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Efficacy of Cabazitaxel in Castration-resistant Prostate Cancer Is Independent of the Presence of *AR-V7* in Circulating Tumor Cells

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Abstract

Background: Androgen receptor splice variant 7 (*AR-V7*) in circulating tumor cells (CTCs) from patients with metastatic castration-resistant prostate cancer (mCRPC) was recently demonstrated to be associated with resistance to abiraterone and enzalutamide. Cabazitaxel might, however, remain effective in *AR-V7*-positive patients.
Objective: To investigate the association between *AR-V7* expression in CTCs and resistance to cabazitaxel.
Design, setting, and participants: We selected patients with mCRPC from the multicenter, randomized, phase 2, randomized, open-label, multicenter study in mCRPC on the pharmacodynamic effects of budesonide on cabazitaxel (Jevtana) (CABARESC). Before the start of the first and third cabazitaxel cycle, CTCs were enumerated using the CellSearch System. In patients with $\geq$10 CTCs in 7.5 ml blood at baseline, the expression of *AR-V7* was assessed by quantitative polymerase chain reaction.
Outcome measures and statistical analysis: The primary end point was the association between the *AR-V7* status and the CTC response rate (decrease to fewer than five CTCs in 7.5 ml blood during treatment). Secondary end points were the prostate-specific antigen (PSA) response rate (RR) and overall survival (OS). Analyses were performed using chi-square and log-rank tests.
Results and limitations: *AR-V7* was detected in 16 of 29 patients (55%) with $\geq$10 CTCs and was more frequently found in abiraterone pretreated patients (5 of 5 [100%] treated vs 7 of 20 [35%] untreated; $p = 0.009$). We found no differences in CTC and PSA RRs. The presence of *AR-V7* in CTCs was not associated with progression-free survival (hazard ratio [HR]: 0.8; 95% confidence interval [CI], 0.4–1.8) or overall survival (HR 1.6; 95% CI, 0.6–4.4).
Conclusions: The response to cabazitaxel seems to be independent of the *AR-V7* status of CTCs from mCRPC patients. Consequently, cabazitaxel might be a valid treatment option for patients with *AR-V7*-positive CTCs.
Patient summary: Tools are needed to select specific treatments for specific patients at specific times. The presence of the gene *AR-V7* in CTCs has been associated with resistance to anti-androgen receptor treatments. We investigated whether this holds true for cabazitaxel, but we found cabazitaxel to be effective independent of the presence of *AR-V7*.

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1. Introduction

Several new treatment options have become available for patients with metastatic castration-resistant prostate cancer (mCRPC). Abiraterone and enzalutamide, both acting on androgen receptor (AR) signaling, improve overall survival (OS) both in the pre- and post-docetaxel setting [1–6]. Cabazitaxel, the next-generation taxane, has been developed to overcome docetaxel resistance and improves OS in mCRPC patients pretreated with docetaxel [3,7]. With the arrival of these treatments, the question of how to optimally sequence treatment lines for mCRPC patients has arisen. Preclinical and clinical data indicate cross-resistance between abiraterone, enzalutamide, and docetaxel [8–12]. However, patients pretreated with abiraterone, enzalutamide, and docetaxel still appear to benefit from cabazitaxel [7,13,14]. Reliable predictive factors reflecting tumor characteristics in real-time are thus urgently needed to guide treatment selection.

A circulating tumor cell (CTC) count from peripheral blood before and during treatment is an independent prognostic factor for progression-free survival (PFS) and OS in mCRPC, and it outperforms prostate-specific antigen (PSA) measurements as an early treatment response marker [15–19]. The presence of the AR splice variant 7 (*AR-V7*), coding for a truncated and constitutively active androgen receptor (AR), in CTCs has been found to be associated with resistance to enzalutamide and abiraterone but not to taxanes, mainly docetaxel [20,21]. We investigated the association of *AR-V7* in CTCs with the response to cabazitaxel in docetaxel-pretreated mCRPC patients. We set up a highly specific reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR) assay to measure messenger RNA (mRNA) expression levels of wild-type AR (*AR-WT*) and *AR-V7* in CTCs enriched by the CellSearch System (Janssen Diagnostics LLC, Raritan, NJ, USA). Extensive and robust data are available concerning the clinical relevance of CTCs enumerated by this relatively widely available US Food and Drug Administration (FDA)-cleared technique. Next, we explored associations between the presence of *AR-V7* in CTCs taken before start of cabazitaxel and the outcome to cabazitaxel.

2. Patients and methods

2.1. Patients

Patients with mCRPC were recruited from an ongoing, multicenter, randomized phase 2 trial investigating the effects of budesonide on cabazitaxel toxicity (CABARESC; Dutch Trial Registry no. NTR2991). All patients had progressive disease after docetaxel (three rising PSA measurements $\geq$2 wk apart, PSA increase $\geq$2.0 μg/l, or radiologic progression). Full inclusion criteria are listed in Supplement 1. All patients received 25 mg/m^2 of cabazitaxel until progression, unacceptable toxicity, or the maximum of 10 cycles. The collection of CTC samples was a side study of the CABARESC trial. For this study, we selected patients who had been included between August 2012 and August 2014 with $\geq$10 CTCs in 7.5 ml blood before the start of cabazitaxel to ensure robust and CTC-specific downstream analysis. The Erasmus Medical Center and local institutional review boards approved the study (METC 11-324). All patients provided written informed consent.

2.2. Sample processing

Before the start of the first and the third cycle of cabazitaxel, CTCs were enumerated from 7.5 ml blood drawn in a CellSave tube using the CellSearch System (Janssen Diagnostics LLC, Raritan, NJ, USA). Characterization of CTCs was done before the first cycle of cabazitaxel from 7.5 ml ethylenediaminetetraacetic acid (EDTA) blood, which was processed using the CellSearch Profile Kit. After RNA isolation, cDNA generation, and preamplification, expression levels of *AR-WT* and *AR-V7* were measured by RT-qPCR in an 11% aliquot of the original starting material using Taqman Gene Expression Assays (Applied Biosystems, Carlsbad, CA, USA) (Supplementary Fig. 1). Details on sample processing are available in Supplement 2.

The performance of the assays was tested through the analysis of 17 healthy blood donors (HBDs) and prostate (22RV1, LNCaP, PC3, and VCaP) and breast (CAMA1, MDA-MB-415, MDA-MB-453, MPE600, SUM185PE, and ZR75.1) cancer cell lines (Supplementary Fig. 1 and 2). A total of 100 cell-line cells were spiked in 7.5 ml HBD blood and CellSearch enriched to serve as negative and positive controls: 22RV1 ($WT_{high}/V7_{high}$), CAMA1 ($WT_{low}/V7_{neg}$), LNCaP ($WT_{high}/V7_{low}$), MDA-MB-415 ($WT_{low}/V7_{neg}$), MDA-MB-453 ($WT_{low}/V7_{neg}$), MPE600 ($WT_{low}/V7_{neg}$), PC3 ($WT_{neg}/V7_{neg}$), SUM185PE ($WT_{low}/V7_{low}$), VCaP ($WT_{high}/V7_{high}$), and ZR75.1 ($WT_{low}/V7_{low}$). All samples were processed in a similar way to the patient blood samples.

2.3. Normalization and statistical analysis

Samples with an average cycle threshold for quantification (C_q) <26.5 for the three reference genes (glucoronidase, beta [*GUSB*]; hydroxymethylbilane [*HMBS*]; and hypoxanthine phosphoribosyltransferase 1 [*HPRT1*]) and an average C_q of the two epithelial genes <26.5 (epithelial cell adhesion molecule [*EPCAM*], keratin 19, type I [*KRT19*]) were considered evaluable. To correct for CTC count and epithelial tumor cell input, C_q values of *AR-V7* and *AR-WT* were normalized to the average C_q value of the epithelial genes (Spearman's r [r_s] with CTC count 0.7; $p < 0.01$) (Supplementary Fig. 3a). Final epithelial tumor cell input in the aliquot of RNA used was calculated using the equation derived from the regression line of the correlation between the epithelial genes and the CTC count, thereby taking into account that only 11% of the originally isolated RNA from all CTCs in the sample was used for the characterization of *AR-V7* status (Supplementary Fig. 3a). A cut-off value for positivity for *AR-V7* was determined based on the cell line and HBD experiments (Supplement 2).

The primary end point of this study was to compare the CTC response rate (CTC RR), defined as a decrease to fewer than five CTCs in 7.5 ml blood during treatment, between patients with *AR-V7*-positive and *AR-V7*-negative CTCs. Secondary objectives were the PSA RR (30% or 50% decline in PSA level from baseline to 12 wk or earlier in the case of treatment discontinuation), best PSA response during treatment, PFS (interval between registration and progression of disease or death), and OS (interval between registration and death). Associations between PFS or OS and the CTC response during treatment were analyzed after the second blood draw. Patients without events were censored at the last date recorded to be progression free and/or alive. Reported end points were based on the Prostate Cancer Working Group 2 guidelines [22].

The main hypothesis stated that there would be no difference in response to cabazitaxel by the presence or absence of *AR-V7*. Since limited data regarding the prevalence of *AR-V7* were available at the time of the study design, no formal sample size calculations were performed. Therefore, our analyses were exploratory. Differences in the primary objective, CTC RR, and secondary objective, PSA RR, were analyzed using the chi-square or Fisher exact tests. Survival was analyzed using Cox regression models and visualized in Kaplan-Meier plots. Other applied tests were the Student t test, the Mann-Whitney U test, and Pearson or