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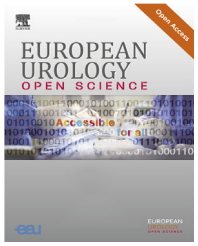

European Association of Urology

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

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Abstract

Lutetium-177 prostate-specific membrane antigen radioligands (^{177}Lu-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 ^{177}Lu-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 ≥50% during treatment. Among 46 patients who provided a blood sample for profiling before ^{177}Lu-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 ^{177}Lu-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.

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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 *TMPRSS2-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) ≥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 ≥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 ≥50% PSA response was confirmed in subsequent measurements. A confirmed ≥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)	132.00 (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 *TMPRSS2-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* (hotspot) 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 ≥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

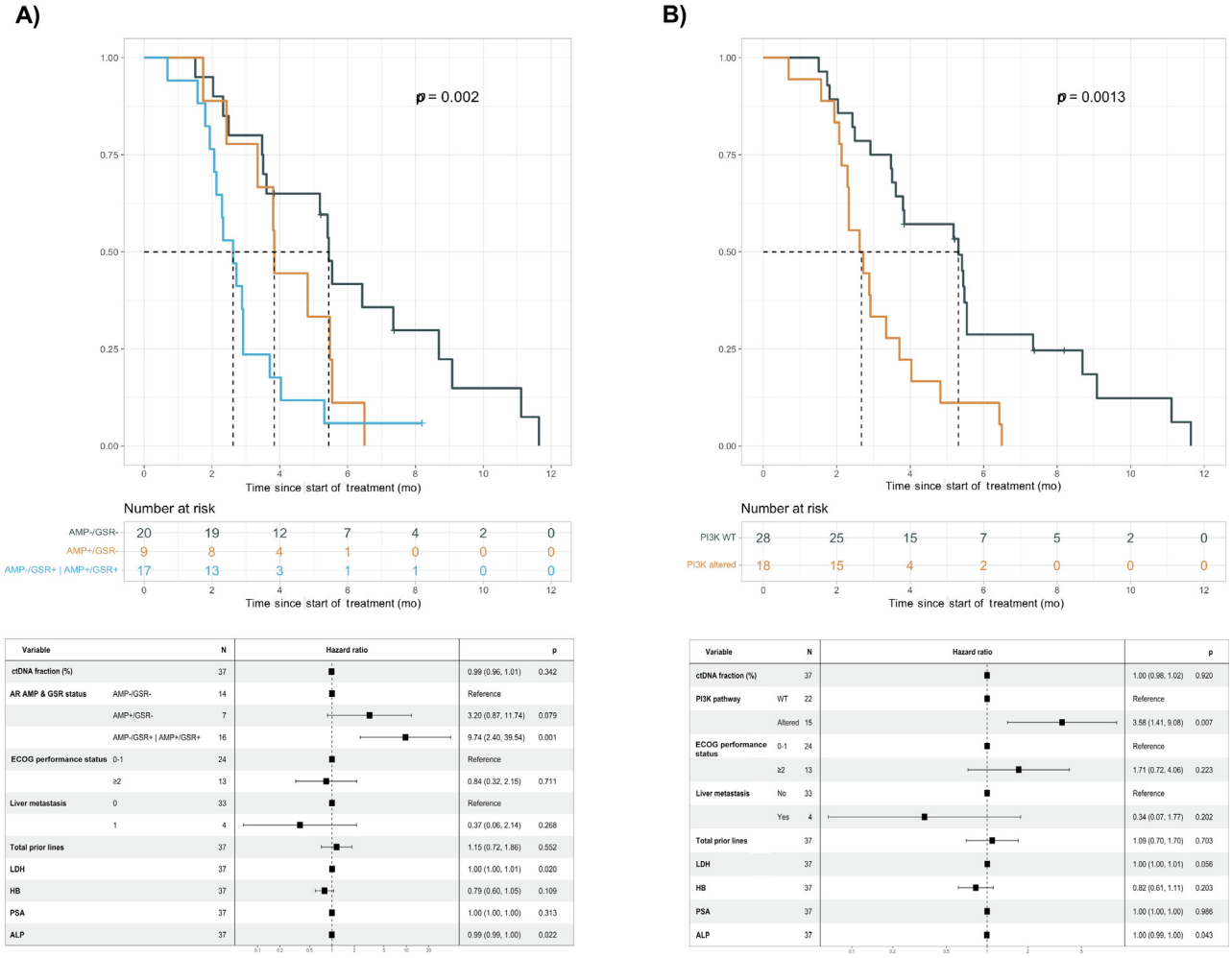

Fig. 1 – Genomic alterations in the *AR* gene and PI3K signalling in plasma cell-free DNA samples at baseline from patients with mCRPC ($n = 46$) in relation to progression-free survival on 177-lutetium-PSMA. Kaplan-Meier (upper) and multivariable Cox regression (lower) analyses of progression-free survival, 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.

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

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Appendix A. Supplementary data

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