimize longer-term responses to PSMA-targeted radionuclide therapy (*5,7*). Although there are limitations in spatial resolution with ^{177}Lu SPECT, its elegant potential as a response biomarker warrants further evaluation. Molecular imaging as an interim response biomarker has been particularly successful in optimizing treatment responses with 12-wk ^{18}F-FDG PET in lymphoma (*15–19*). More recently, Gafita et al. proposed a 12-wk interim PSMA PET scan on the basis of its predictive value for early disease progression in a multicenter ^{177}Lu-PSMA-617 therapy trial (RECIP 1.0) (*5*). Being able to identify treatment-resistant phenotypes early could allow either intensification with the addition of synergistic drugs or a change in treatment, thereby maximizing opportunities for treatment response in individuals and avoiding the clinical and financial costs of continuing futile treatment. Generally, this has been done in mCRPC by monitoring serum PSA response (*20*). Similarly to PSMA, there is heterogeneity of PSA expression, meaning it is not an accurate measure of disease volume in a significant proportion of men with mCRPC (*21*). In this study, both SPECT TTV and PSA progression were predictive of PFS. However, 21% of those in this study with no PSA progression had SPECT TTV progression. Gafita et al. had a similar finding, with 14% demonstrating PSMA PET progression before PSA progression (*5*). Larger numbers are required to determine whether use of SPECT TTV in combination with PSA can more effectively identify disease progression, but this study provides strong preliminary evidence, with ^{177}Lu SPECT identifying progression in a subset of patients who had not yet experienced a PSA rise. These results raise the question of whether ^{177}Lu SPECT or PSMA PET should be the preferred interim imaging response biomarker for ^{177}Lu-PSMA-617 therapy.

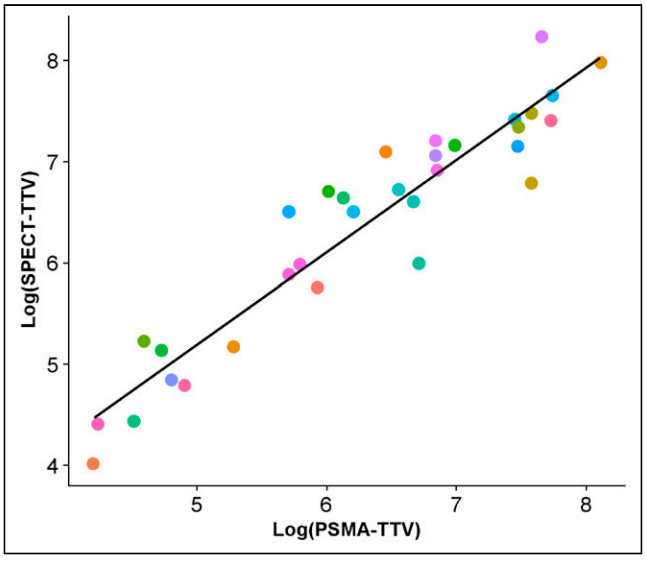

FIGURE 3. Scatterplot of log(PSMA TTV) at baseline vs. log(SPECT TTV) at cycle 1.

Posttherapy imaging both with planar imaging and with ^{177}Lu SPECT after radionuclide therapy has traditionally been used for dosimetric calculations to determine the dose to nontarget organs and tumor (*22–25*). Because of its significantly lower spatial resolution and inability to detect small lesions relative to PET imaging, ^{177}Lu SPECT has not been considered for treatment response. In our direct comparison of quantitative findings between ^{68}Ga-PSMA PET and ^{177}Lu SPECT within 2 wk, TTV was very similar between ^{177}Lu SPECT and PET, with a high correlation between the 2 modalities, although theoretically ^{177}Lu SPECT will underestimate small-volume disease (*26*). However, evaluating disease progression requiring treatment change or intensification should not depend on identifying small-volume disease. A lesion that is below the spatial resolution for detection on ^{177}Lu SPECT will become visible as its size increases. Although the findings from this trial confirm that ^{177}Lu SPECT has potential for identifying clinically significant disease progression, the opposite may be a more difficult issue. ^{177}Lu SPECT may struggle to confirm complete resolution of all sites of disease in men with exceptional responses to ^{177}Lu-PSMA-617 therapy. Confirmation of an exceptional response may indeed require the spatial resolution of PSMA PET, and further research is required to more precisely define the limitations of ^{177}Lu SPECT and appropriate minimal volume changes required to identify progression.

This study relied on quantitation of ^{177}Lu SPECT data, rather than visual assessment, to determine an increase in TTV. It is becoming clear that assessment of treatment response using PSMA-based

TABLE 4
Repeatability of ^{177}Lu SPECT Quantitation Measures

Parameter	Absolute test–retest difference	Repeatability estimate
SUV_{max}	1.47 (−1.46 to 4.39)	0.99 (0.97–0.99); $P < 0.001$
SUV_{mean}	−0.29 (−1.06 to 0.48)	0.90 (0.81–0.95); $P < 0.001$
Tumor volume (cm^3)	34.21 (−14.62 to 83.03)	0.99 (0.98–0.99); $P < 0.001$

Data are estimate and 95% CIs.

imaging for PSMA-targeted therapy must focus on changes in volume rather than measures of intensity (*27,28*). Accurate visual assessment of changes in tumor volume can be difficult, especially in the presence of large-volume metastatic bone disease. We found that the repeatability of tumor volume on ^{177}Lu SPECT was high and comparable to PSMA PET/CT (*29*). However, quantitation remains outside routine reporting guidelines and is time-intensive. Further work is needed both to evaluate the accuracy of quantitation over visual assessment and to streamline quantitation to be more user-friendly for integration into routine clinical practice (*9*).

Changes in SPECT TTV were predictive of progression-free but not overall survival in this study. Although this finding may be due to the small patient cohort, it may also be due to the many patients with progressive disease on 12-wk posttherapy SPECT who were taken off trial and changed to an agent that proved effective. Further work is required to evaluate the benefit of ^{177}Lu SPECT as a prognostic biomarker.

There were several limitations to this study. First, the patient numbers were small, and a larger cohort is needed to validate these findings. This was a single-center study, and ^{177}Lu SPECT quantitative measures can vary significantly between centers and systems (*30*). Further evaluation is required to harmonize image acquisition and reconstruction across centers and imaging systems for results to be reproducible. Finally, appropriate volume cutoffs for a significant increase in SPECT TTV need to be defined. This will require trials with larger patient numbers and outcome data. However, this study provided a strong foundation on which to build further work.

CONCLUSION

Increasing SPECT TTV on quantitative ^{177}Lu SPECT predicted a short PFS and identified progression in some men who had yet to demonstrate PSA progression. This tool shows promise as an imaging response biomarker.

DISCLOSURE

This investigator-initiated study was sponsored by St. Vincent's Hospital, Sydney, and supported by a Cancer Institute NSW prostate translational research grant. Noxopharm Limited provided funding for drug and PET scans, and AAA/Novartis provided the PSMA-617 ligand. Louise Emmett has an advisory role with Noxopharm Limited, received grant funding support through St. Vincent's Clinic Foundation, and received trials support from Novartis and Astellas. Anthony Joshua has an advisory role with Noxopharm Limited and received institutional funding from Novartis. Peter Wilson and Remy Niman are salaried employees of MIM Software, Inc. Sarennya Pathmanandavel received funding through the Cancer Institute NSW and St. Vincent's Clinic Foundation. No other potential conflict of interest relevant to this article was reported.

ACKNOWLEDGMENTS

We thank the patients, as well as the clinical trials teams at the Department of Theranostics and Nuclear Medicine and the Kinghorn Cancer Centre, St. Vincent's Hospital, for their support.

KEY POINTS

QUESTION: Do the SPECT images acquired 24 h after ^{177}Lu PSMA-617 therapy provide predictive information on patient outcomes?

PERTINENT FINDINGS: A change in SPECT TTV between dose 1 and dose 3 ^{177}Lu PSMA-617 is predictive of PFS.

IMPLICATIONS FOR PATIENT CARE: SPECT images early in treatment have the potential to predict the response to therapy, potentially allowing adjustment of treatment combinations or changes in therapy to improve patient outcomes.

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