



European Association of Urology



Brief Correspondence

AR and PI3K Genomic Profiling of Cell-free DNA Can Identify Poor Responders to Lutetium-177-PSMA Among Patients with Metastatic Castration-resistant Prostate Cancer

Jan Vanwelkenhuyzen^{a,b,c,†}, Eva Van Bos^{d,†}, Siska Van Bruwaene^{d,†}, Karl Lesage^d, Alex Maes^e, Sezgin Üstmert^e, Filip Lavent^e, Laurence Beels^e, Henrik Grönberg^f, Piet Ost^{a,b,g}, Johan Lindberg^{f,‡}, Bram De Laere^{a,b,f,‡,*}

Article info

Article history:

Accepted May 9, 2023

Associate Editor:

Guillaume Ploussard

Keywords:

Metastatic castration-resistant prostate cancer
Lutetium-177-PSMA
Circulating tumour DNA
Biomarker
Liquid biopsy
Cell-free DNA

Abstract

Lutetium-177 prostate-specific membrane antigen radioligands (¹⁷⁷Lu-PSMA) are new therapeutic agents for the treatment of metastatic castration-resistant prostate cancer (mCRPC). We evaluated the prognostic value of circulating tumour DNA (ctDNA) profiling in patients with mCRPC starting treatment with ¹⁷⁷Lu-PSMA I&T. Between January 2020 and October 2022, patients with late-stage mCRPC ($n = 57$) were enrolled in a single-centre observational cohort study. Genomic alterations in the AR gene, PI3K signalling pathway, *TP53*, and *TMPRSS2-ERG* were associated with progression-free survival (PFS) on Kaplan-Meier and multivariable Cox regression analyses. Median PFS of 3.84 mo (95% confidence interval [CI] 3.3–5.4) was observed, and 21/56 (37.5%) evaluable patients experienced a prostate-specific antigen response of $\geq 50\%$ during treatment. Among 46 patients who provided a blood sample for profiling before ¹⁷⁷Lu-PSMA treatment, ctDNA was detected in 39 (84.8%); higher ctDNA was correlated with shorter PFS. Genomic structural rearrangements in the AR gene (hazard ratio [HR] 9.74, 95% confidence interval [CI] 2.4–39.5; $p = 0.001$) and alterations in the PI3K signalling pathway (HR 3.58, 95% CI 1.41–9.08; $p = 0.007$) were independently associated with poor ¹⁷⁷Lu-PSMA prognosis on multivariable Cox regression. Prospective evaluation of these associations in biomarker-driven trials is warranted.

Patient summary: We examined cell-free DNA in blood samples from patients with advanced metastatic prostate cancer who started treatment with lutetium-177-PSMA, a new radioligand therapy. We found that patients with genetic alterations in the androgen receptor gene or PI3K pathway genes did not experience a lasting benefit from lutetium-177-PSMA.

© 2023 The Author(s). Published by Elsevier B.V. on behalf of European Association of Urology. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

[†] These authors contributed equally to this work.

[‡] These authors jointly supervised this work.



The therapeutic armamentarium for metastatic castration-resistant prostate cancer (mCRPC) has most recently expanded with the introduction of radioligand-based therapy with lutetium-177-labelled ligands for prostate-specific membrane antigen (^{177}Lu -PSMA) for advanced PSMA-positive disease [1,2]. To date, information on detection of genomic events and their association with ^{177}Lu -PSMA therapeutic responses and outcomes is lacking [3–5]. Here we report a retrospective translational analysis for the most common altered genes or signalling pathways (occurring in >30% patients), including the androgen receptor gene (*AR*), phosphoinositide 3-kinase (*PI3K*) signalling, and *TP53* and *TPRSS2-ERG* genes, in baseline circulating tumour DNA (ctDNA) samples from ^{177}Lu -PSMA-treated patients with mCRPC in terms of their association with prostate-specific antigen (PSA) responses and outcomes.

A detailed description of the patients and methods is provided in the [Supplementary material](#). In brief, from January 2020 to October 2022 we enrolled 57 patients with mCRPC in a single-centre noninterventional observational cohort study at AZ Groeninge Hospital (Kortrijk, Belgium; EC registration number: B670201941650). All patients had previously received at least one chemotherapy and/or one novel AR signalling inhibitor regimen for mCRPC. All patients had ^{68}Ga -PSMA or ^{18}F -PSMA uptake by metastases on positron emission tomography/computed tomography (PET/CT) and were eligible for treatment with ^{177}Lu -PSMA I&T. After obtaining informed consent, data on clinicopathological characteristics, PSA responses, and outcomes were prospectively collected ([Table 1](#)). In addition, liquid biopsy samples were collected before and during ^{177}Lu -PSMA treatment for comprehensive genomic profiling of plasma-derived circulating tumour DNA (ctDNA) as previously described [6]. The cell-free DNA genomic profiling assay is custom designed for metastatic prostate cancer and can comprehensively detect all genomic alterations relevant to metastatic prostate cancer ([Supplementary material](#)) [6]. Treating physicians were blinded to ctDNA results during treatment follow-up. PFS was defined as the time until patients were no longer clinical benefiting according to Prostate Cancer Working Group 3 guidelines, which is a composite time-to-event measure defined as the date and specific reason(s) for discontinuation of a therapy, triple assessed in terms of biochemical, radiological, and clinical progression. The (confirmed) $\geq 50\%$ PSA response rates throughout the course of ^{177}Lu -PSMA treatment were a secondary outcome measure.

Median PFS in our cohort was 3.84 mo (95% confidence interval [CI] 3.3–5.4 mo); 53/57 patients (93.0%) had experienced disease progression at the time of analysis. PSA response data were available for 56/57 patients (98.2%). In total 21/56 (37.5%) patients experienced a PSA response of $\geq 50\%$ throughout the course of their treatment, which was associated with superior PFS (median 2.9 vs 7.3 mo; $p < 0.0001$), especially when the $\geq 50\%$ PSA response was confirmed in subsequent measurements. A confirmed $\geq 50\%$ PSA response remained independently associated with PFS (hazard ratio [HR] 0.10, 95% CI 0.04–0.30; $p < 0.001$) on multivariable Cox regression analysis ([Supplementary Fig. 1](#)).

Table 1 – Patient characteristics ($n = 57$) and baseline blood chemistry

Parameter	Result
Median age, yr (IQR)	70.51 (64.88–74.85)
ECOG performance status, n (%)	
0–1	41 (74.5)
≥ 2	14 (25.5)
Gleason score, n (%)	
Gleason 5–7	24 (42.1)
Gleason 8–10	30 (52.6)
Unknown	3 (5.3)
Metastasis stage at diagnosis, n (%)	
M0	36 (64.3)
M1	18 (32.1)
Mx	2 (3.6)
PSMA PET/CT findings, n (%)	
Lymph node metastases	37 (64.9)
Bone metastases	52 (91.2)
Visceral metastases	26 (45.6)
Liver metastases, n (%)	5 (8.8)
Median haemoglobin, g/dl (IQR)	11.1 (10.0–12.3)
Median PSA, ng/ml (IQR)	132.0 (35.8–396.0)
Median ALP, IU/liter (IQR)	116.0 (82.5–205.5)
Median LDH IU/liter (IQR)	264.0 (214.50–437.0)
Prior radical prostatectomy, n (%)	30 (52.6)
Prior prostate radiotherapy, n (%)	27 (48.2)
Median prior lines of systemic therapy, n (IQR)	4 (3–4)
Prior ARSI, n (%)	
None	1 (1.8)
1 regimen	35 (61.4)
≥ 2 regimens	21 (36.9)
Prior taxane-based chemotherapy, n (%)	
None	3 (5.3)
1 regimen	15 (26.3)
≥ 2 regimens	39 (68.5)
Other prior systemic therapy, n (%)	
Radium-223	22 (38.6)
PARP inhibitor	6 (10.5)
Platinum-based chemotherapy	3 (5.3)

ARSI = androgen receptor signalling inhibitor; CT = computed tomography; ECOG = Eastern Cooperative Oncology Group; IQR = interquartile range; LDH = lactate dehydrogenase; PET = positron emission tomography; PSA = prostate-specific antigen; PSMA = prostate-specific membrane antigen.

A peripheral blood sample was collected from 46/57 patients (80.7%) at the start of ^{177}Lu -PSMA treatment. Targeted DNA sequencing using the Prostate Biomarker (ProBio) panel [6,7] detected ctDNA in 39/46 patients (84.8%). Quartile index stratification of ctDNA levels identified three prognostic groups (low/undetectable, intermediate, and high ctDNA) with different Kaplan-Meier PFS estimates (median 7.3 vs 4.3 vs 2.4 mo; $p = 0.0023$; [Supplementary Fig. 2](#)). As a well-recognised prognostic biomarker, the ctDNA fraction was included as a continuous variable in all subsequent multivariable Cox regression analyses. Genomic alterations were most frequently detected in the *AR*, *PTEN*, *TP53*, and *TPRSS2-ERG* genes, with prevalence estimates in line with the literature [8] ([Supplementary Fig. 3](#)).

There are different classes of AR gene-body alterations that warrant comprehensive profiling [6]. Here, AR (hot-spot) mutations, amplifications, and genomic structural rearrangements (GSRs) were detected in 10/46 (21.7%), 24/46 (52.2%), and 22/46 (47.8%) patients, respectively. Correlation analysis for individual AR alteration classes revealed that AR mutations were not associated with outcomes. PFS was shorter for patients with AR amplifications

(median 2.9 vs 5.4 mo; $p = 0.0097$) or intra-AR GSRs (median 2.7 vs 5.5 mo; $p = 0.0012$) than for patients with a copy number-neutral wild-type AR gene (Supplementary Fig. 4). Seventeen patients harboured GSRs within coding or cryptic exon regions of the AR gene body, representing a unique subpopulation with worse PFS than for patients with amplified-only or wild-type AR (median 2.6 vs 3.8 vs 5.4 mo; $p = 0.002$). On multivariable Cox regression analysis, AR GSRs remained independently associated with poor PFS (HR 9.74, 95% CI 2.4–39.5; $p = 0.001$; Fig. 1A).

PI3K pathway alterations were detected in 18/46 patients (39.1%), the most common of which was homozygous *PTEN* deletion (14/18, 77.8 %). Patients with PI3K pathway alterations had shorter PFS (median 2.7 vs 5.3 mo; $p = 0.0013$), which remained independently associated with poor prognosis on multivariable Cox regression analysis (HR 3.58, 95% CI 1.41–9.08; $p = 0.007$; Fig. 1B). Finally, we observed that alterations in *TP53*, a well-established biomarker of poor prognosis in the context of AR signalling inhibitors [9], and *TMPRSS2-ERG* were not associated with

^{177}Lu -PSMA outcomes (Supplementary Fig. 5). Although genomic alterations in the AR gene and the PI3K signalling pathway were associated with PFS, none of the molecular biomarkers assessed were associated with a PSA response of $\geq 50\%$ (Supplementary Fig. 6).

In conclusion, we demonstrated the prognostic value of baseline ctDNA profiling in ^{177}Lu -PSMA-treated mCRPC, with high ctDNA levels associated with inferior outcomes. Specific AR (GSRs and amplifications) and PI3K pathway signalling alterations (mostly *PTEN* loss) were associated with inferior outcomes. For AR amplifications, this finding is in line with the literature [4]; however, we also demonstrated that the prognostic value of AR alterations may be driven by intragenic structural variants, which frequently co-occur with AR gene amplifications in late-stage disease [9].

Our study has some limitations. First, this post hoc analysis was performed in a relatively small, but real-life, all-comer cohort representing a heavily pretreated patient population. This may be explained in part by the initial introduction of ^{177}Lu -PSMA for compassionate use or systemic

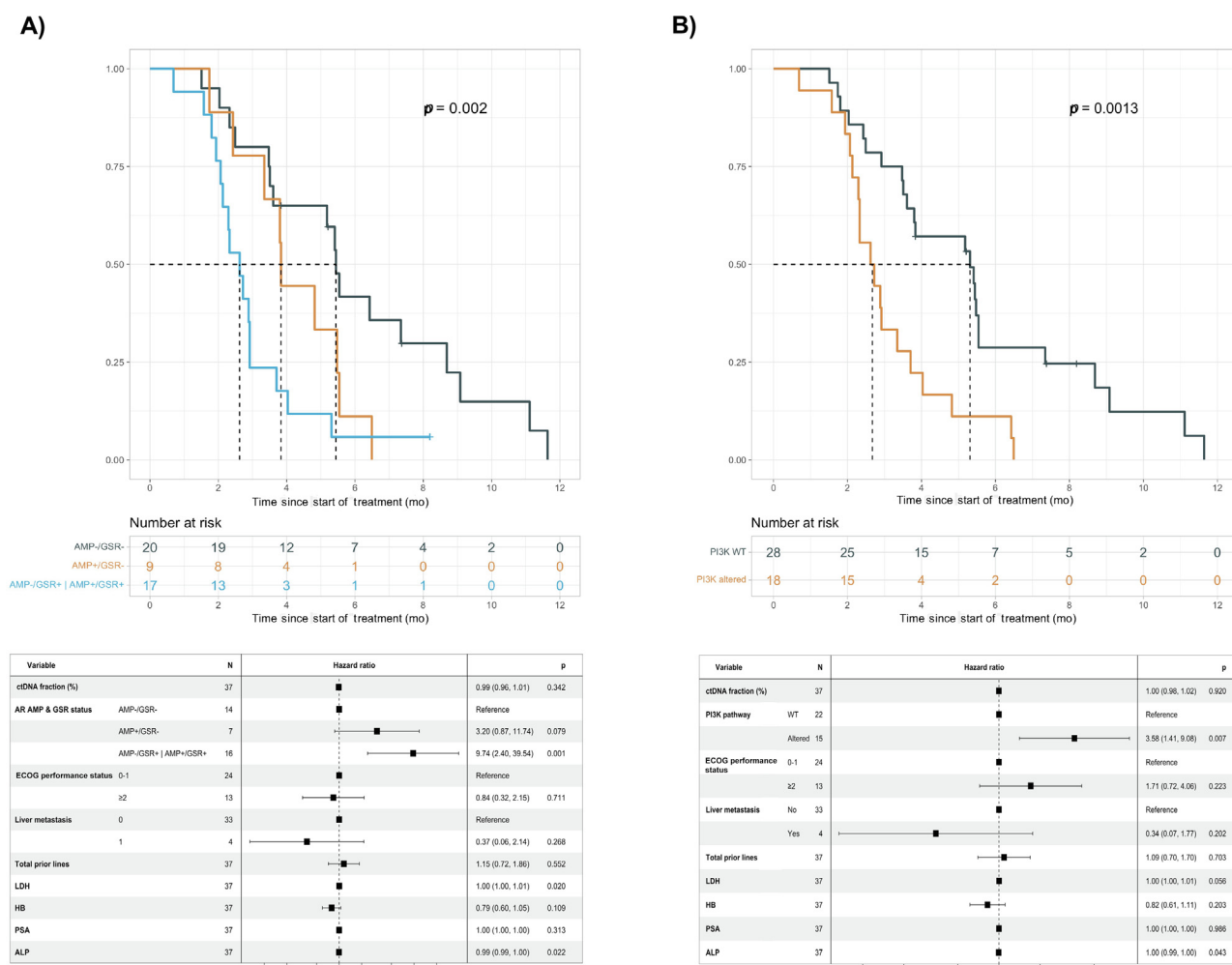


Fig. 1 – Genomic alterations in the AR gene and PI3K signalling in plasma cell-free DNA samples at baseline from patients with mCRPC ($n = 46$) in relation to progression-free survival on ^{177}Lu -PSMA. Kaplan-Meier (upper) and multivariable Cox regression (lower) analyses of progression-free survival, stratified according to baseline detection of genomic alterations in (A) AR and (B) PI3K signalling. The p values in the Kaplan-Meier plots were calculated via a log-rank test. ALP = alkaline phosphatase; AMP = gene amplification; ctDNA = circulating tumour DNA; ECOG = Eastern Cooperative Oncology Group; GSR = genomic structural rearrangements; HB = haemoglobin; LDH = lactate dehydrogenase; PSA = prostate-specific antigen; WT = wild type.

therapy in the third or later lines in Belgium. Second, fluorodeoxyglucose (FDG) PET/CT was not routinely performed in all patients given the molecular imaging reimbursement criteria in Belgium. Whereas limited FDG uptake did not preclude treatment with ^{177}Lu -PSMA, adequate PSMA uptake was mandatory for study eligibility. Finally, prognostic PSMA-related variables outside of our standard practice (eg, the number of PSMA-positive lesions and mean standardised uptake values) were not included [10]. Current patient numbers precluded analysis of other genomic aberrations observed in relation to outcomes. Although hypothesis-generating, these data warrant prospective evaluation of AR and PI3K pathway genomic alterations for patient selection for ^{177}Lu -PSMA treatment, which we will investigate in the ProBio trial (NCT03903835) [7].

Author contributions: Jan Vanwelkenhuyzen had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Ost, Van Bruwaene, De Laere.

Acquisition of data: Vanwelkenhuyzen, Van Bos, Van Bruwaene, Lesage, Üstmert, Lindberg.

Analysis and interpretation of data: Vanwelkenhuyzen, Van Bos, Van Bruwaene, Lindberg, De Laere.

Drafting of the manuscript: Vanwelkenhuyzen, Van Bos, De Laere.

Critical revision of the manuscript for important intellectual content: All authors.

Statistical analysis: Vanwelkenhuyzen, Lindberg, De Laere.

Obtaining funding: None.

Administrative, technical, or material support: Maes, Üstmert, Lavent, Beels, Van Bruwaene, Grönberg, Lindberg.

Supervision: Lindberg, Van Bruwaene, De Laere.

Other (patient accrual and treatment): Van Bos, Lesage, Van Bruwaene.

Financial disclosures: Jan Vanwelkenhuyzen certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: None.

Funding/Support and role of the sponsor: This study was performed with the support of SU2C (Belgium) (STI.VLK.2020.0006.01 & STI.VLK.2022.0005.01). The Funding agencies had no role in data collection and analysis, study design and preparation of the manuscript.

Acknowledgments

We are grateful to the patients and staff of AZ Groeninge for their valuable contributions and for the support of CRIG's PrIO-MiC network.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.euros.2023.05.008>.

References

- [1] Sartor O, de Bono J, Chi KN, et al. Lutetium-177-PSMA-617 for metastatic castration-resistant prostate cancer. *N Engl J Med* 2021;385:1091–103. <https://doi.org/10.1056/NEJMoa2107322>.
- [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. [https://doi.org/10.1016/S0140-6736\(21\)00237-3](https://doi.org/10.1016/S0140-6736(21)00237-3).
- [3] Fettke H, Ng N, Hauser C, et al. Circulating tumor DNA and outcomes with lutetium-PSMA in advanced prostate cancer: preliminary results from an Australian study. *Cancer Res* 2022;82 (12 Suppl):532. <https://doi.org/10.1158/1538-7445.AM2022-532>.
- [4] De Giorgi U, Sansovini M, Severi S, et al. Circulating androgen receptor gene amplification and resistance to ^{177}Lu -PSMA-617 in metastatic castration-resistant prostate cancer: results of a phase 2 trial. *Br J Cancer* 2021;125:1226–32. <https://doi.org/10.1038/s41416-021-01508-5>.
- [5] Privé BM, Slootbeek PHJ, Laarhuis BI, et al. Impact of DNA damage repair defects on response to PSMA radioligand therapy in metastatic castration-resistant prostate cancer. *Prostate Cancer Prostat Dis* 2022;25:71–8. <https://doi.org/10.1038/s41391-021-00424-2>.
- [6] Mayrhofer M, De Laere B, Whittington T, et al. Cell-free DNA profiling of metastatic prostate cancer reveals microsatellite instability, structural rearrangements and clonal hematopoiesis. *Genome Med* 2018;10:85. <https://doi.org/10.1186/s13073-018-0595-5>.
- [7] De Laere B, Crippa A, Discacciati A, et al. Clinical trial protocol for ProBio: an outcome-adaptive and randomised multiarm biomarker-driven study in patients with metastatic prostate cancer. *Eur Urol Focus* 2022;8:1617–21. <https://doi.org/10.1016/j.euf.2022.03.005>.
- [8] Abida W, Cyrta J, Heller G, et al. Genomic correlates of clinical outcome in advanced prostate cancer. *Proc Natl Acad Sci U S A* 2019;116:11428–36. <https://doi.org/10.1073/pnas.1902651116>.
- [9] De Laere B, Oeyen S, Mayrhofer M, et al. TP53 outperforms other androgen receptor biomarkers to predict abiraterone or enzalutamide outcome in metastatic castration-resistant prostate cancer. *Clin Cancer Res* 2019;25:1766–73. <https://doi.org/10.1158/1078-0432.ccr-18-1943>.
- [10] Gafita A, Calais J, Grogan TR, et al. Nomograms to predict outcomes after ^{177}Lu -PSMA therapy in men with metastatic castration-resistant prostate cancer: an international, multicentre, retrospective study. *Lancet Oncol* 2021;22:1115–25. [https://doi.org/10.1016/S1470-2045\(21\)00274-6](https://doi.org/10.1016/S1470-2045(21)00274-6).

^a Department of Human Structure and Repair, Ghent University, Ghent, Belgium

^b Cancer Research Institute Ghent, Ghent, Belgium

^c Centre for Medical Biotechnology (CMB) VIB, Zwijnaarde, Belgium

^d Department of Urology, AZ Groeninge, Kortrijk, Belgium

^e Department of Nuclear Medicine, AZ Groeninge, Kortrijk, Belgium

^f Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden

^g Department of Radiotherapy, GZA Sint-Augustinus, Antwerp, Belgium

* Corresponding author. Department of Human Structure and Repair, Ghent University, Ghent 9000, Corneel Heymanslaan 1, Belgium
E-mail address: bramdlae.delaere@ugent.be (B. De Laere).