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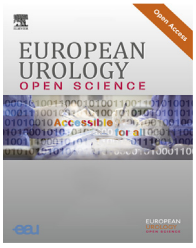

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Prostate Cancer

Circulating Tumour DNA Biomarkers Associated with Outcomes in Metastatic Prostate Cancer Treated with Lutetium-177-PSMA-617

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Abstract

Background: Lutetium-177-prostate-specific membrane antigen-617 (Lu-PSMA) is an effective therapy for metastatic castration-resistant prostate cancer (mCRPC). However, treatment responses are heterogeneous despite stringent positron emission tomography (PET)-based imaging selection criteria. Molecularly based biomarkers have potential to refine patient selection and optimise outcomes.
Objective: To identify circulating tumour DNA (ctDNA) features associated with treatment outcomes for men treated with Lu-PSMA.
Design, setting, and participants: ctDNA from men treated with Lu-PSMA in combination with idronoxil for progressive mCRPC were analysed using an 85-gene customised sequencing assay. ctDNA fractions, molecular profiles, and the presence of alterations in aggressive-variant prostate cancer (AVPC) genes were analysed at baseline, cycle 3 and at disease progression.
Intervention: Men received Lu-PSMA with idronoxil every 6 wk for up to six cycles.
Outcome measurements and statistical analysis: Baseline and exit PSMA and fluorodeoxyglucose PET/computed tomography (CT) imaging was conducted at baseline and study exit. Single-photon emission CT (SPECT) scans were performed 24 h after Lu-PSMA. Blood samples were collected at baseline, cycle 3 and at disease progression. Cox proportional-hazards models were used to assess associations and derive hazard ratios (HRs) and confidence intervals (CIs) for associations between molecular factors, imaging features, and clinical outcomes.
Results and limitations: Sixty samples from 32 men were sequenced (32 at baseline, 24 at cycle 3, four from patients with disease progression); two samples (baseline, on-treatment) from one individual were excluded from analysis owing to poor quality of the baseline sequencing data. Alterations in AVPC genes were associated

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with shorter prostate-specific antigen (PSA) progression-free survival (PFS) and overall survival (OS) in univariate (HR 3.4, 95% CI 1.5–7.7; p = 0.0036; and HR 3.3, 95% CI 1.4–7.7; p = 0.0063, respectively) and multivariate analyses (HR 4.8, 95% CI 1.8–13; p = 0.0014; and HR 4.1, 95% CI 1.6–11; p = 0.004).

Conclusions: ctDNA alterations in AVPC genes were associated with shorter PSA PFS and OS among men treated with Lu-PSMA and intermittent idronoxil. These candidate molecular biomarkers warrant further study to determine whether they have predictive value and potential to guide synergistic combination strategies to enhance outcomes for men treated with Lu-PSMA for mCRPC.

Patient summary: Certain DNA/gene changes detected in the blood of men with advanced prostate cancer were associated with shorter benefit from lutetium PSMA, a targeted radioactive therapy. This information may be useful in determining which men may benefit most from this treatment, but additional research is needed.

1. Introduction

When added to standard of care (SOC) therapy, lutetium-177-prostate-specific membrane antigen-617 (Lu-PSMA) radioligand therapy improves imaging-based progression-free survival and overall survival among men with prostate cancer previously treated with at least one prior line of novel androgen-receptor pathway inhibition and one to two lines of taxane-based chemotherapy [1,2]. However, more than one-third of men treated with Lu-PSMA monotherapy fail to achieve a prostate-specific antigen (PSA) response of at least 50% despite stringent positron emission tomography (PET)-based selection criteria [2] and the duration of benefit is heterogeneous among those who do experience a response [1,2].

^{68}Gallium (Ga)-PSMA PET imaging is used to identify the patients most suitable for Lu-PSMA therapy, but criteria have yet to be standardised. A Ga-PSMA PET mean standardised uptake value (SUVmean) of $\geq$10 was predictive of a higher PSA response rate to Lu-PSMA versus cabazitaxel in the TheraP study [3] and compared to SOC in the VISION trial [4]. Only a small proportion of men meet this criterion and a poor response may still be seen in patients with high SUVmean. It is hypothesised that radiation resistance is one mechanism underlying treatment resistance to Lu-PSMA, and thus synergistic combinations may improve outcomes. Therefore, we conducted the prospective phase 1/2 LuPIN trial of Lu-PSMA in combination with idronoxil [5], a synthetic flavonoid derivative of genistein that may have radiosensitising properties [6].

Metastatic castration-resistant prostate cancer (mCRPC) is characterised by molecular and phenotypic heterogeneity, so it is unlikely that a single predictive biomarker will adequately predict meaningful treatment outcomes [7]. Our previous analysis of 18 men revealed that variants in genes related to aggressive-variant prostate cancer (AVPC) were associated with worse outcomes among men treated with Lu-PSMA [5]. To build on this knowledge and identify potential molecular biomarkers associated with Lu-PSMA treatment response, we performed a molecular analysis for a subset of patients enrolled in the LuPIN trial and treated with Lu-PSMA in combination with idronoxil. Here we report results for serial analyses of cell-free DNA (cfDNA) from 31 patients treated in the LuPIN study in the context of their imaging findings and therapeutic outcomes.

2. Patients and methods

2.1. Study design and participants

The patient population included 32 men with mCRPC who had blood samples prospectively collected while enrolled in the phase 1/2 LuPIN clinical trial of Lu-PSMA in combination with idronoxil [8]. The study protocol was approved by the St. Vincent's Hospital institutional review board (HREC/17/SVH/19, ACTRN12618001073291) and all patients provided informed written consent. The LuPIN trial required high PSMA expression on Ga-PSMA PET/computed tomography (CT) without discordant disease on fluorodeoxyglucose (FDG) PET/CT imaging and progressive mCRPC following at least two lines of taxane-based chemotherapy and one novel androgen signalling inhibitor (ASI). Patients received up to six doses of ^{177}Lu-PSMA-617 (7.5 GBq) intravenously once every 6 wk in combination with 400–1200 mg of idronoxil suppositories on the day of and for 9 d following Lu-PSMA treatment. Additional details on the study treatment procedures have been published previously [8].

Blood was collected for circulating tumour DNA (ctDNA) analysis on day 1 of treatment, at 12 wk on treatment (day 1, cycle 3), and 6 wk after the sixth cycle of treatment (end of treatment, EOT) and/or on disease progression (PD). Thirty-one of 56 (55%) men on the study had suitable baseline samples available for this analysis, of whom 23 had on-treatment samples and four had EOT/PD samples. Ten cfDNA samples from presumed healthy males aged <30 yr (BioIVT, Westbury, NY, USA) were used as a normal reference for detecting copy number alterations (CNAs).

2.2. Sample processing and cfDNA assay methods

Samples were analysed using a customised 85-gene hydrid capture–based cfDNA assay with unique molecular identifiers (UMIs) designed to detect prostate cancer–relevant single-nucleotide variants (SNVs), small insertions and deletions (indels), copy number alterations (CNAs), and *TMPRSS2-ERG* gene fusions. Assay sensitivity was confirmed in a reference sample, for which 13/14 (93%) known SNVs at 0.5% variant allele frequency were detected.

cfDNA was extracted from double-spun plasma derived from EDTA whole blood tubes using a QIAmp circulating nucleic acid kit (Qiagen, Hilden, Germany) and quantified using a Qubit dsDNA HS Assay kit (ThermoFisher, Waltham, MA, USA). Library preparation was performed with 50 ng of cfDNA and xGen Prism DNA Library Preparation kits (Integrated DNA Technologies, USA). The libraries were amplified with xGen unique dual index primers (Integrated DNA Technologies, Coralville, IA, USA) and checked using ThermoFisher LabChip GX Touch. Hybrid capture was performed with 500 ng of library cfDNA pooled in six-plex, the 10 301 customised xGen discovery probe set (Integrated DNA Technologies), and an xGen Hybridization and Capture kit (Integrated DNA Technologies). Captured library pools were sequenced with 150-bp paired-end reads on an Illumina NovaSeq 6000 platform at the Garvan Institute of Medical Research.

Sequence data were processed and analysed using a customised pipeline following Integrated DNA Technologies recommendations with modifications (Supplementary material). In brief, demultiplexed FASTQ files were converted to unmapped BAM files, and UMIs were extracted and added to the RX tag. Reads were aligned to the human reference genome hg38 using BWA-MEM. Sequence error correction was performed using a combined read family approach. Consensus reads were realigned, filtered, and clipped. Variants were called using VarDict [9] and copy number ratios were obtained with CNVkit [10]. ctDNA fractions were estimated from allele fractions observed for heterozygous germline single-nucleotide polymorphisms (SNPs). Copy number loss and gain events were called on the basis of copy number ratios after adjusting for the ctDNA fraction. *TMPRSS2-ERG* gene fusions were called on the basis of split reads or copy number loss.

2.3. Imaging procedures and analyses

Ga-PSMA and FDG PET/CT scans were performed at baseline and trial exit (either after completion of 6 cycles of treatment or at cessation of treatment because of progressive disease [11]). In addition to baseline and exit PET imaging, Lu-PSMA single-photon emission CT (SPECT)/CT scans were acquired 24 h after each dose of Lu-PSMA for quantitative analyses of total tumour volume, SUVmax, and SUVmean using MIM software (Cleveland, OH, USA) [11,12].

2.4. Study endpoints

The primary endpoint for the LuPIN trial was safety and tolerability assessed using National Cancer Institute Common Terminology Criteria for Adverse Events v5.0. Secondary endpoints used to correlate molecular characteristics to outcomes included the PSA decline from baseline (any decline and a decline $\geq$50% [PSA_{50}]) at any time point, Prostate Cancer Working Group 3–defined PSA progression-free survival (PFS), and overall survival (OS), defined as time from day 1 of treatment to death.

Exploratory endpoints included associations between treatment outcomes (PSA response, PSA PFS, OS) and baseline ctDNA fraction, change in ctDNA fraction (difference between baseline and on-treatment ctDNA fraction), presence of variants, including AVPC-associated genes (*TP53, PTEN, RB1*), and changes in variants in serial samples.

2.5. Statistical analysis

Owing to the exploratory nature of this molecular substudy, the sample size was based on the availability of suitable samples from men enrolled in the LuPIN trial rather than formal power calculations. Patient characteristics are reported as the median and interquartile range (IQR) or as the absolute frequency and proportion. A two-sided exact binomial 95% confidence interval (CI) was calculated for PSA response rates. Time-to-event outcomes (PSA PFS, OS) were analysed using the Kaplan-Meier method and 95% CIs were calculated (SPSS; SPSS Inc., Chicago, IL, USA). Kaplan-Meier survival and univariate and multivariate Cox proportional-hazards models were used to assess associations between molecular factors, PET/SPECT imaging features, and clinical outcomes and derive hazard ratios (HRs) using R version 4.3.0 (R Foundation for Statistical Computing, Vienna, Austria). Multivariate analysis included known prognostic factors recorded just before cycle 1 of treatment (elevated alkaline phosphatase, anaemia [haemoglobin below the lower limit of normal], and volume of disease [high volume and/or visceral metastases]), with p values adjusted for testing of multiple genes using the Benjamini-Hochberg method [13].

3. Results

3.1. Patient characteristics and outcomes

A total of 60 samples (32 baseline, 24 on-treatment, four EOT/PD) from 32 patients were initially sequenced, but two samples (baseline, on-treatment) from one individual were excluded from analysis owing to poor quality of the baseline sequencing data. The baseline characteristics for the men included in the final analysis are summarised in Table 1. Median follow-up was 19.1 mo for the trial population. For the overall trial population (n = 56), the PSA_{50} response rate was 61% (95% CI 47–74%), median PSA PFS was 7.5 mo (95% CI 5.9–9.0), and median OS was 19.7 mo (95% CI 9.5–30). For the 31 men included in this analysis,

Table 1 – Baseline characteristics for the 31 patients included in the analysis

Parameter	Result
Median age, yr (IQR)	68 (64–75)
ECOG performance status, *n* (%)	
0–1	30 (97)
2	1 (3)
Median PSA at cycle 1, µg/l (IQR)	329 (55–6)
Median haemoglobin, g/l (IQR)[a]	121 (110–127)
Median alkaline phosphatase, U/l (IQR)[b]	162 (90–324)
Disease volume on PSMA PET, *n* (%)	
<20 metastases, no visceral metastasis	6 (19)
≥20 metastases and/or visceral metastasis	25 (81)
PSMA PET results	
Median SUVmean (IQR)	8.3 (7.3–9.3)
Median SUVmax (IQR)	39 (27–53)
Median tumour volume, ml (IQR)	935 (314–1375)
FDG PET results	
Median SUVmax (IQR)	8.1 (5.4–10.0)
Median SUVmean (IQR)	4.0 (3.5–4.3)
Median tumour volume, ml (IQR)	68.0 (19.8–352.8)
Prostate cancer history	
Gleason score, *n* (%)	
≤7	5 (16)
8–10	19 (61)
Unknown/not available	7 (23)
Prior systemic treatments, *n* (%)	
LHRH agonist/antagonist	31 (100)
Chemotherapy	31 (100)
Docetaxel	31 (100)
Cabazitaxel	31 (100)
Other chemotherapy agent	3 (9)
Novel androgen signalling inhibitor	31 (100)

ECOG = Eastern Cooperative Oncology Group; FDG = fluorodeoxyglucose; IQR = interquartile range; LHRH = luteinising hormone–releasing hormone; PET = positron emission tomography; PSA = prostate-specific antigen; PSMA = prostate-specific membrane antigen; SUV = standardised uptake value.
[a] Normal range 130–180 g/l.
[b] Normal range 30–100 U/l.

the PSA_{50} response rate was 65%, median PSA PFS was 6.3 mo, and median OS was 15.8 mo.

3.2. ctDNA fraction

The ctDNA fraction was estimated in 27/31 baseline samples (27%, IQR 21.5–37.5%), 16/23 cycle 3 samples, and three of four EOT/PD samples based on the allele fractions observed for SNPs subject to loss of heterozygosity (LOH; Supplementary material). The remaining samples did not include informative SNPs, consistent with either the absence of ctDNA or a lack of SNPs with LOH in the regions of the genome analysed. Changes in estimated ctDNA fraction between baseline and cycle 3 were correlated with changes in tumour volume on PSMA SPECT/CT (ρ = 0.48, p = 0.06; Supplementary Fig. 1). A reduction in ctDNA fraction or tumour volume showed a trend for better PSA PFS or OS (Supplementary Fig. 1).

3.3. ctDNA molecular profiles

Commonly altered genes and pathways in baseline samples (n = 31) are summarised in Figure 1 and included *AR* (65%), *TP53* (23%), *RB1* (26%), *TMPRSS2-ERG* (42%), PI3K pathway genes (42%), WNT pathway genes (35%), and DNA repair genes (58%). Overall findings for the patient cohort are summarised in Supplementary Figure 2. These findings are generally consistent with previous studies in mCRPC [14]. A longitudinal analysis comparing baseline and cycle 3 time points did not show treatment-associated changes for individual genes.

3.4. ctDNA variants of interest and outcome correlates

3.4.1. AVPC genes

One or more alterations in AVPC genes (*PTEN, RB1, TP53*) were associated with shorter PSA PFS and OS on both univariate and multivariate analysis (Fig. 2); in particular, *TP53* alterations were strongly associated with worse outcomes.

3.4.2. Other potential biomarkers associated with outcomes

Associations between molecular changes, including SNVs and CNAs, in individual genes and PSA PFS outcomes were analysed in univariate analyses and in multivariate analyses adjusted for known prognostic factors. Results are summarised as volcano plots of false discovery rate–adjusted p values and log(HR) in Figure 3. The gene most strongly associated with PSA PFS on multivariate analysis was *TP53* (HR 21.7, 95% CI 3.9–119.3; adjusted p = 0.015). *NCOA2*, *MTOR*, *PIK3R1*, and *NBN* were also associated with shorter PSA PFS (adjusted p = 0.015, 0.017, 0.023, and 0.025, respectively; HRs included in Supplementary Fig. 3). *BRIP1* alterations were associated with short PSA PFS (adjusted p = 0.018) but all of these alterations co-occurred with *NCOA2* amplifications.

In a gene set analysis, PI3K pathway alterations shown in Figure 1 were associated with shorter PSA PFS (HR 3.7; p = 0.0022) and OS (HR 4.1; p = 0.0025) on univariate analysis and on multivariate analysis (HR 3.7; p = 0.0058; and HR 3.6; p = 0.013, respectively).

3.4.3. Outcomes for participants with SUVmean >10

Five men (16%) had SUVmean >10 on baseline PSMA PET. There were no significant differences in median PSA PFS

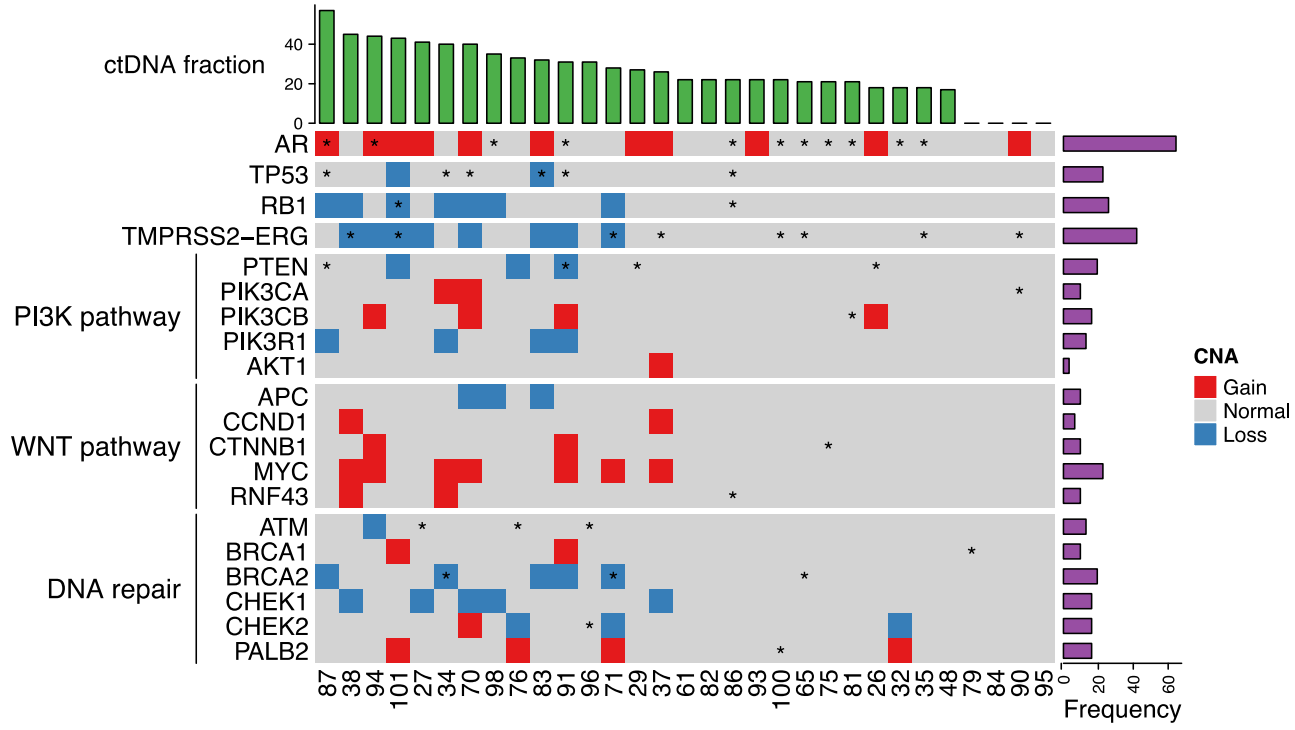

Fig. 1 – Commonly altered genes and pathways in baseline samples sorted by descending estimated ctDNA fraction. Asterisks indicate a single-nucleotide variation, indel, or gene fusion (*TMPRSS2-ERG*).

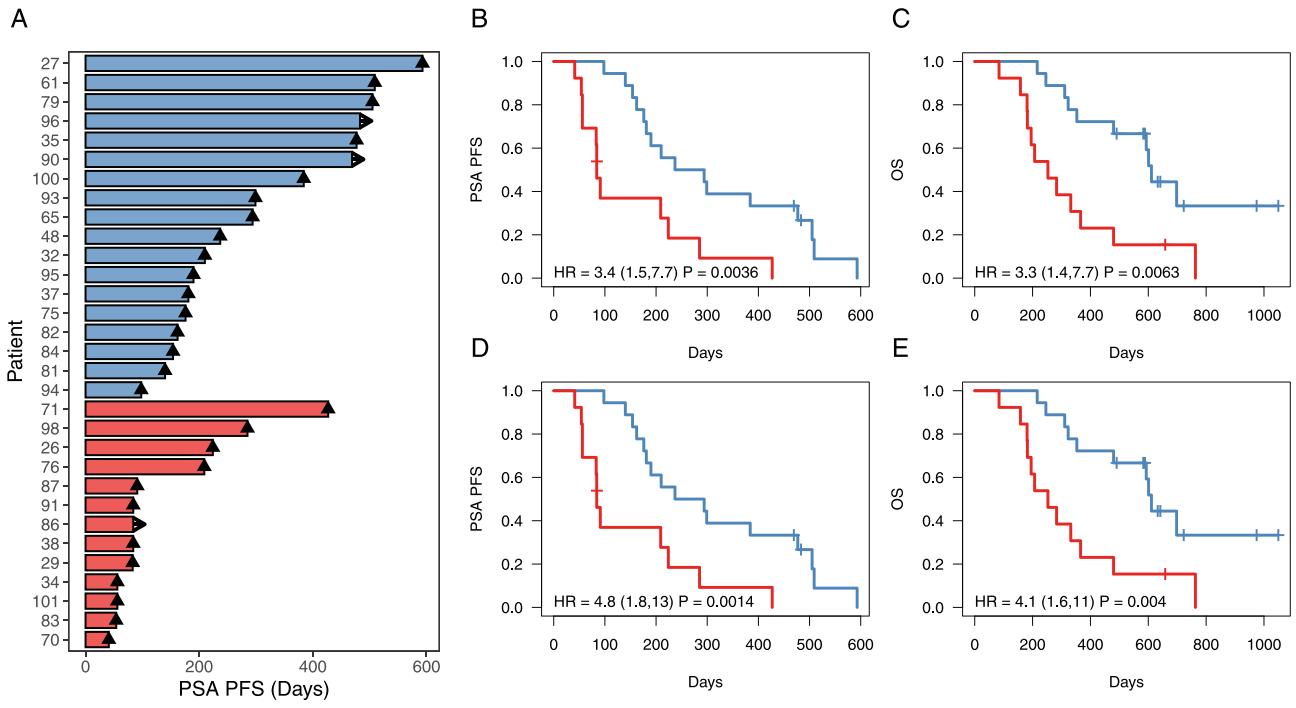

Fig. 2 – (A) Swimmer plot for prostate-specific antigen (PSA) progression-free survival (PFS). Triangles indicate progression. Kaplan-Meier curves for survival outcomes stratified by the presence (red) or absence (blue) of aggressive-variant prostate cancer gene alterations: (B) univariate analysis for PSA PFS, (C) univariate analysis for overall survival (OS), (D) multivariate analysis for PSA PFS, and (E) multivariate analysis for OS. HR = hazard ratio with 95% confidence interval in parentheses.

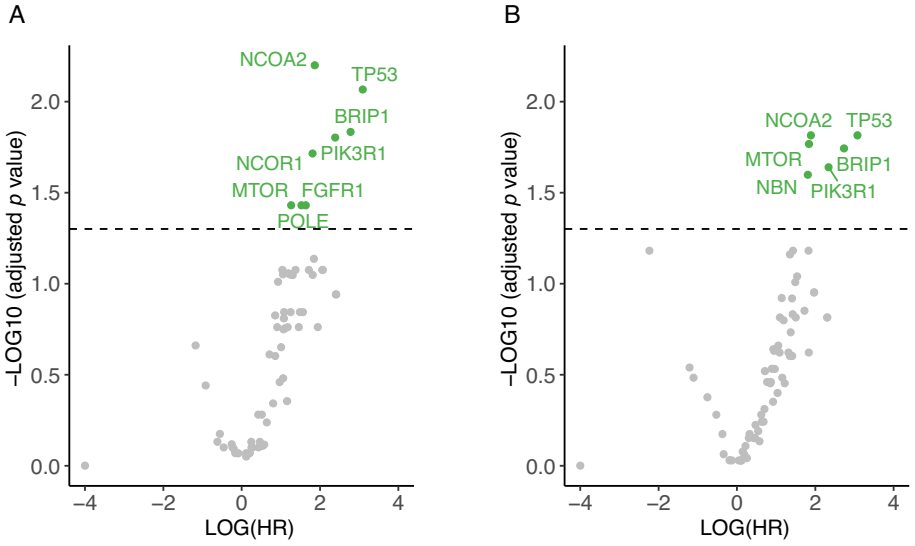

Fig. 3 – Volcano plots of genes associated with prostate-specific antigen progression-free survival outcomes on (A) univariate analysis and (B) multivariate analysis incorporating baseline clinical prognostic factors. Associations with a *p* value <0.05 after adjustment for the false discovery rate are highlighted in green. HR = hazard ratio.

(p = 0.62) and OS (p = 0.96) between the SUV <10 and >10 groups.

4. Discussion

In this study we explored molecular features of ctDNA and their associations with treatment outcomes among men with mCRPC treated with Lu-PSMA in combination with intermittent idronoxil as a radiosensitiser. Alterations in at least one AVPC gene were associated with shorter PSA PFS and OS. Activating mutations in the PI3K pathway and individual alterations in the AR-associated gene *NCOA2* were also correlated with shorter survival outcomes. Baseline PSMA PET SUVmean, ctDNA fraction, and PSMA PET tumour volume were not associated with outcomes, but a decrease

in tumour volume at 12 wk (cycle 3) and a reduction in ctDNA fraction may be associated with better outcomes, although the numbers were small in these groups.

With the success of the VISION trial, Lu-PSMA therapy is increasing in popularity globally, but treatment outcomes are heterogeneous, with only a small portion of patients experiencing a prolonged response. Information on biomarkers associated with Lu-PSMA treatment outcomes is limited, but our study identified potential biomarkers for further study that may help in refining patient selection for this efficacious but costly treatment. It was previously found that SUVmean was associated with better outcomes, but this generally applied to only one-third of men treated with Lu-PSMA [3,4]. In addition, correlation between changes in total tumour volume on PET and SPECT imaging and Lu-PSMA outcomes has been observed [11,12]. However, in an ideal scenario a predictive biomarker would already be present at baseline or could be used in combination with imaging parameters to improve the predictive value. ctDNA evaluation has the added benefit of potentially identifying alternative therapeutic options (eg, PARP inhibitors for *BRCA2* mutations) and mechanisms of treatment resistance while being relatively noninvasive.

Notably, PI3K pathway–activating mutations and AR-associated gene alterations were associated with worse outcomes in our study. There is significant crosstalk between the PI3K and AR pathways, which may serve as a mechanism of treatment resistance when either pathway is targeted individually [15]. PSMA downregulation, as seen with PSMA-targeting agents such as Lu-PSMA, is associated with AR signalling activation. Potentially, aberrant AR signalling induced by *NCOA2* alterations [16,17] may reduce tumour reliance on PI3K signalling and therefore PSMA expression, with a reduction in Lu-PSMA uptake intracellularly [18]. In addition, PI3K activation has been identified as a mechanism underlying radiotherapy resistance [19,20]. Therefore, co-administration of Lu-PSMA with a PI3K pathway inhibitor and/or more potent AR pathway inhibitor may represent a strategy to overcome Lu-PSMA resistance.

While this exploratory analysis has highlighted several therapeutic possibilities, the study is limited by the relatively small sample size and single-arm design. The association of AVPC alterations with poorer outcomes may be prognostic rather than predictive, although the strongest correlation was observed for PSA PFS rather than OS. Further work is needed to analyse larger data sets for men treated with Lu-PSMA to clarify these potential molecular biomarkers and ultimately improve patient outcomes.

5. Conclusions

We identified biomarkers associated with treatment outcomes among men with mCRPC treated with Lu-PSMA that may be evaluated in future studies to ascertain their predictive value in refining patient selection for this therapy. AVPC gene alterations strongly associated with shorter PSA PFS and OS and PI3K activating mutations may represent a therapeutically targetable mechanism of treatment resistance.

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

Study concept and design: Emmett, Crumbaker.
Acquisition of data: Emmett, Crumbaker, Pathmanandavel, Tao, Boulter, Goldstein, Murray, Kummerfeld.
Analysis and interpretation of data: Emmett, Crumbaker, Pathmanandavel, Tao, Goldstein, Murray.
Drafting of the manuscript: Crumbaker, Goldstein.
Critical revision of the manuscript for important intellectual content: Emmett, Crumbaker, Pathmanandavel, Tao, Goldstein, Murray, Joshua.
Statistical analysis: Goldstein, Crumbaker, Pathmanandavel.
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Other (*provision of treatments*): Emmett, Crumbaker, Joshua.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.euros.2023.08.007.

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