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Abstract

Background

The androgen receptor (AR) pathway-associated gene *NCOA2* has an established oncogenic role in early prostate cancer, and likewise is a driver of metastatic disease and castration-resistant prostate cancer. However, its significance as a biomarker in metastatic castration-resistant prostate cancer (mCRPC), both alone and in conjunction with co-occurring *AR* alterations using a liquid biopsy approach has not been investigated.

Methods

Ninety-one patients were included in this study, (n=68 receiving an androgen receptor pathway inhibitor (ARPI) and n=23 receiving taxane chemotherapy). Up to 30mL of peripheral blood was collected prior to commencing treatment from each patient. Plasma cell-free DNA, along with a matched germline sample, underwent targeted next-generation sequencing using a validated, highly sensitive in-house prostate cancer panel. Variants in *AR* and *NCOA2* were identified and correlated with clinical outcomes.

Results

Plasma *AR* and *NCOA2* aberrations were identified in 35% and 13% of the cohort, respectively, whilst 8% had concurrent *AR* and *NCOA2* alterations. *NCOA2* copy number gain and any *NCOA2* aberration predicted for lower PSA response rates. Likewise, median overall survival was shorter for *NCOA2* gain (10.1 months vs 18.3 months, $p = 0.004$), remaining significant after adjusting for covariates including circulating tumour DNA fraction and tumour suppressor gene alterations. Importantly, dual *AR* and *NCOA2* aberrations were also associated with inferior outcomes, including no PSA responses in patients treated with ARPIs (0% vs 64%, $p = 0.02$).

Conclusions

These data highlight the importance of identifying multiple markers of AR pathway modulation in mCRPC and represent the first instance of the assessment of plasma *NCOA2* status as a prognostic biomarker for standard-of-care therapies. Further assessment is

warranted to determine if *NCOA2* aberrations are a marker of primary resistance to AR pathway inhibitors.

Keywords: biomarker, castrate-resistant, cell-free DNA, liquid biopsy, prostate cancer, AR pathway inhibitor

Introduction

Systemic treatment options for metastatic castration-resistant prostate cancer (mCRPC) have rapidly expanded in recent years. Among the options now available for mCRPC are androgen receptor pathway inhibitors (ARPI) (abiraterone acetate and enzalutamide) and taxane cytotoxics (docetaxel and cabazitaxel). Although use of these agents has clearly improved survival for men with mCRPC, treatment outcomes are variable and both primary and acquired resistance pose major ongoing challenges. Many gene alterations have been linked to shorter therapeutic responses and overall survival in mCRPC, including the tumour suppressor genes (TSGs) *PTEN*, *RB1* and *TP53*.[1] However, a high proportion of biomarker-positive patients still respond well to therapy.[2-4] Therefore, there remains a pressing need to identify both predictive and prognostic biomarkers for mCRPC[5]. Due to challenges in obtaining timely metastatic biopsy tissue, liquid biopsy approaches to biomarker research have taken on increasing importance in advanced prostate cancer,[6, 7] In particular, cell-free DNA (cfDNA), consisting of both tumour-derived (circulating tumour DNA; ctDNA) and non-tumour-derived DNA,[8] is readily detectable in the majority of patients, and has been shown to be a pragmatic and high-fidelity substitute for tumour biopsies in mCRPC, although identifying low frequency somatic variants of clinical significance remains challenging using existing high-throughput technologies.[6]

Molecular alterations in the androgen receptor (*AR*) gene have been detected as a key driver of primary and acquired resistance to systemic mCRPC therapy,[9-11] with considerable efforts being made to profile the *AR* using circulating tumour nucleic acids.[12,13] In particular, *AR* copy number gain, detected in the cfDNA of 30-50% of patients, has been associated with shorter overall survival and worse treatment responses in both enzalutamide and abiraterone-treated patients.[13-15] Similarly, in some studies, *AR* copy number gain has been associated with

poor outcomes in patients receiving chemotherapy.[16] However, like TSG alterations, the presence of an *AR* aberration does not preclude response to these systemic therapies, with *AR* status likely only explaining a proportion of the phenotypic responses observed clinically. Other mechanisms of AR pathway activation in the castration-resistant state have been identified, including aberrant expression of AR transcriptional coactivators.[10, 17, 18]

The nuclear receptor coactivator 2 (NCoA2; also known as SRC-2, GRIP1 or TIF2) protein, a member of the p160 transcriptional coregulators, is recruited to the enhancer regions of AR-responsive genes.[19,20] It does not directly bind DNA but rather interacts with the AR to modulate its transcriptional activity.[19] In metastatic prostate cancer, *NCOA2* alterations have been observed in 15-25% of cases,[4, 18] with higher NCoA2 levels being linked to increased AR protein expression, AR pathway signalling, and faster progression to a castration-resistant state.[21, 22] Additionally, there is considerable evidence suggesting *NCOA2* aberrations may be associated with a more aggressive phenotype of localised prostate cancer (*i.e.* shorter time to biochemical recurrence, poor prognosis and increased morbidity).[18, 21-23] However, the utility of *NCOA2* alterations as a biomarker is still poorly understood in mCRPC, and has not previously been investigated using cfDNA.

In this study, a high-sensitivity next-generation sequencing (NGS) assay that detects variants down to 0.25% variant allele frequency (VAF) was utilised to interrogate plasma cfDNA and matched germline DNA samples from mCRPC patients commencing standard-of-care systemic therapies. Analysis of AR pathway aberrations (in the *AR* and *NCOA2* genes) revealed *NCOA2* to be a novel marker of poor prognosis and of shorter response to ARPI therapy, independently of plasma tumour fraction and presence of TSG alterations. The occurrence of either an *AR* or *NCOA2* alteration, as well as dual *AR* and *NCOA2* alterations, was a stronger predictor of clinical outcomes than *AR* aberrations alone, with our data suggesting that broader analysis of AR pathway-associated loci may have high clinical value in mCRPC.

Materials and Methods

Study cohorts and sample processing

Ninety-one patients with mCRPC were included in the primary analysis. Patients were prospectively recruited from Monash Health (Melbourne, Australia) and Eastern Health (Melbourne, Australia) between September 2016 and August 2018. All patients provided written informed consent, with ethics approval obtained from each institution's human research ethics committee. Peripheral blood (up to 30 mL) was collected in EDTA-containing or dedicated cfDNA-stabilising tubes (Streck) immediately prior to commencing systemic therapy. Following blood collection, cfDNA and matched fragmented genomic DNA were isolated from plasma and buffy coat leukocytes, with short double-stranded DNA fragments ≤500 bp being selectively retained for library preparation as described previously.[24] Data from a secondary mCRPC cohort was downloaded from cBioportal (http://www.cbioportal.org).

Hybridisation capture, sequencing and variant identification

Molecularly-tagged cfDNA and germline DNA libraries were generated and underwent hybridisation capture using a custom library of biotinylated RNA baits and double-captured sequencing (dCAP-Seq) methodology to improve on-target read fraction as described previously.[24] Performance of this prostate cancer panel has been previously established, with a sensitivity and specificity of 93% and 99.5% for detecting variants at ≥0.5% VAF.[24] The full list of targeted genes is provided in Supplemental Table 1. For the purposes of this study, only the *AR*, *NCOA2*, *PTEN*, *RB1* and *TP53* genes have been reported. Ultra-deep, paired-end sequencing of DNA libraries was performed on the Illumina HiSeq. 2500, with barcode-directed error-correction and somatic and germline variant detection occurring as previously described, with clonal haematopoiesis variants of indeterminate potential (CHIP) removed using matched leukocytes.[24-26] Somatic alterations with VAFs above the lower limit of detection (0.25%) were identified in the *AR* and *NCOA2* genes. Copy number alterations (CNAs) were identified in patients with at least 2% tumour fraction using VarSeq CNV caller (v2, Golden Helix).[24, 27] ctDNA fractions were calculated using heterozygous germline polymorphisms in regions without detectable copy number gain.[6, 24]

Clinical outcomes analysis and statistical methodology

Follow-up time was calculated from date of sample acquisition to date of last patient contact. Kaplan-Meier survival estimates (log-rank test) and Cox regression models (univariable and multivariable) were then used to assess the association between *AR* and/or *NCOA2* aberrations and clinical outcomes, including overall survival (OS; time from treatment start until death from any cause), and progression-free survival (PFS; time from treatment commencement to either confirmed PSA [prostate-specific antigen], clinical or radiographic progression, or death from prostate cancer, whichever occurred first). PSA progression was defined as an increase of ≥25% greater than the nadir and an absolute increase of at least 2 ng/ml in serum PSA.[28] Clinical covariates for multivariable analysis were ctDNA (as a continuous variable), the presence of an aberration in a known driver tumour-suppressor gene (TSG),[3, 4, 29] prior taxane chemotherapy and/or ARPI therapy, ECOG (Eastern Cooperative Oncology Group) performance status and presence of cancer related pain at enrolment. Associations with PSA response (which required ≥12 weeks follow-up) were assessed using Pearson's chi-squared testing. All time-to-event outcomes were censored at date of last patient contact if the event had not yet occurred. Unless otherwise stated, statistical significance was defined as $p<0.05$.

Results

Study cohort

A total of 91 pre-treatment blood samples were collected from 68 patients commencing ARPI therapy (abiraterone or enzalutamide) and 23 patients commencing taxane chemotherapy (docetaxel or cabazitaxel). Baseline clinical characteristics and prior systemic therapy exposure are presented in Table 1 and Figure 1. Median follow-up for non-deceased patients was 35.9 months (mo). Median OS and PFS was 17.1 mo (range 1.6-51) and 3.7 mo (range 0.6-51), respectively. The PSA response rate for patients with at least 12 weeks follow-up was 53%. Median ctDNA fraction was 12.4%, and was detectable (≥2%) in 57 (63%) patients. As a continuous variable, tumour fraction was associated with shorter PFS and OS times (Table 2).

Distribution of plasma alterations

cfDNA sequencing metrics and detected genomic aberrations for each patient are provided in Supplemental Tables 2 and 3. A median de-duplicated sequencing depth of 1,242X and 1,292X was achieved for all point mutations identified in *AR* and *NCOA2,* respectively, with allelic frequencies of 0.31-44% and 0.31-48% for each gene. *AR* and *NCOA2* alterations (single-nucleotide variant [SNV] and/or CNA) were identified in 35% and 13% of the cohort, respectively, with amplifications more common than SNVs for both genes (Figure 1). All *AR* gains were reported as focal whilst one *NCOA2* gain was broad, encompassing regions from 8p12-8q22.1. In total, 37 patients had at least one gene alteration, with seven samples displaying dual *AR* and *NCOA2* aberrations. Twenty-three (62%) of these 37 patients were treatment-naïve (Figure 1). There was no association between prior therapy and the presence of *AR/NCOA2* alterations (Supplementary Table 3).

The landscape of tumour-suppressor gene alterations in mCRPC

Alterations in either *PTEN, RB1* and/or *TP53* genes were present in 46% of the cohort, with *PTEN* being the most commonly aberrant TSG (30%). In line with previous reports, the presence of a TSG alteration was associated with shorter survival times (Table 2), with *RB1* also being predictive of lower PSA response (29% vs 60%, $p = 0.01$; Table 3). Patients with a TSG alteration were no more likely to have an *NCOA2* alteration than their wild-type counterparts (Supplemental Table 4).

NCOA2 aberrations associated with poor outcomes in mCRPC

In total, out of the 57 patients with detectable ctDNA fraction, seven patients (12%) harboured an *NCOA2* copy number gain, whilst missense variants were observed in five patients, including two patients with a missense germline SNV (Figure 1). Patients with a plasma *NCOA2* alteration were more likely to experience cancer-related pain and higher levels of total cfDNA at the beginning of treatment (Supplemental Table 4). Presence of an *NCOA2* SNV alone was not independently associated with clinical outcomes, however the presence of any *NCOA2* DNA alteration (SNV or CNA) was associated with shorter OS, which remained significant upon multivariable analysis when adjusting for baseline clinicopathologic factors and other poor prognostic variables, including ctDNA fraction and

presence of a TSG alteration (Table 2). Additionally, any *NCOA2* alteration and *NCOA2* copy number gain alone were both significantly associated with lower PSA response rates to systemic therapy (25% vs 57%, $p = 0.04$; and 14% vs 56%, $p = 0.046$, respectively; Table 3 and Figure 2). *NCOA2* gain was also associated with inferior PFS and OS (median PFS 2.7 mo vs 4.3 mo, $p = 0.005$; and median OS 10.1 mo vs 18.3 mo, $p = 0.004$; Figure 3), whilst any *NCOA2* alteration was independently associated with shorter OS (HR 3.0, $p = 0.02$; Table 2). The significance of *NCOA2* alterations as a marker of poor outcomes was largely driven by the ARPI-treated cohort (Supplemental Tables 5-8). Notably, no patients harbouring an *NCOA2* gain attained a PSA response to ARPI therapy (0% vs 64%, $p = 0.02$).

Prognostic impact of *NCOA2* alterations in a secondary cohort

A second, independent dataset was assembled using a publicly available dataset of mCRPC patients who underwent whole exome sequencing of a solid tumour biopsy prior to receiving an ARPI (with or without a combination therapy).[4] Of these patients, 128 of 429 had accompanying overall survival data and were thus included in this analysis. The median follow-up time for non-deceased patients in this cohort was 32.8 months. In total, an *NCOA2* alteration was detected in 25/128 (20%) biopsies. Of these, only one was an SNV. In this cohort, the presence of *NCOA2* copy number gain was significantly associated with shorter overall survival (median gain 17.7 mo vs neutral 28.8 mo, $p = 0.04$; Supplemental Figure 1).

AR aberrations and prognosis in mCRPC

Of the patients with sufficient ctDNA fraction to detect plasma CNAs, 20 (35%) had an *AR* copy number gain (Figure 1). An additional 12 patients had an *AR* SNV, whilst a further three men had dual *AR* aberrations (SNV and CNA). The most common somatic missense *AR* variants were the promiscuity-conferring L702H, V716M and H875Y ligand-binding domain (LBD) mutations (Supplemental Figure 2). There was no association between prior AR-targeted therapy and *AR* LBD mutations (Fisher's exact test $p = 1.0$), and no correlation was observed between clinical outcomes and the presence of an *AR* SNV alone. In line with other reports, the presence of *AR* copy number gain was independently associated with worse PFS, which was most apparent in the ARPI-treated cohort (HR 3.1, $p = 0.003$; Supplemental Table

5). However, *AR* alterations alone were not independently linked to OS (Table 2), or to PSA response (Table 3).

NCOA2 increases discriminatory capacity of *AR* as a prognostic factor

Since the NCoA2 protein is an AR pathway coactivator, we hypothesised that dual alterations in AR pathway-associated loci (*AR* and *NCOA2*) might identify patients with particularly aggressive disease phenotypes. Analysis of copy number status of these two loci demonstrated that the presence of either an *AR* or an *NCOA2* gain was independently predictive of shorter PFS and OS (PFS HR 2.5, $p = 0.002$; OS HR 2.0, $p = 0.049$; Table 2). Once again, this relationship appeared to be primarily driven by the ARPI-treated subgroup rather than the chemotherapy-treated cohort (Supplemental Tables 5 and 6).

We next analysed outcomes in patients with any aberration (CNA and/or SNV) in both genes. Dual-aberrant patients experienced inferior OS (10.1 mo vs 18.3 mo, $p = 0.002$) and PFS (2.7 mo vs 4.3 mo, $p = 0.01$; Figure 4), a finding that remained significant after adjustment for other clinicopathological variables (Table 2). Of note, this dual-aberrant grouping appeared to be a stronger predictor of poor prognosis than *AR* aberrations alone (univariable HR for OS 2.0 vs 4.1, Table 2). The presence of concurrent *AR* and *NCOA2* aberrations was also associated with significantly lower PSA response rates on systemic therapy (14% vs 56%, $p = 0.046$; Table 3). Importantly, no patients with dual AR pathway-associated aberrations responded to ARPI therapy, although subgroup numbers were small (Supplemental Table 7).

Discussion

Large scale efforts to characterise the genomic landscape of metastatic prostate cancer have shown *AR* to be the most commonly aberrant gene in mCRPC.[6, 10] However, it has become apparent that *AR* exonic aberrations are only one mechanism of AR pathway re-activation in mCRPC, and are not the only marker of increased pathway activity.[11, 30, 31] Other mechanisms modulating AR signalling appear to contribute to the clinical phenotype of disease observed, and may include aberrant expression of AR coactivators such as *NCOA2*.

Our study is notable for being the first investigation into the clinical significance of plasma *NCOA2* status in mCRPC. In a prospectively collected cohort incorporating 91 mCRPC

patients receiving contemporary standard-of-care therapies, we showed that *NCOA2* aberrations were readily detectable using plasma cfDNA, and at 13% population prevalence, are sufficiently common to support further investigation as a biomarker. Both germline and somatic *NCOA2* alterations were observed in this mCRPC cohort, highlighting the importance of performing matched cfDNA and germline DNA analysis when assessing potentially clinically-relevant loci in metastatic prostate cancer. Additionally, by utilising a highly-sensitive targeted assay, we were able to include ultra-low frequency somatic variants in this analysis that may otherwise remain unidentified using less sensitive approaches. However, due to small cohort size we were unable to conclusively discern the significance of these (potentially subclonal) specific variants, and future studies will need to determine their relative import in mCRPC.

We observed that *NCOA2* copy number gain, or the presence of any *NCOA2* alteration were both independently associated with shorter OS and with a lower PSA response rate. These results were observed in ARPI-treated patients but not in chemotherapy-treated patients, perhaps reflecting different mechanisms of treatment resistance, or alternatively the smaller size of the chemotherapy cohort. The association between *NCOA2* gain and shorter OS was confirmed in a second cohort of mCRPC patients who underwent tissue whole exome sequencing in a previous study[4]. In this cohort, there was a higher prevalence of *NCOA2* gains (20% vs 13%), potentially due to the limitations of CNA identification in patients with low ctDNA purity.[32, 33]

Interestingly, *AR* alterations were not independently associated with inferior OS in either the overall cohort or the ARPI-treated cohort. This is in contrast to previous reports,[13-15] and could again be attributable to low ctDNA purity, or the contribution of other factors that can act to up-regulate AR-mediated transcription. In view of the latter, we subsequently examined outcomes in patients harbouring alterations in both *AR* and *NCOA2*.

Importantly, we found that patients with dual *AR* and *NCOA2* alterations had significantly shorter PFS and OS on univariable and multivariable analyses. Moreover, no patients with this dual-aberrant signature exhibited a biochemical response to ARPI therapy, suggesting it

may be a marker of primary resistance to AR targeted therapies in mCRPC. This relationship was not observed for *AR* aberrations alone. Together, these observations suggest dual-aberrant patients have an aggressive disease phenotype and particularly poor outcomes, and highlights the value of multi-gene assessment when identifying markers of treatment resistance and response in mCRPC, in particular in the AR signalling pathway.

Interestingly, it was CNAs across these two genes, rather than SNVs, that appeared to drive associations with poor outcomes in mCRPC. No clinical relevance was observed for the presence of *AR* SNVs nor *NCOA2* SNVs in either the overall cohort or the ARPI-treated subgroup. The lack of predictive utility of *AR* missense mutations has been reported previously.[34, 35] In this study, known benign variants and missense variants with low likelihood of pathogenicity, as predicted by *in-silico* tools, were removed from analysis.[24, 36] However, variants with unknown clinical significance were included in analysis, a standard approach in the genomics field.[37, 38] The significance of such variants will only become clear in the future with extensive functional studies paired with consistent reporting to publicly-available databases.

Whilst NCoA2 plays a fundamental role in the activation of AR pathway signalling, it has also been implicated in other oncogenic pathways in prostate cancer models.[21, 39] Specifically, hyperactivation of the PI3K/AKT pathway in *NCOA2* over-expressed murine models of prostate cancer has been reported, mediated through direct transcriptional regulation of key PI3K pathway genes, including *PHLPP1* and *FKPB5*.[21] This knowledge, taken together with the observation that patients with *NCOA2* copy number gains have poor outcomes, suggests that *NCOA2* aberrations may also have clinical relevance in patients treated with Akt inhibitors, which have demonstrated benefit in phase III trials in mCRPC.[40]

In line with other reports, higher ctDNA fraction was also a marker of poor prognosis in this study. There is not yet a clear consensus as to what fraction of plasma tumour content identifies high-risk patients with advanced prostate cancer, with efforts to use ctDNA fraction in the clinic hampered by the abundance of methods currently being implemented and a lack of consensus on the most appropriate and accurate approach for prostate cancer.[8, 41]

We acknowledge several limitations of our study. Lack of copy number data for patients with low ctDNA fractions is a limitation of many current cfDNA assays, and may lead to the underestimation of CNAs in this cohort. Additionally, sample size may limit the confidence and generalisability of our findings.

Conclusion

Using plasma cfDNA from a prospectively collected cohort paired with a highly-sensitive targeted sequencing assay, we found that any *NCOA2* aberration or *NCOA2* gain alone were independent negative prognostic factors. Moreover, we identified a novel subgroup of patients harbouring dual *AR/NCOA2* aberrations who had poor outcomes on systemic therapy, including the absence of any PSA responses to ARPI therapy. Our study is noteworthy for being the first to examine the clinical impact of *NCOA2* alterations in mCRPC patients, with results supporting future studies in larger independent cohorts to further investigate the clinical utility of *NCOA2* as a biomarker.

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Figure legends

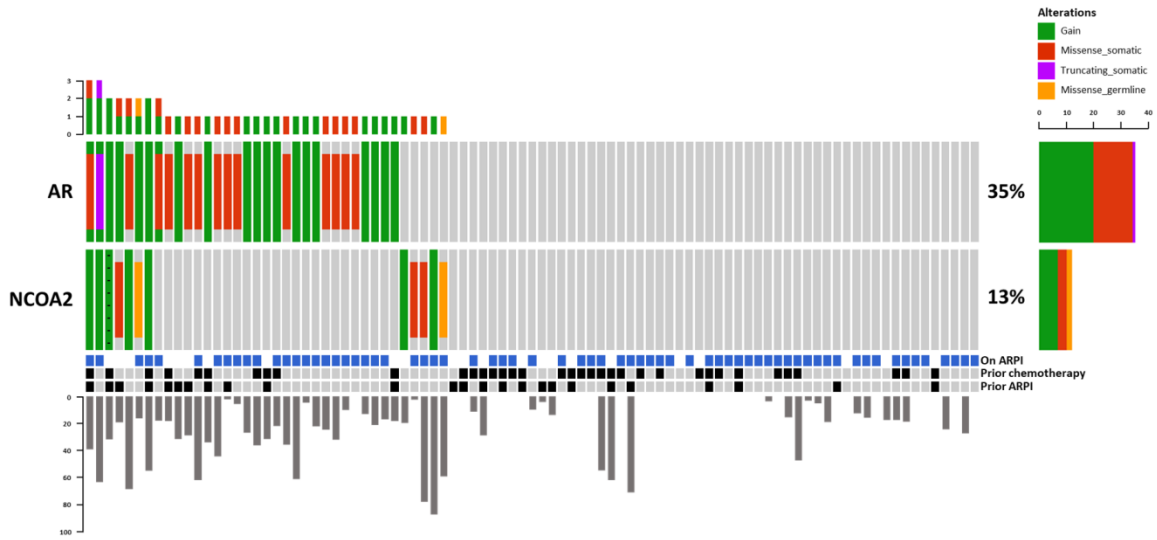

Figure 1: Landscape of *AR* and *NCOA2* aberrations identified in cell-free DNA. Total variants (top) and circulating tumour DNA fraction (%, bottom) per patient sample shown, along with therapy received after sample collection (androgen receptor pathway inhibitor; ARPI in blue squares), and prior therapies for metastatic prostate cancer (black squares). Dashed lines indicate broad 8q gain rather than focal *NCOA2* gain.

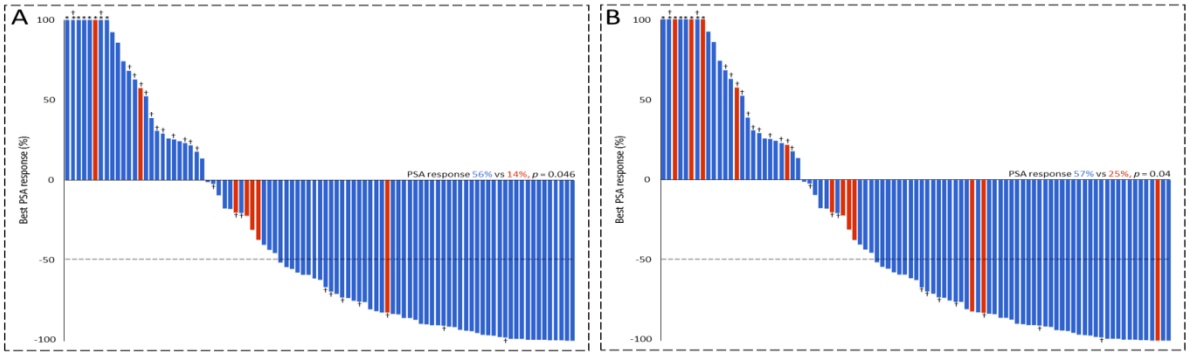

Figure 2: Waterfall plot of best prostate-specific antigen (PSA) response according to (A) *NCOA2* copy number status and (B) The presence of any *NCOA2* aberration (somatic and

germline point mutations included). Red bars indicate the presence of a particular *NCOA2* aberration. Asterisk indicates an increase of >100% in PSA from baseline, daggers denote patients who received taxane chemotherapy. *p*-values represent Pearson's chi-squared testing.

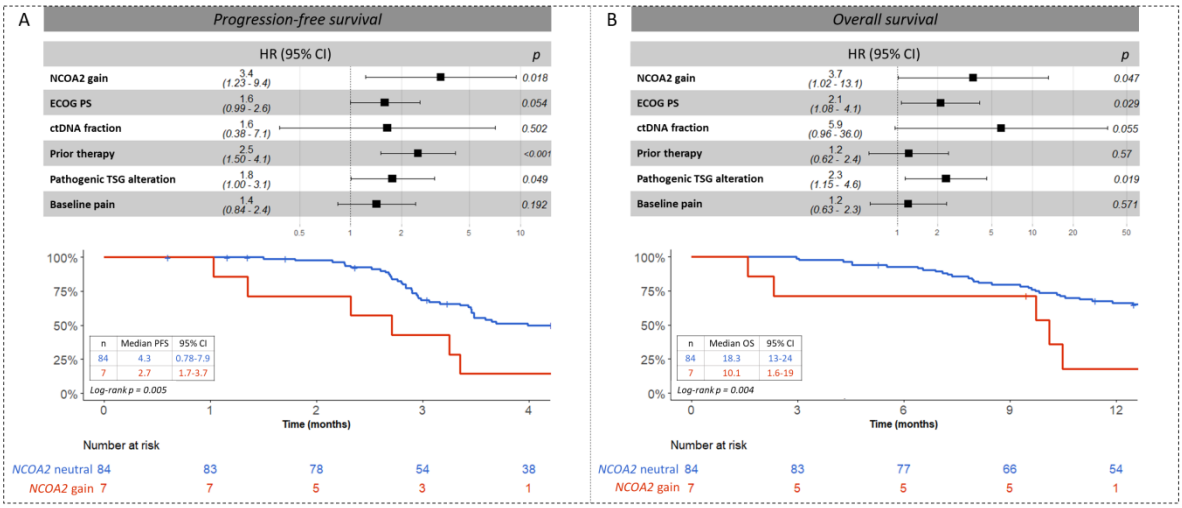

Figure 3: Multivariable Cox regression analysis (top) and Kaplan-Meier analysis (bottom) of (A) progression-free survival and (B) overall survival according to *NCOA2* copy number status.

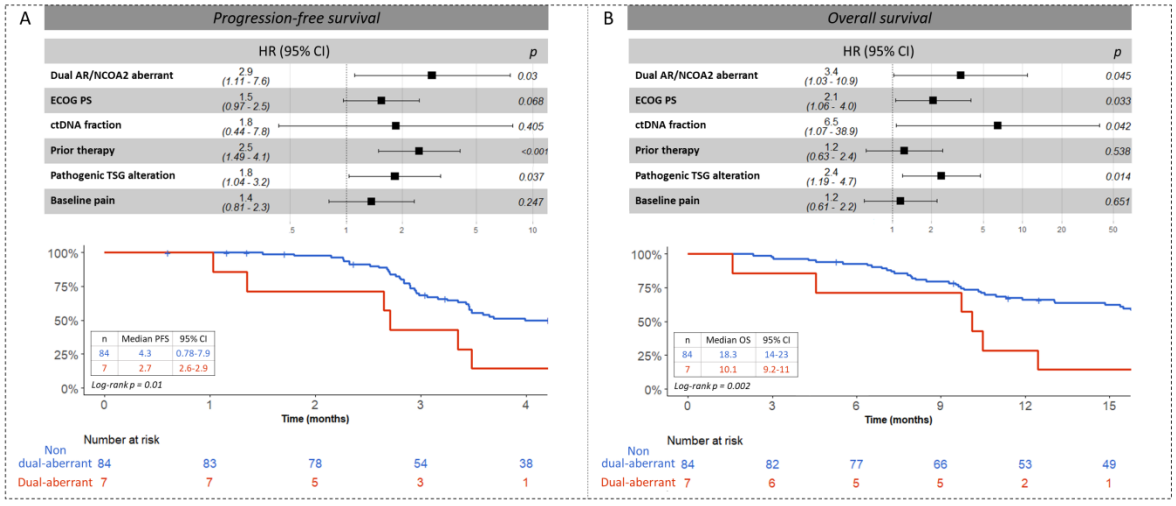

Figure 4: Multivariable Cox regression analysis (top) and Kaplan-Meier analysis (bottom) of (A) progression-free survival and (B) overall survival according to dual *AR* and *NCOA2* aberration status.

Table 1: Patient characteristics at baseline

	Study cohort n=91
TREATMENT ALLOCATION, N (%)	
ARPI	68 (75)
Taxane chemotherapy	23 (25)
AGE	
Median (range)	73 (46–91)
RACE, N (%)	
White	81 (89)
Asian	5 (5.5)
Other	5 (5.5)
CANCER-RELATED PAIN AT BASELINE, N (%)	
Absent	37 (41)
Present	54 (59)
ECOG PERFORMANCE SCORE, N (%)	
0-1	79 (87)
2	12 (13)
DISTRIBUTION OF DISEASE, N (%)	
Bone	84 (92)
Nodal	39 (43)
Visceral	9 (10)
GLEASON GRADE GROUP, N (%)	
≤7	24 (26)
≥8	45 (50)
No biopsy/unknown	22 (24)
PRIMARY TREATMENT, N (%)	
Surgery	15 (17)
Radiation	19 (21)
Metastatic disease at diagnosis	51 (56)
Primary ADT	3 (3)
None	3 (3)
PRIOR USE OF CHEMOTHERAPY, N (%)	
Yes	36 (40)
No	55 (60)
PRIOR USE OF ARPI, N (%)	
Yes	25 (27)
No	66 (73)
BASELINE PSA (ng/ml)	
Median (range)	28.6 (0.51–2,719)
CTDNA FRACTION (%)	
Median (range)	12.4 (0-87.6)

ECOG = Eastern Cooperative Oncology Group; ADT = androgen deprivation therapy; ARPI = androgen receptor pathway inhibitor; PSA = prostate-specific antigen; ctDNA = circulating tumour DNA.

Table 2: Uni- and multivariable Cox regression analyses of progression-free survival and overall survival, stratified according to gene aberration status and known poor prognostic markers (n=91)

		Progression-free survival			*Overall survival*		
Variable	**n**	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***

UNIVARIABLE ANALYSIS[a]							
AR gain	20	2.1	1.2-3.6	**0.008**	2.4	1.3-4.7	**0.008**
AR SNV	15	1.2	0.65-2.2	0.6	1.2	0.60-2.6	0.5
Any *AR* aberration	32	1.7	1.1-2.8	**0.03**	2.0	1.1-3.6	**0.02**
NCOA2 gain	7	3.2	1.4-7.6	**0.008**	4.8	1.7-14	**0.004**
NCOA2 SNV	5	0.88	0.28-2.8	0.8	3.3	1.2-9.3	**0.03**
Any *NCOA2* aberration	12	1.8	0.87-3.6	0.1	4.4	2.0-9.7	**<0.001**
AR or *NCOA2* gain	23	2.1	1.2-3.6	**0.006**	2.9	1.5-5.4	**0.001**
AR or *NCOA2* aberration	37	1.6	1.0-2.7	**0.04**	2.5	1.4-4.5	**0.002**
AR and *NCOA2* aberration	7	2.9	1.2-6.9	**0.02**	4.1	1.4-12	**0.009**
PTEN aberration	27	1.5	0.90-2.5	0.2	2.3	1.3-4.2	**0.006**
RB1 aberration	21	1.6	0.90-2.7	0.1	3.3	1.8-6.2	**<0.001**
TP53 aberration	18	2.6	1.5-4.8	**0.001**	3.3	1.7-6.3	**<0.001**
Any TSG alteration	42	1.9	1.2-3.1	**0.005**	3.1	1.8-5.5	**<0.001**
ctDNA fraction	-	6.0	1.9-19	**0.002**	23	5.6-92	**<0.001**
Prior therapy for mCRPC	45	2.0	1.3-3.2	**0.003**	0.86	0.47-1.6	0.6
ECOG PS	-	1.2	0.83-1.8	0.3	1.5	0.89-2.5	0.1
Baseline pain	54	2.0	1.2-3.2	**0.005**	1.8	1.0-3.2	**0.043**
MULTIVARIABLE ANALYSIS[a,b]							
AR gain	20	2.4	1.4-4.3	**0.003**	1.7	0.84-3.4	0.1
Any *AR* aberration	32	1.9	1.1-3.3	**0.03**	1.1	0.53-2.1	0.9
NCOA2 gain	7	3.4	1.2-9.4	**0.02**	3.7	1.02-13	**0.047**
NCOA2 SNV	5	-	-	-	2.1	0.66-6.7	0.2
Any NCOA2 aberration	12	-	-	-	3.0	1.2-7.6	**0.02**
AR or *NCOA2* gain	23	2.5	1.4-4.5	**0.002**	2.0	1.0-4.0	**0.049**
AR or *NCOA2* aberration	37	1.9	1.1-3.5	**0.03**	1.3	0.65-2.8	0.4
AR and *NCOA2* aberration	7	2.9	1.1-7.6	**0.03**	3.4	1.03-11	**0.045**

[a] *p*-values <0.05 are indicated in **bold**.

[b] Denotes that each aberration type/gene class is entered individually in a multivariable Cox regression model, including the following covariates: ctDNA fraction, prior chemotherapy and/or androgen receptor pathway inhibition, the presence of cancer-related pain at baseline, ECOG performance status and the presence of a tumour suppressor gene alteration (in PTEN, RB1 or TP53).

AR = androgen receptor; CI = confidence interval; ctDNA = circulating tumour DNA; ECOG = Eastern Cooperative Oncology Group; HR = hazard ratio; mCRPC = metastatic castration-resistant prostate cancer; NCOA2 = nuclear receptor coactivator 2; SNV = single nucleotide variant.

Table 3: Pearson's χ^2 analysis of PSA response rate according *AR* and/or *NCOA2* status for the entire mCRPC cohort (n=91)

Variable	PSA response rate no vs yes (%)	*p*[a]
AR gain	40/71 (56) vs 8/20 (40)	0.2[#]
AR SNV	40/76 (53) vs 8/15 (53)	1.0[#]
Any *AR* aberration	33/59 (56) vs 15/32 (47)	0.4[#]
NCOA2 gain	47/84 (56) vs 1/7 (14)	**0.046***
NCOA2 SNV	46/86 (53) vs 2/5 (40)	0.7*

Any *NCOA2* aberration	45/79 (57) vs 3/12 (25)	**0.04**[#]
AR or *NCOA2* gain	39/68 (57) vs 9/23 (39)	0.1[#]
AR or *NCOA2* aberration	31/54 (57) vs 17/37 (46)	0.3[#]
AR and *NCOA2* aberration	47/84 (56) vs 1/7 (14)	**0.046***
PTEN aberration	37/64 (58) vs 11/27 (41)	0.1[#]
RB1 aberration	42/70 (60) vs 6/21 (29)	**0.01**[#]
TP53 aberration	41/73 (56) vs 7/18 (39)	0.2[#]
Any TSG alteration	33/49 (67) vs 15/42 (36)	**0.003**[#]
ctDNA fraction > 0.02	21/34 (62) vs 27/57 (47)	0.2[#]
Prior therapy for mCRPC	32/46 (70) vs 16/45 (36)	**0.001**[#]
ECOG PS ≥2	42/69 (61) vs 6/12 (50)	0.8[#]
Baseline pain	25/37 (68) vs 23/54 (43)	**0.02**[#]

[a]*p*-values <0.05 are indicated in **bold.**

[#]Pearson's χ^2 *test.*

*Fisher's exact test.

AR = androgen receptor; ARPI = androgen receptor pathway inhibitor; NCOA2 = nuclear receptor coactivator 2; PSA = prostate-specific antigen; SNV = single nucleotide variant.