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Comprehensive Profiling of the Androgen Receptor in Liquid Biopsies from Castration-resistant Prostate Cancer Reveals Novel Intra-AR Structural Variation and Splice Variant Expression Patterns

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Abstract

Background: Expression of the androgen receptor splice variant 7 (AR-V7) is associated with poor response to second-line endocrine therapy in castration-resistant prostate cancer (CRPC). However, a large fraction of nonresponding patients are AR-V7–negative.
Objective: To investigate if a comprehensive liquid biopsy–based AR profile may improve patient stratification in the context of second-line endocrine therapy.
Design, setting, and participants: Peripheral blood was collected from patients with CRPC ($n = 30$) before initiation of a new line of systemic therapy. We performed profiling of circulating tumour DNA via low-pass whole-genome sequencing and targeted sequencing of the entire *AR* gene, including introns. Targeted RNA sequencing was performed on enriched circulating tumour cell fractions to assess the expression levels of seven AR splice variants (ARVs).
Outcome measurements and statistical analysis: Somatic AR variations, including copy-number alterations, structural variations, and point mutations, were combined with ARV expression patterns and correlated to clinicopathologic parameters.
Results and limitations: Collectively, any AR perturbation, including ARV, was detected in 25/30 patients. Surprisingly, intra-AR structural variation was present in 15/30 patients, of whom 14 expressed ARVs. The majority of ARV-positive patients expressed multiple ARVs, with AR-V3 the most abundantly expressed. The presence of any ARV was associated with progression-free survival after second-line endocrine treatment (hazard ratio 4.53, 95% confidence interval 1.424–14.41; $p = 0.0105$). Six out of 17 poor responders were AR-V7–negative, but four carried other AR perturbations.
Conclusions: Comprehensive AR profiling, which is feasible using liquid biopsies, is necessary to increase our understanding of the mechanisms underpinning resistance to endocrine treatment.
Patient summary: Alterations in the androgen receptor are associated with endocrine treatment outcomes. This study demonstrates that it is possible to identify different types of alterations via simple blood draws. Follow-up studies are needed to determine the effect of such alterations on hormonal therapy.

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1. Introduction

Prostate cancer is the most common cancer diagnosed in men, with nearly 410 000 diagnoses in Europe each year. Approximately 20–25% will develop metastatic disease, which inevitably progresses to lethal castration-resistant prostate cancer (CRPC). CRPC is characterized by progressive disease under maximal androgen blockade. Nonetheless, continued targeting of the androgen receptor (AR) has demonstrated that this signalling pathway remains one of the main drivers of progressive disease, even in the CRPC setting [1]. Besides taxane-based chemotherapy regimens, next-generation androgen deprivation therapies, encompassing both the CYP17 inhibitor abiraterone acetate and novel antiandrogens such as enzalutamide, have become available. However, up to 20–40% of patients have resistant disease at the start of these second-line AR therapies [2–5].

Various AR perturbations, such as mutations [6–8], amplifications [9–11], and splice variants [12–15], have been associated with resistance to androgen deprivation therapies. The emergence of mutations is affected by the treatment history, as individual mutations have different clinical consequences [16,17]. Amplifications occur in 29–45% of CRPC patients before starting a new antiandrogen therapy [9,11,18] and increase the expression of AR, which is associated with resistance to next-generation androgen deprivation therapies [11]. Furthermore, AR splice variants (ARVs) can act as constitutively active transcription factors, bypassing the need for activating ligands and therefore stimulating ligand-independent growth and progression of the disease [19–22].

Prostate cancer metastasises primarily to bone [2] and there are low success rates for obtaining adequate material for profiling, even in the research setting [18]. The application of liquid biopsies, in the form of circulating tumour cells (CTCs), circulating tumour DNA (ctDNA), or exosomes, has potential for biomarker profiling without access to metastatic tissue. Consequently, AR-V7 has recently been linked to resistance to abiraterone acetate and enzalutamide in multiple studies that applied various forms of liquid biopsy [12–15].

However, there is ongoing discussion about the discriminatory value of detecting AR-V7 expression [23]. As other AR perturbations have been associated with endocrine treatment outcome, it is likely that a combination test at both the DNA and RNA levels will improve patient stratification. Previous work pioneered by Li and colleagues demonstrated a connection between structural AR variation and the generation of noncanonical transcripts [24,25]. We hypothesized that at least a subset of CRPC patients may carry relevant intra-AR variations.

Therefore, we performed a pilot study in a selected cohort of patients with CRPC involving thorough AR profiling at both the DNA and RNA levels in liquid biopsies ($n = 34$) from 30 patients. Our profiling combined mutations, copy-number variations (CNVs), and sequencing of the entire AR gene, including introns, in combination with expression information from the full-length AR and seven ARVs (AR45, AR-V1, AR-V2, AR-V3, AR-V5, AR-V7, and AR-V9). The aim of the study was to investigate if a comprehensive AR profile could provide additional information to stratify patients beyond AR-V7 expression in the context of endocrine treatment.

2. Patients and methods

The Supplementary material provides a detailed description of all the methods. In brief, we collected blood samples from chemotherapy pretreated and chemo-naïve patients with CRPC in a non-interventional clinical study. Ethical approval was obtained from the institutional review and ethics board of GZA Sint-Augustinus. All patients provided a written informed consent document. Blood collections included samples for germline DNA extraction, CTC enumeration, CTC enrichment, and extraction of cell-free DNA from plasma.

ARV expression levels were assessed by performing cDNA synthesis, multiplex exon-junction–specific PCR (MASTR, Multiplicom NV), and Illumina sequencing on RNA derived from CellSearch-enriched CTC fractions. DNA-based library preparation was performed using a ThruPLEX DNA-seq kit (Rubicon Genomics). Low-pass whole-genome sequencing (1×50 bp) was performed for identification of copy-number alterations. Targeted sequencing was performed using a SeqCap EZ system (Roche Nimblegen) for detection of point mutations and intra-AR structural variations (2×100 bp). Sequencing was conducted on a Hiseq2500 instrument in rapid mode. Details on sequence data processing and statistical analysis are available in the Supplementary material. To identify intra-AR structural variations, we developed an in-house structural variant–calling algorithm, *svcaller*, that is publicly available (https://github.com/tomwhi/svcaller).

3. Results

From October 2013 to June 2015, liquid biopsies ($n = 34$) were collected from 30 patients with CRPC. Clinicopathologic and radiologic data for the cohort are given in Supplementary Table 1. The selected cohort encompasses patients with poor prognosis, with 17/30 (56.7%) patients having M1 disease at initial diagnosis. The goal was to thoroughly investigate the AR molecular status in the context of endocrine treatment (Fig. 1).

Cell-free DNA (cfDNA) was successfully extracted from 33 plasma samples and sequencing libraries were constructed. The libraries were subjected to low-pass whole-genome sequencing to determine the copy-number AR status as well as genome-wide somatic CNVs (Supplementary Table 2, Supplementary Fig. 1). AR amplifications were detected in 20/30 patients, with high-level amplifications in 11 patients.

Subsequently, targeted sequencing was performed via in-solution hybridisation capture on the same sequencing libraries used for low-pass whole-genome sequencing. The target region contained baits complementary to 112 genes (Supplementary Table 3), including all coding exons and nonrepetitive intronic regions of AR (Supplementary Fig. 2). The overall average coverage was 1169× (interquartile range [IQR] 904.5×–2180×; Supplementary Table 2). Somatic mutations were detected in all profiled samples (Supplementary Fig. 3, Supplementary Table 4). Genes previously reported to be over-represented in CRPC compared to primary prostate cancer [18], such as *TP53*,

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