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

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Running title: SPECT quantitation as response biomarker with LuPSMA-617.

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ABSTRACT

Background: ^{177}LuPSMA-617 is an effective and novel treatment in metastatic castrate-resistant prostate cancer (mCRPC). Our ability to assess response rates and therefore efficacy may be improved using predictive tools. This study investigates the predictive value of serial ^{177}Lu- PSMA SPECT imaging in monitoring treatment response. **Methods:** 56 men with progressive mCRPC previously treated with chemotherapy and novel androgen signaling inhibitor (ASI) enrolled in LuPIN trial receiving up to 6 doses of LuPSMA-617 and a radiation sensitizer (NOX66). ^{68}Ga-PSMA-11 and ^{18}FDG PET/CT were performed at study entry and exit, and ^{177}Lu-SPECT/CT (SPECT) vertex to mid thighs was acquired 24hrs following each treatment. SPECT quantitative analysis was undertaken at cycles 1 (baseline) and 3 (week-12) of treatment (C1 and C3). **Results:** 32/56 men had analyzable serial ^{177}Lu-SPECT/CT imaging at both C1 and C3. In this subgroup, median PSA-PFS was 6.3 months (95%CI 5-10) and median OS 12.3 months (95%CI 12-24). PSA 50% response rate was 63% (20/32). SPECT total tumor volume (SPECT-TTV) was reduced in 68% (22/32, median -0.20L (-1.4 to -0.001) and increased in 31% (10/32, median 0.36 (0.1-1.4)). Any increase in SPECT-TTV was associated with shorter PSA-PFS (HR 4.1 (95% CI 1.5-11.2), p 0.006). A ≥30% increase in SPECT-TTV was also associated with shorter PSA-PFS (HR 3.3 (95%CI 1.3-8.6), p 0.02). Tumoral SUVmax was reduced in 91% (29/32) and SUVmean in 84% (27/32); neither was associated with PSA-PFS or OS outcomes. PSA progression by week-12 was also associated with shorter PSA-PFS (HR 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 months (95% CI 2.8-5.6),

and 5/10 men had both PSA and SPECT TTV progression at week 12 (median PSA-PFS 2.8 months (95% CI 1.8-3.7). **Conclusion:** Increasing PSMA-TTV on quantitative ^{177}Lu-SPECT/CT predicts short progression free survival and may play a future role as an imaging response biomarker.

INTRODUCTION

^{177}LuPSMA-617 is an effective therapy in metastatic castrate resistant prostate cancer although treatment resistance and short response duration remains common(*1-4*). Accurate monitoring of response to ^{177}LuPSMA-617 may improve patient outcomes by enabling treatment escalation, change in treatment, or treatment 'holiday', dependent on imaging results. Interim and serial PSMA PET has recently been shown to be predictive of progression free survival with PSMA targeted radionuclide therapy (*5*). Quantitative ^{177}Lu-single photon emission computed tomography /CT (^{177}Lu-SPECT) imaging following each ^{177}LuPSMA-617 dose may also be valuable in response monitoring in addition to providing dosimetric information. This LuPIN trial sub-study aimed to determine if quantitative parameters on serial ^{177}Lu-SPECT imaging 24 hours post ^{177}LuPSMA-617 therapy were predictive of treatment response and progression free survival.

MATERIALS AND METHODS

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

Screening

Men with progressive mCRPC, based on either conventional imaging (computed tomography [CT] and bone scan) or a rising PSA based on Prostate Cancer Working Group 3 (PCWG3) criteria (*8*), were eligible for screening. Prior treatment with at least one line of taxane chemotherapy (docetaxel and/or cabazitaxel) and an ASI (abiraterone and/or enzalutamide) were required for inclusion. Men underwent screening with ^{18}F-fluorodeoxyglucose (FDG) and ^{68}Ga-HBEDD-PSMA-11 (PSMA) PET/CT, bone scan 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 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

Men received up to 6 doses of ^{177}LuPSMA-617 at 6-week intervals with 3 dose escalated cohorts of NOX66 (400mg, 800mg, 1200mg). NOX66 suppositories were administered as a radio-sensitizer on days 1-10 post each ^{177}LuPSMA-617 injection. All cohorts were administered 7.5 GBq of ^{177}LuPSMA-617 on day 1 via slow intravenous injection. The PSMA-617 precursor (AAA Novartis) was radiolabelled to no-carrier-added ^{177}lutetium chloride according to 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 prior

to assess adverse events and biochemical responses. Patients were treated on trial until they were no longer clinically benefitting from treatment.

Imaging procedures and analysis

PSMA and FDG PET/CT scans were performed at baseline and trial exit (after completing 6 cycles or when treatment was ceased) with imaging acquisition and analysis parameters previously published(*9*). ^{177}Lu-SPECT (vertex to mid thighs) were acquired 24 hours after ^{177}LuPSMA-617 injection using a Discovery 670 system (GE Healthcare, Milwaukee, USA) with the following parameters: medium energy collimators, 3 bed positions, 60 projections over 360 degrees with an acquisition time of 10 seconds per frame, 128 x 128 matrix and 4.42 x 4.42 mm^2 pixel size. An energy window centered on 208 keV +/- 10% with a 165 keV +/- 6.5 % scatter window were used. A non-contrast low dose CT scan was performed immediately after using the following parameters: pitch = 1, tube voltage of 120 kV, automatic mAs control (reference mAs 90), slice thickness of 3.7mm, matrix of 512 x 512, field of view of 40cm. The SPECT projection images were reconstructed with an iterative Ordered Subset Estimation Maximum (OSEM) algorithm that used 4 iterations and 10 subsets using SPECTRA Quant™ (MIM Software, Inc, Cleveland, USA). No pre- or post-reconstruction 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 calculated based on a cylinder phantom with known activity.

Quantitative analysis

^{177}Lu-SPECT and ^{68}Ga PSMA PET/CT were analysed semi-quantitatively by a nuclear medicine physician utilising MIM (LesionID™, MIM Software Inc., Cleveland, US) software and a standardised semi-automated workflow to delineate regions of interest with a minimum SUV cut-off of 3. All lesions identified quantitatively were manually reviewed and physiologic activity removed. Whole body quantitation derived total tumor volume (TTV), SUVmax and SUVmean for both PSMA-PET and PSMA-SPECT (*10*).

Statistical Analyses

We measured PSA decline from baseline (≥50% (PSA50)) at any time-point, PSA progression-free survival (PSA-PFS) as defined by PCWG3 criteria, and overall survival (OS) (*8,11*). The Kaplan-Meier method was used to characterise time-to-event endpoints and estimate medians (presented with 95% CIs). We correlated changes in TTV, PSMA intensity, clinical and biochemical parameters with time-to-event outcomes, using univariate Cox proportional hazards regression models (*12,13*). Continuous variables included increase in TTV, SUVmax, SUVmean, time since diagnosis. P-values below 5% were considered significant. We compared TTV from PSMA-PET/CT with C1 ^{177}Lu-SPECT derived tumour volume using scatter plots, Pearson's correlation coefficient. Reproducibility of ^{177}Lu-SPECT TTV and PSMA- SUVmax was undertaken using repeatability statistics calculated from a hierarchical linear mixed model that accounted for variance in score at the patient level. (*14*). A 95% confidence interval for

the repeatability statistics was derived via bootstrapping. Analyses were performed using R (version 4.0.5).

RESULTS

Baseline Patient Characteristics

57% (32/56) men enrolled in LuPIN had ^{177}Lu-SPECT imaging suitable for analysis. 30% (17/56) had incomplete SPECT data precluding analysis and 13% (7/56) did not reach C3 of treatment. Baseline characteristics are summarized in Table 1. In this LuPIN sub-study, 53% (17/32) completed 6 cycles of treatment while 47% (15/32) completed between 3 and 5 cycles. 63% (20/32) achieved PSA50 at any time point. At time of analysis, 84% (27/32) were deceased. There was no difference in either PSA-PFS or OS based on NOX66 dose. Overall, median OS was 12.3 months (95%CI 11.7-23.6). Median PSA PFS was 6.3 months (95% CI 5.1-9.8).

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 -0.20L (-1.4 to -0.001)) and increased in 31% (10/32, median 0.36 (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, (-195 to +42)) and SUVmean reduced in 84% (27/32, median -2.6 (-12 to +10)).

Correlation with Patient Outcomes

Increase in SPECT-TTV between baseline and week-12 was associated with significantly worse PSA-PFS (HR 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 months (95%CI 2.8-5.6), compared to 7.1 months (95%CI 6.3-10.7) for those with no increase in TTV. A ≥ 30% increase in SPECT-TTV was also associated with shorter PSA-PFS (HR 3.3 (95%CI 1.3-8.6), p 0.02). (Figure 1). Increased SUVmax or SUVmean between baseline and week-12 were not associated with PSA-PFS or OS (Table 3). 25% (8/32) patients demonstrated PSA progression by Week-12. PSA progression at week-12 was associated with significantly worse PSA-PFS (HR 26.5 (95%CI 5.4-131), p<0.001). Pts with PSA progression at week-12 had a median PSA-PFS of 3.5 months (95%CI 1.1-4.5) vs 7.9 months (95%CI 6.3-10.7) 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 months (95% CI 2.8-5.6)), and 5/10 men had both PSA and SPECT TTV progression at week 12 (median PSA-PFS 2.8 months (95% CI 1.8-3.7)) (Figure 2).

Reproducibility

TTV was compared between ^{68}Ga PSMA-11 PET/CT at screening and the baseline ^{177}Lu-SPECT/CT (median days between scans 15 (range 6-56)). There was a strong correlation between PSMA-PET TTV and C1 ^{177}Lu-SPECT TTV (R = 0.87 (95% CI 0.74-0.93), p < 0.001) (Figure 3). Mean TTV was similar on PSMA PET compared to SPECT [PET-TTV 925mls (± 856mls) and SPECT-TTV 949mls (± 852mls)].

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 SUVmax, SUVmean or TTV. Repeatability estimate for SUVmax was 0.99 (95%CI 0.97-0.99), SUV mean 0.90 (95%CI 0.81-0.95) and TTV 0.99 (95%CI 0.98-0.99) (Table 4).

DISCUSSION

This study has found that quantified changes in SPECT-TTV between baseline and 12-week ^{177}LuPSMA-617 predict progression free survival in men treated on a prospective PSMA targeted therapy trial. This is the first study that has evaluated SPECT parameters for response biomarker capability, a potentially valuable development that utilizes a readily available tool to potentially enhance personalized treatment by directly assessing treatment response. ^{177}LuPSMA-617 has proven an effective therapy for mCRPC with randomised trials demonstrating both improved overall survival and radiographic progression free survival compared to standard of care(*3*) , and higher PSA 50% response rates and improved patient reported outcomes compared to cabazitaxel (*2*). However, responses can be heterogenous and progression free survival remains relatively short(*2,3*). Combination trials with ^{177}LuPSMA-617 are underway to investigate whether combining ^{177}LuPSMA-617 with other agents may deepen and prolong responses (NCT04419402, NCT03658447, NCT03874884)(*15*). Predictive and interim response biomarkers, both imaging and genomic, will be critical in personalising treatments to optimise longer term treatment responses to PSMA targeted radionuclide therapy (*5,9*). Although there are limitations in spatial resolution with ^{177}Lu-SPECT, its elegant potential as a response biomarker warrants further evaluation.Molecular

imaging as interim response biomarker has been particularly successful in optimising treatment responses with the use of a 12-week ^{18}F-FDG PET in lymphoma (*16,17*) (*18-20*). More recently, Gafita et al have proposed a 12-week interim PSMA PET scan based on its predictive value for early disease progression in a multi-centre ^{177}LuPSMA-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 change in treatment, thereby maximising opportunities for treatment response in individual patients and avoiding the clinical and financial costs of continuing futile treatment. Generally, this has been done in mCRPC by monitoring serum PSA response (*21*). Similar 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 (*22*). In this study, both SPECT TTV and PSA progression were predictive of progression free survival. However, 21% of those in this study with no PSA progression had ^{177}Lu-SPECT TTV progression. Gafita et al had a similar finding with 14% demonstrating PSMA PET progression prior to PSA progression (*5*). Larger numbers are required to determine if use of ^{177}Lu-SPECT-TTV in combination with PSA can more effectively identify disease progression, but this study provides strong preliminary evidence, with SPECT identifying progression in a subset of patients that had not yet had a PSA rise. These results raise the question as to whether ^{77}LuPSMA-SPECT or PSMA PET should be used as preferred interim imaging response biomarker for ^{177}LuPSMA-617 therapy.

Post therapy imaging both with planar and SPECT following radionuclide therapy has been traditionally utilized for dosimetric calculation to determine dose to non-target

organs and tumor(*23-26*). Due to its significantly lower spatial resolution and inability to detect small lesions relative to PET imaging, it has not been considered for treatment response. In our direct comparison of quantitative findings between Ga PSMA-PET and SPECT within 2 weeks, TTV was very similar between SPECT and PET with a high correlation between the two modalities, although theoretically SPECT will underestimate small volume disease (*27*). However, evaluating disease progression requiring treatment change or intensification should not depend on identifying small volume disease. A lesion that is below spatial resolution for detection on SPECT, will become visible as its size increases. While the findings from this trial confirm that SPECT has potential for identifying clinically significant disease progression, the opposite may be a more difficult issue. SPECT may struggle to confirm complete resolution of all sites of disease in men with exceptional responses to ^{177}LuPSMA-617 therapy. Confirmation of exceptional response may indeed require the spatial resolution of PSMA PET and further research in this is required to more precisely define the limitations of Lu-SPECT and appropriate minimal volume changes required to identify progression.

This study relied on quantitation of SPECT data to determine an increase in TTV rather than visual assessment. 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 (*28,29*). Accurately assessing changes in tumour volume visually can be difficult, especially in the presence of large volume metastatic bone disease. We found repeatability of tumour volume on ^{177}Lu-SPECT was high and comparable to PSMA PET/CT (*30*). However, quantitation remains outside of routine reporting guidelines and is time intensive to undertake.

Further work needs to be done both to evaluate accuracy of quantitation over visual assessment, and streamline quantitation to be more user friendly for integration into routine clinical practice (*31*).

Changes in SPECT TTV were predictive of progression free but not overall survival in this study. While this may be due to the small patient cohort, it may also be because many patients with progressive disease on the 12-week post therapy SPECT were taken off trial and changed therapy to an agent which proved effective. Further work is required to evaluate the benefit of SPECT as a prognostic biomarker.

There are several limitations to this study. Firstly, patient numbers are small, and a larger cohort is needed to validate these findings. This is a single centre study and ^{177}Lu-SPECT quantitative measures can vary significantly between centres and systems (*32*). Further evaluation is required to harmonise image acquisition and reconstruction across centres and imaging systems for results to be reproducible. Finally, further research is necessary to better define appropriate volume cut-offs for significant increase in TTV that should be used to identify disease progression. This will require trials with larger patient numbers and outcome data. However, this study has provided a strong foundation on which to build further work.

CONCLUSION

Increasing PSMA -TTV on quantitative ^{177}Lu-SPECT/CT predicts short progression free survival and identified progression in some men that had yet to demonstrate PSA progression. This tool shows promise as an imaging response biomarker.

FINANCIAL DISCLOSURES

The investigator-initiated study was sponsored by St Vincent's Hospital and was 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.

LE -Advisory Role: Noxopharm Limited. Trials support – Novartis, Astellas, grant funding support from St Vincent's Clinic Foundation

AMJ – Advisory Role: Noxopharm Limited. Institutional funding – Novartis.

PW, RN – salaried employees of MIM Software, Inc.

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KEY POINTS

Question: Do the SPECT images acquired 24 hours following ^{177}Lu PSMA-617 therapy provide predictive information on patient outcomes?

Pertinent Findings: Change in SPECT total tumor volume between dose 1 and dose 3 Lu PSMA is predictive of progression free survival.

Implications for patient care: The post therapy SPECT images early in treatment have the potential to predict patient responses to therapy. This may allow adjustments in treatment combinations or change in therapy to improve patient outcomes.

REFERENCES

1. Emmett L, Crumbaker M, Ho B, et al. Results of a Prospective Phase 2 Pilot Trial of (177)Lu-PSMA-617 Therapy for Metastatic Castration-Resistant Prostate Cancer Including Imaging Predictors of Treatment Response and Patterns of Progression. *Clin Genitourin Cancer.* 2018.

2. Hofman MS, Emmett L, Sandhu S, et al. [(177)Lu]Lu-PSMA-617 versus cabazitaxel in patients with metastatic castration-resistant prostate cancer (TheraP): a randomised, open-label, phase 2 trial. *Lancet.* 2021;397:797-804.

3. Sartor O, de Bono J, Chi KN, et al. Lutetium-177-PSMA-617 for Metastatic Castration-Resistant Prostate Cancer. *N Engl J Med.* 2021.

4. Violet J, Sandhu S, Iravani A, et al. Long term follow-up and outcomes of re-treatment in an expanded 50 patient single-center phase II prospective trial of Lutetium-177 ((177)Lu) PSMA-617 theranostics in metastatic castrate-resistant prostate cancer. *J Nucl Med.* 2019.

5. Gafita A, Rauscher I, Weber M, et al. Novel framework for treatment response evaluation using PSMA-PET/CT in patients with metastatic castration-resistant prostate cancer (RECIP 1.0): an international multicenter study. *J Nucl Med.* 2022.

6. Crumbaker M, Pathmanandavel S, Yam AO, et al. Phase I/II Trial of the Combination of (177)Lutetium Prostate specific Membrane Antigen 617 and Idronoxil (NOX66) in Men with End-stage Metastatic Castration-resistant Prostate Cancer (LuPIN). *Eur Urol Oncol.* 2020.

7. Pathmanandavel S, Crumbaker M, Yam AO, et al. 177Lutetium PSMA-617 and idronoxil (NOX66) in men with end-stage metastatic castrate-resistant prostate cancer (LuPIN): Patient outcomes and predictors of treatment response of a Phase I/II trial. *Journal of Nuclear Medicine.* 2021.

8. Scher HI, Morris MJ, Stadler WM, et al. Trial Design and Objectives for Castration-Resistant Prostate Cancer: Updated Recommendations From the Prostate Cancer Clinical Trials Working Group 3. *J Clin Oncol.* 2016;34:1402-1418.

9. Pathmanandavel S, Crumbaker M, Yam AO, et al. (177)Lutetium PSMA-617 and idronoxil (NOX66) in men with end-stage metastatic castrate-resistant prostate cancer (LuPIN): Patient outcomes and predictors of treatment response of a Phase I/II trial. *J Nucl Med.* 2021.

10. Niman R, Buteau JP, Kruzer A, Turcotte Ãr, Nelson A. Evaluation of a semi-automated whole body PET segmentation method applied to Diffuse Large B Cell Lymphoma. *J Nucl Med.* 2018;59:592.

11. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer.* 2009;45:228-247.

12. Halabi S, Lin CY, Kelly WK, et al. Updated prognostic model for predicting overall survival in first-line chemotherapy for patients with metastatic castration-resistant prostate cancer. *J Clin Oncol.* 2014;32:671-677.

13. Gafita A, Calais J, Grogan TR, et al. Nomograms to predict outcomes after 177Lu-PSMA therapy in men with metastatic castration-resistant prostate cancer: an international, multicentre, retrospective study. *The Lancet Oncology.* 2021.

14. Stoffel MA, Nakagawa S, Schielzeth H, Goslee S. rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods in Ecology and Evolution.* 2017;8:1639-1644.

15. Emmett L, Subramaniam S, Joshua AM, et al. ENZA-p trial protocol: a randomized phase II trial using prostate-specific membrane antigen as a therapeutic target and prognostic indicator in men with metastatic castration-resistant prostate cancer treated with enzalutamide (ANZUP 1901). *BJU Int.* 2021.

16. Gallamini A, Barrington SF, Biggi A, et al. The predictive role of interim positron emission tomography for Hodgkin lymphoma treatment outcome is confirmed using the interpretation criteria of the Deauville five-point scale. *Haematologica.* 2014;99:1107-1113.

17. Eertink JJ, Burggraaff CN, Heymans MW, et al. Optimal timing and criteria of interim PET in DLBCL: a comparative study of 1692 patients. *Blood Adv.* 2021;5:2375-2384.

18. Radford J, Illidge T, Counsell N, et al. Results of a trial of PET-directed therapy for early-stage Hodgkin's lymphoma. *N Engl J Med.* 2015;372:1598-1607.

19. Borchmann P, Goergen H, Kobe C, et al. PET-guided treatment in patients with advanced-stage Hodgkin's lymphoma (HD18): final results of an open-label, international, randomised phase 3 trial by the German Hodgkin Study Group. *The Lancet.* 2017;390:2790-2802.

20. Johnson P, Federico M, Kirkwood A, et al. Adapted Treatment Guided by Interim PET-CT Scan in Advanced Hodgkin's Lymphoma. *N Engl J Med.* 2016;374:2419-2429.

21. Halabi S, Vogelzang NJ, Ou S-S, Owzar K, Archer L, Small EJ. Progression-free survival as a predictor of overall survival in men with castrate-resistant prostate cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2009;27:2766-2771.

22. Balk SP, Ko YJ, Bubley GJ. Biology of prostate-specific antigen. *J Clin Oncol.* 2003;21:383-391.

23. Fendler WP, Reinhardt S, Ilhan H, et al. Preliminary experience with dosimetry, response and patient reported outcome after 177Lu-PSMA-617 therapy for metastatic castration-resistant prostate cancer. *Oncotarget.* 2017;8:3581-3590.

24. Jackson PA, Hofman MS, Hicks RJ, Scalzo M, Violet J. Radiation Dosimetry in (177)Lu-PSMA-617 Therapy Using a Single Posttreatment SPECT/CT Scan: A Novel Methodology to Generate Time- and Tissue-Specific Dose Factors. *J Nucl Med.* 2020;61:1030-1036.

25. Violet J, Jackson P, Ferdinandus J, et al. Dosimetry of (177)Lu-PSMA-617 in Metastatic Castration-Resistant Prostate Cancer: Correlations Between Pretherapeutic Imaging and Whole-Body Tumor Dosimetry with Treatment Outcomes. *J Nucl Med.* 2019;60:517-523.

26. Yadav MP, Ballal S, Tripathi M, et al. Post-therapeutic dosimetry of 177Lu-DKFZ-PSMA-617 in the treatment of patients with metastatic castration-resistant prostate cancer. *Nucl Med Commun.* 2017;38:91-98.

27. Khalil MM, Tremoleda JL, Bayomy TB, Gsell W. Molecular SPECT Imaging: An Overview. *Int J Mol Imaging.* 2011;2011:796025.

28. Kurth J, Kretzschmar J, Aladwan H, et al. Evaluation of [68Ga]Ga-PSMA PET/CT for therapy response assessment of [177Lu]Lu-PSMA radioligand therapy in metastasized castration refractory prostate cancer and correlation with survival. *Nucl Med Commun.* 2021;42:1217-1226.

29. Grubmuller B, Senn D, Kramer G, et al. Response assessment using (68)Ga-PSMA ligand PET in patients undergoing (177)Lu-PSMA radioligand therapy for metastatic castration-resistant prostate cancer. *Eur J Nucl Med Mol Imaging.* 2019;46:1063-1072.

30. Seifert R, Sandach P, Kersting D, et al. Repeatability of (68)Ga-PSMA-HBED-CC PET/CT-derived total molecular tumor volume. *J Nucl Med.* 2021.

31. Niman R, Buteau JP, Kruzer A, Turcotte Ãr, Nelson A. Evaluation of a semi-automated whole body PET segmentation method applied to Diffuse Large B Cell Lymphoma. *Journal of Nuclear Medicine.* 2018;59:592.

32. Peters SMB, Meyer Viol SL, van der Werf NR, et al. Variability in lutetium-177 SPECT quantification between different state-of-the-art SPECT/CT systems. *EJNMMI Phys.* 2020;7:9.

FIGURES

Figure 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

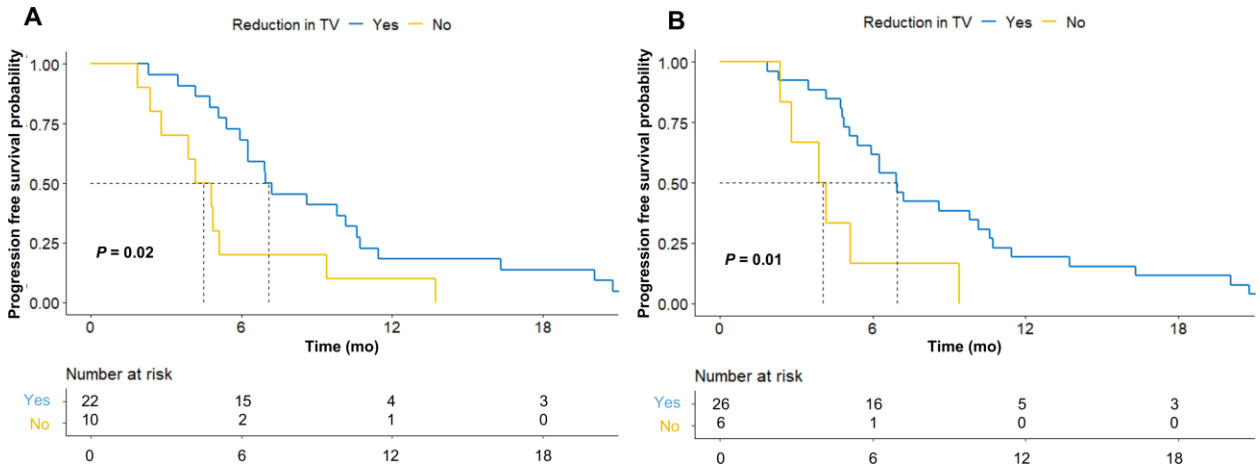
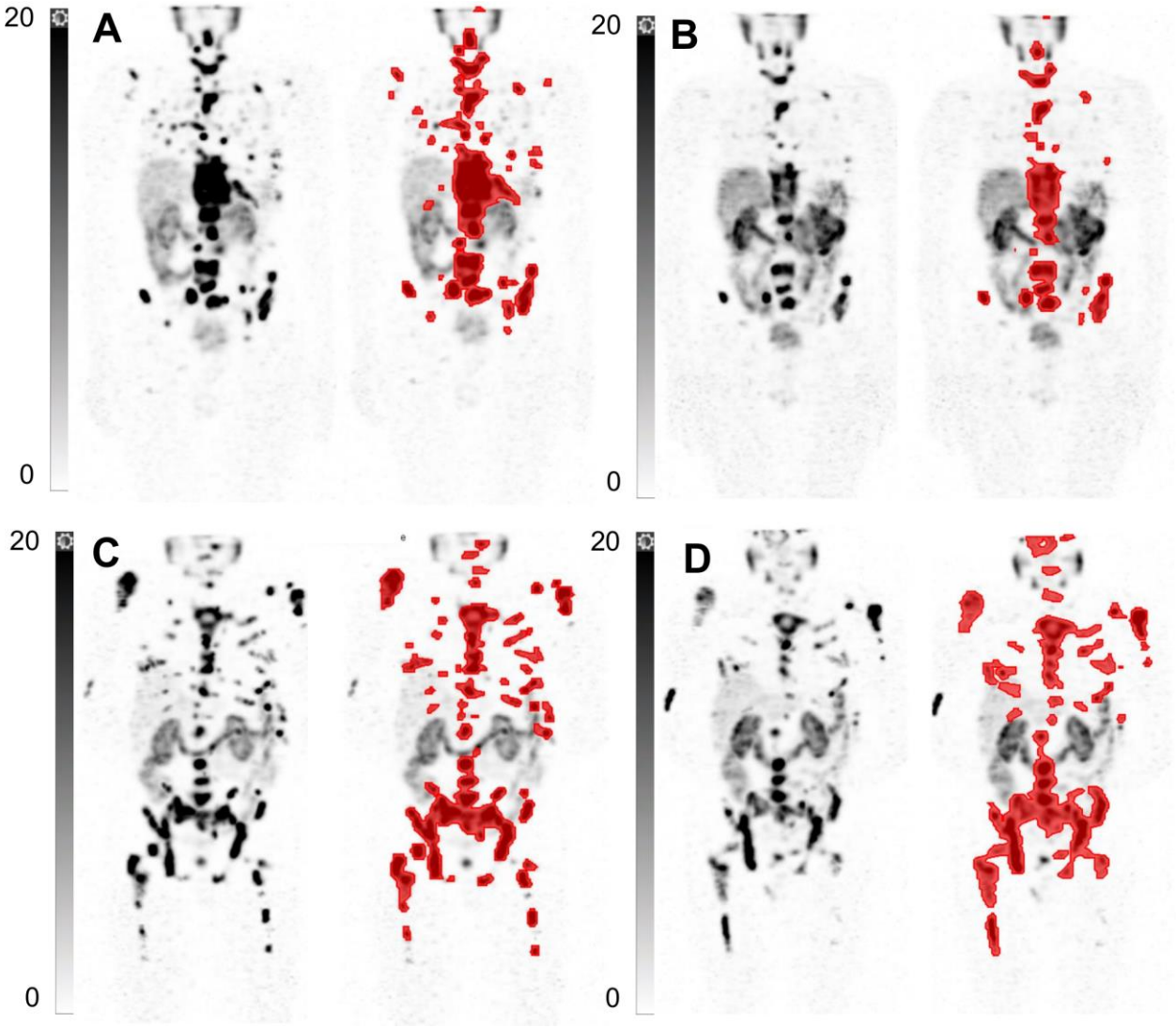
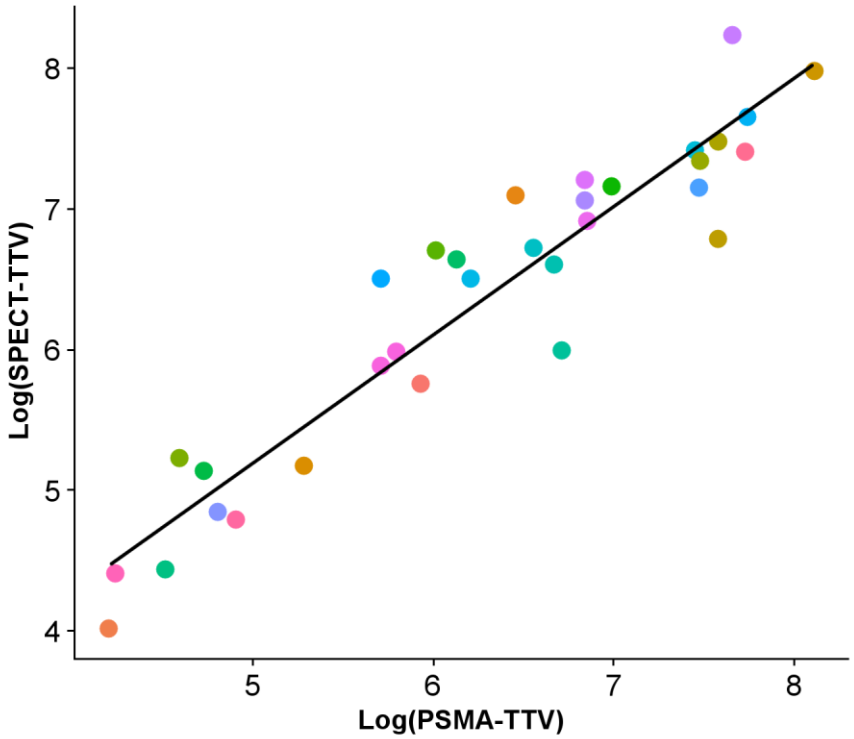


Figure 2. Maximum intensity projection (MIP) and quantitation of ^{177}Lu-SPECT at cycle 1 (A) and cycle 3 (B) for a patient with reduction in SPECT-TTV and PSA and a PSA-PFS 22 months. MIP and quantitation of ^{177}Lu-SPECT at cycle 1 (C) and cycle 3 (D) for a patient with increase in SPECT-TTV > 30%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



459 Figure 3. Scatterplot of log(PSMA-TTV) at baseline versus log(SPECT-TTV) at cycle 1



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TABLES

Table 1. Baseline patient characteristics.

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

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

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

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

	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]

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

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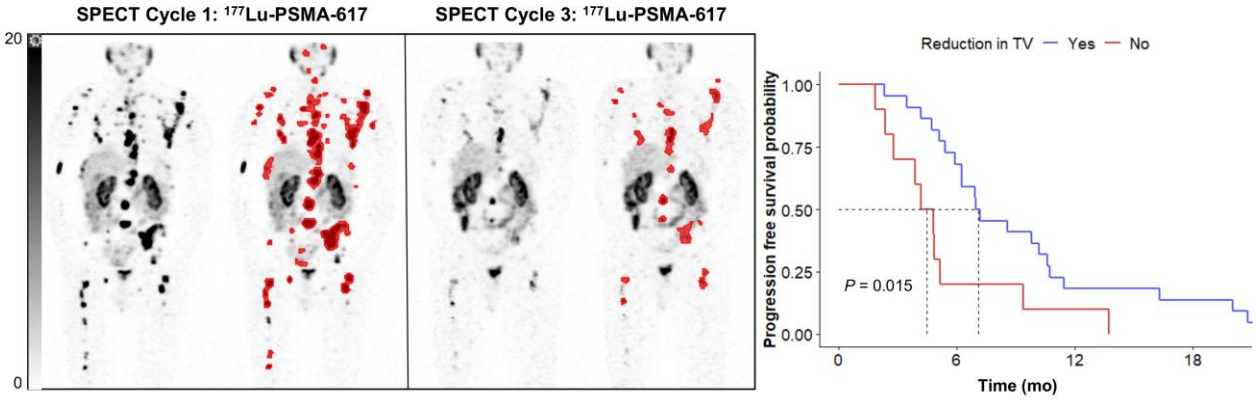
GRAPHICAL ABSTRACT



Change in ^{177}Lu-SPECT total tumor volume between cycle 1 and cycle 3 of therapy predicts PSA progression free survival