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Loss of SLCO1B3 drives taxane resistance in prostate cancer

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Background: Both taxanes, docetaxel and cabazitaxel, are effective treatments for metastatic castration-resistant prostate cancer (mCRPC). However, resistance to taxanes is common. Our objective was to investigate mechanisms of taxane resistance in prostate cancer.

Methods: Two docetaxel-resistant patient-derived xenografts (PDXs) of CRPC were established (PC339-DOC and PC346C-DOC) in male athymic nude mice by frequent intraperitoneal administrations of docetaxel. Next-generation sequencing was performed on PDX tissue pre- and post-docetaxel resistance and gene expression profiles were compared. [¹⁴C]-docetaxel and [¹⁴C]-cabazitaxel uptake assays *in vitro* and cytotoxicity assays were performed to validate direct involvement of transporter genes in taxane sensitivity.

Results: Organic anion-transporting polypeptide (SLCO1B3), an influx transporter of docetaxel, was significantly downregulated in PC346C-DOC tumours. In accordance with this finding, intratumoural concentrations of docetaxel and cabazitaxel were significantly decreased in PC346C-DOC as compared with levels in chemotherapy-naïve PC346C tumours. In addition, silencing of SLCO1B3 in chemo-naïve PC346C resulted in a two-fold decrease in intracellular concentrations of both taxanes. Overexpression of SLCO1B3 showed higher sensitivity to docetaxel and cabazitaxel.

Conclusions: The SLCO1B3 determines intracellular concentrations of docetaxel and cabazitaxel and consequently influences taxane efficacy. Loss of the drug transporter SLCO1B3 may drive taxane resistance in prostate cancer.

To date, ~25% of patients diagnosed with prostate cancer will eventually progress to metastatic castration-resistant prostate cancer (mCRPC) (Rane *et al*, 2012). The mCRPC patients are generally treated with docetaxel as standard first-line chemotherapy. Unfortunately, there is a large variability in response to docetaxel treatment among patients (Petrylak *et al*, 2004; Tannock *et al*, 2004; de Bono *et al*, 2010). Whereas some patients show prolonged responses, others respond poorly and progress rapidly. Among new options for docetaxel-resistant patients, cabazitaxel is a strong alternative taxane with proven

efficacy in docetaxel-resistant patients (de Bono *et al*, 2010). We previously showed that higher intratumoural concentrations of cabazitaxel in docetaxel-resistant tumours might lead to stronger antitumour activity (De Morree *et al*, 2016). Hence, it is highly relevant to determine tumour factors that may be linked to docetaxel resistance and cabazitaxel sensitivity. Such predictive biomarkers of taxane resistance will allow to define patients who are less likely to benefit from continued treatment with docetaxel, and thus might be candidates for treatment with cabazitaxel.

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In order to mimic the development of docetaxel resistance in patients, we set out to develop docetaxel resistance in a selection of patient-derived xenografts (PDXs) of prostate cancer. The PDX model has been demonstrated to largely resemble the complexity of prostate cancer including molecular diversity, cellular heterogeneity and histology (Kopetz *et al*, 2012). To investigate mechanisms of docetaxel resistance, we performed next-generation sequencing (NGS) and compared gene expression profiles of the created docetaxel-resistant tumours vs their parental tumours. We identified downregulation of SLCO1B3 as a potential mechanism of taxane resistance in CRPC. We show that silencing of SLCO1B3 resulted in decreased uptake of both docetaxel and cabazitaxel, whereas SLCO1B3 overexpression enhanced taxane sensitivity.

MATERIALS AND METHODS

Drugs. Docetaxel and cabazitaxel (Sanofi, Vitry-sur-Seine, France) formulations were prepared in polysorbate-80-ethanol (1:1, v/v) and further diluted in 5% (w/v) glucose solution to a final concentration of 2.5 mg ml⁻¹ for *in vivo* experiments. In uptake assays, [¹⁴C]-docetaxel and [¹⁴C]-cabazitaxel (specific activity: 83–86 mCi⁻¹mmol⁻¹; Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany) were used.

Development of docetaxel-resistant PDXs. The PC339, PC346C and PC374 xenografts with respectively low, moderate and high sensitivity to docetaxel were selected from the Erasmus MC prostate cancer PDX panel (van Weerden *et al*, 2009). The three selected PDXs were derived from patients progressive under standard androgen depletion therapy (ADT). Some molecular characteristics of the PDX models are listed in Supplementary Table 1. Docetaxel resistance was developed as described previously (De Morree *et al*, 2016). In short, tumour fragments of PDXs were subcutaneously transplanted on 8-week-old non-castrated male NMRI nude mice (NMRI-Foxn1^{nu}; Taconic, Hudson, NY, USA) and when tumours were established, mice received one bolus injection of docetaxel (33 mg kg⁻¹) intraperitoneally (i.p.) every 14 days until tumours progressed. Mice were not castrated, as it was previously shown that androgen levels in male mice resemble the androgen levels of chemically castrated men, originating from adrenal androgens (Sedelaar *et al*, 2013). Tumour fragments were consecutively passaged until growth rates of docetaxel-treated tumours were similar to those of the chemo-naive original tumour. All experiments were approved by the Animal Experiments Committee under the Dutch Experiments on Animals Act in adherence of the European Convention for Protection of Vertebrate Animals used for Experimental Purposes (Directive 2010/63/EU).

Assessment of antitumour activity. Antitumour activity of taxanes in chemo-naive and docetaxel-resistant PDXs was determined after a single i.p. injection of docetaxel 33 mg kg⁻¹ or cabazitaxel 33 mg kg⁻¹, or 0.9% (w/v) NaCl at a tumour volume of 300 mm³ as described previously (De Morree *et al*, 2016). In short, tumour volumes were measured twice a week by caliper. Antitumour activity of taxanes was monitored using the log cell kill formula (formula 1). This formula theoretically models the working mechanism of taxanes, assuming that a proportion of cells are killed after a single treatment while taking into account tumour doubling time.

$$\text{Log cell kill} = TC(\text{days}) / (3.32 * Td)$$

Formula 1: Log cell kill closely mimics clinical end points such as disease progression and takes into account how fast the tumour grows (tumour doubling time) in relation with the tumour growth delay induced by treatment according to the formula. *T* is the

median time in days to reach a tumour volume of 1000 mm³ in the treated mice. *C* is the median time in days to reach the same tumour volume in the control mice. *Td* is the tumour doubling time, derived from a log-linear growth plot of the control tumours in exponential growth phase. Log cell kill values are translated to antitumour activity as follows (Lloyd, 1975; Schabel *et al*, 1977; Corbett *et al*, 2003): 0.7–1.2 = +; 1.3–1.9 = ++; 2.0–2.8 = +++; and >2.8 = ++++. Resistance to taxanes was defined as log cell kill <0.7.

NGS of PDXs. Three independent tumours of each PDX, docetaxel-naive PC339, PC346C and PC374, and of docetaxel-resistant PC339-DOC and PC346C-DOC, were analysed by NGS/RNA seq. In brief, total RNA was extracted using RNA-Bee reagent (Tel-Test, Inc. Friendswood, TX, USA) according to the manufacturer's instructions. The RNA quantity and quality were analysed using Agilent Laboratory-on-Chip analysis (Agilent Bioanalyzer 2100, Agilent Technologies, Santa Clara, CA, USA). RNA samples with RNA Integrity Number (RIN) ≥7 were included. Library and paired-end RNA sequencing were executed by AROS (Applied Biotechnologies, Aarhus, Denmark) on a Illumina HiSeq 2000 with a sequencing depth of minimum 35 Mio reads.

mRNA expression validation using real-time PCR. Total RNA was isolated from PC346C and PC346C-DOC tumours to validate the expression levels of SLCO1B3. In addition, RNA from abiraterone- and enzalutamide-resistant tumours PC346Enza and PC346Abl101 that were previously developed (van Soest *et al*, 2013; Van Soest *et al*, 2015) was isolated. The SLCO1B3 mRNA (Taqman Assay On Demand, Hs00251986_m1, Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) was measured using a 7500 Fast Real-Time PCR System (Applied Biosystems). Gene expression was normalised using the ΔC_t method against housekeeping genes hypoxanthineguanine phosphoribosyltransferase (HPRT, Taqman assay 4310890E) and Porphobilinogen Deaminase (PBGD, Taqman assay Hs00609297_m1).

Bioinformatics analysis. A detailed description of the bioinformatics approach and analysis is provided in the Supplementary Methods. Gene expression profiles of chemotherapy-naive and docetaxel-resistant tumours were compared. The mRNA gene expression profiles were generated of which the expression was significantly altered in the docetaxel-resistant tumours. A false discovery rate (FDR) of <0.05 was used.

Gene silencing of taxane-related drug transporters. To evaluate the involvement of drug transporters in taxane efficacy, the top 10 of transporters that correlated with docetaxel and the top 10 transporters that correlated with cabazitaxel sensitivity were selected (see Supplementary Methods for details on the selection process). Genes were silenced in parental PC346C cells using the Sigma Transporter Silencing Bank (Sigma Mission siRNA of Ion Channel and Transporter Panel S106100, Sigma-Aldrich, St Louis, MO, USA) focussing on adenosine triphosphate-binding cassette (ABC) transporters and solute carrier (SLCs) families that were correlated with taxane sensitivity (for further details see Supplementary Methods). In short, PC346C cells were transfected with siRNA pools targeting various transporters. After 48 h, a [¹⁴C]-docetaxel and [¹⁴C]-cabazitaxel uptake assay was performed. Retained [¹⁴C]-cabazitaxel and [¹⁴C]-docetaxel levels were counted with a scintillation counter and normalised to levels observed in control cells transfected with a nontargeting siRNA pool (Zimmerman *et al*, 2016).

Intratumoural taxane concentration. Mice bearing docetaxel-naive or docetaxel-resistant PDXs received a single intraperitoneal injection of docetaxel 33 mg kg⁻¹ or cabazitaxel 33 mg kg⁻¹ at a tumour volume of 300 mm³ and were killed after 7 days. Tumour tissue was homogenised in lithium-heparinised human plasma

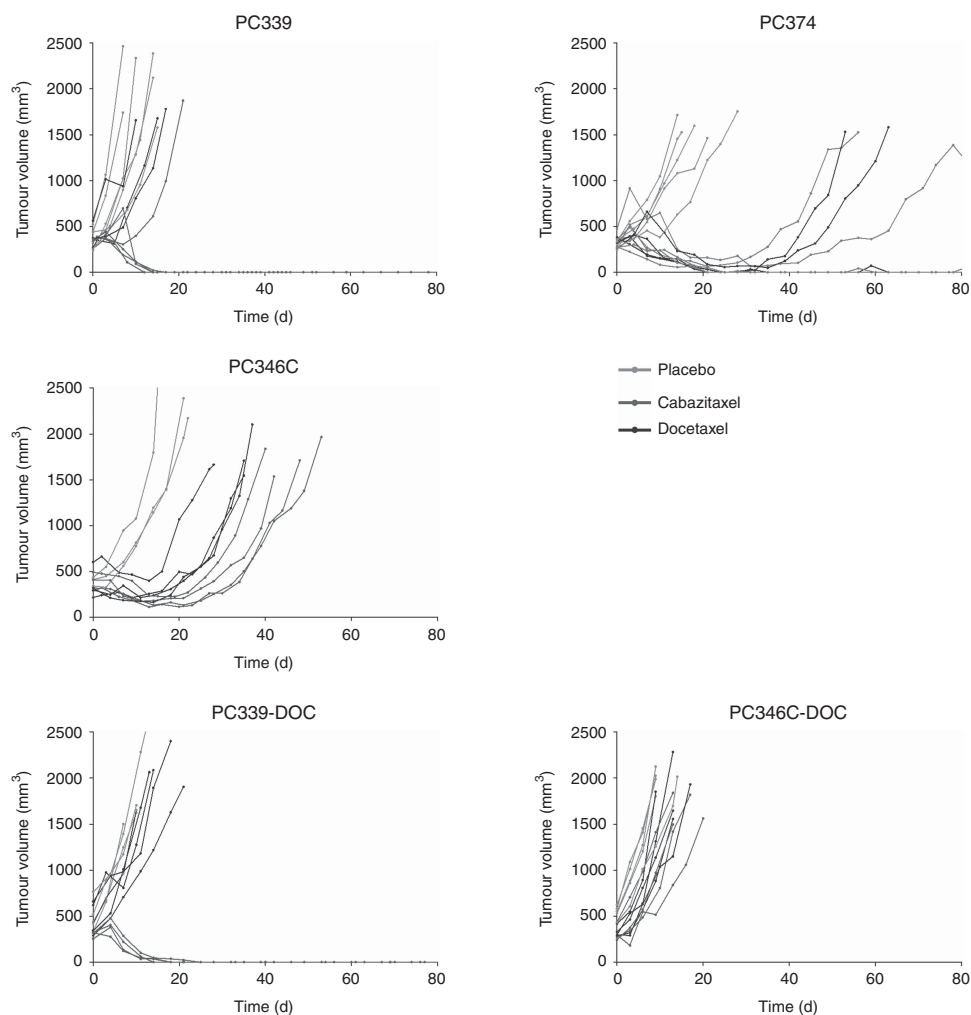
(1:5 w/v) and processed for further analysis by a validated liquid chromatography/tandem mass spectrometry (LC/MS-MS) assay to determine intratumoural docetaxel and cabazitaxel concentrations as previously described (Engels *et al*, 2006; de Bruijn *et al*, 2012; de Morree *et al*, 2016).

Cell proliferation assays. To substantiate the role of SLCO1B3 in taxane sensitivity, SLCO1B3-negative PC339C and PC346C-DCC-G cells were stably transfected with a SLCO1B3 or GFP (control) expression construct (see Supplementary Methods). The SLCO1B3-overexpressing cells and GFP-control cells were seeded in a 96-well plate at a concentration of 2500 cells per well and incubated for 10 days with docetaxel or cabazitaxel (serial concentration range 0–10 nM). Proliferation was assessed using PrestoBlue Cell Viability reagent (Thermo Fisher Scientific,

Waltham, MA, USA). Data are expressed as mean \pm s.e.m. of three independent experiments with at least six replicates per condition. The IC₅₀ values were determined using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA). The IC₅₀ values were compared using the extra sum-of-squares *F*-test with a boundary for significance of $P \leq 0.05$.

RESULTS

Generation of docetaxel-resistant PDXs of prostate cancer. In order to develop docetaxel resistance *in vivo*, PC339, PC346C and PC374 tumours were serially passaged under docetaxel pressure. The chemotherapy-naive PC339 PDX was characterised by a poor response to docetaxel with log cell kill values of 0.42 (log cell kill



PDX	Docetaxel		Cabazitaxel	
	Log cell kill	Antitumour activity	Log cell kill	Antitumour activity
Chemotherapy-naive				
PC339	0.42	--	>2.8	++++
PC374	1.6	++	1.8	++
PC346C	1.0	+	1.2	+
Docetaxel-resistant				
PC339-DOC	0.18	--	>2.8	++++
PC346C-DOC	0.20	--	0.23	--

Figure 1. Taxane response in docetaxel-naive and docetaxel-resistant PDXs. Tumour-bearing mice were treated with a single injection of either placebo (green), docetaxel 33 mg kg⁻¹ (black) or cabazitaxel 33 mg kg⁻¹ (red). Tumour volume was measured twice a week. Each line represents a single mouse. Log cell kill <0.7 was considered as resistant. Table insert summarises log cell kill values and antitumour activity of docetaxel and cabazitaxel in the various PDX models. A full colour version of this figure is available at the British Journal of Cancer journal online.

>0.7 is considered therapy responsive) (Figure 1). Within 4 mouse passages, a docetaxel-resistant variant, PC339-DOC, was established translating in a log cell kill value of 0.18. In contrast, the chemotherapy-naïve PC346C was relatively sensitive to docetaxel treatment (log cell kill value of 1.0) and remained responsive for almost 1 year after the first docetaxel treatment before acquiring resistance (log cell kill 0.20). Finally, the chemotherapy-naïve PC374 was extremely sensitive to docetaxel treatment (log cell kill 1.6) and we were unable to create a resistant counterpart of this PDX. Cabazitaxel was at least as potent as docetaxel with corresponding log cell kill values of >2.8, 1.2 and 1.8 for the docetaxel-naïve PC339, PC346C and PC374, respectively. Interestingly, cabazitaxel exhibited high sensitivity in PC339-DOC (log cell kill >2.8), whereas it showed cross-resistance with docetaxel in PC346C-DOC (log cell kill 0.23) (Figure 1).

Significant downregulation of SLCO1B3 expression in docetaxel-resistant PDX. To unravel potential mechanisms of resistance, we performed NGS/RNA seq between parental chemo-naïve and docetaxel-resistant PDX tumours and identified differentially expressed genes based on a FDR of 0.05 (see Table 1 for the top 15 gene list). The docetaxel uptake transporter SLCO1B3 was the most significantly downregulated gene in PC346C-DOC. The SLCO1B3 was neither expressed in the docetaxel-naïve PC339 nor in the docetaxel-resistant PC339-DOC. Validation with qPCR confirmed the substantial downregulation of SLCO1B3 in PC346C-DOC (Figure 2). To rule out that SLCO1B3 downregulation was caused by serial passaging of PC346C rather

than by docetaxel treatment, we showed that SLCO1B3 expression was retained in parental PC346C tumours during propagation (Supplementary Figure 1).

Downregulation of SLCO1B3 in abiraterone- and enzalutamide-resistant cells. We have previously shown cross-resistance between AR-targeted therapies, such as abiraterone and enzalutamide, and docetaxel *in vitro* and *in vivo* (van Soest *et al*, 2013; van Soest *et al*, 2015). Therefore, SLCO1B3 expression was also evaluated in PC346C cell lines with acquired resistance to abiraterone (PC346abi101) and enzalutamide (PC346enza). Similar to PC346C-DOC, SLCO1B3 expression was low in both PC346abi101 and PC346enza cells (Figure 2).

Decreased intratumoural taxane concentrations in docetaxel-resistant PDXs. As SLCO1B3 is a known transporter of docetaxel, downregulation of SLCO1B3 most likely result in reduced intratumoural taxane concentrations, inferring the observed resistant phenotype of PC346C-DOC. Indeed, intratumoural concentrations of docetaxel were significantly lower in PC346C-DOC as compared with chemo-naïve PC346C tumours (respectively $P=0.003$ and $P=0.0006$; Figure 3). As expected from the observed cross-resistance between docetaxel and cabazitaxel, intratumoural cabazitaxel levels were also reduced and not different from docetaxel in PC346C-DOC. In contrast, and in line with the efficacy of cabazitaxel in PC339 and PC339-DOC tumours, cabazitaxel levels were significantly higher than docetaxel levels in both PC339 ($P=0.005$) and PC339-DOC tumours ($P=0.01$; Figure 3). The significance of SLCO1B3 in the regulation of effective intratumoural levels of taxanes is provided in Figure 4.

Table 1. Top 15 genes significantly^a up- or down-regulated in the parental vs the docetaxel-resistant PDX

PC346C vs PC346C-DOC	Function ^b	Log fold change	PC339 vs PC339-DOC	Function ^b	Log fold change
SLCO1B3	Organic anion transmembrane transporter activity	-6.03	SPATA21	Calcium ion binding	-2.90
RBM24	3'-UTR binding and nucleotide binding	-5.07	MSMB	Member of the immunoglobulin binding factor family	2.69
HES7	Protein dimerisation activity and transcription factor binding	4.02	GRIP2	Undefined	-2.46
TNFRSF18	tumour necrosis factor-activated receptor activity	3.71	EGFLAM	Glycosaminoglycan binding	2.38
VANGL2	Regulation of planar cell polarity	3.70	POTEM	Undefined	2.29
SLC30A4	Zinc ion transmembrane transporter activity	-3.06	AFAP1	Actin binding and phospholipid binding	-2.20
ATP2B4	Calmodulin binding and sodium channel regulator activity	2.61	USH2A	Collagen binding and myosin binding	2.08
FADS2	Stearoyl-CoA 9-desaturase activity and iron ion binding	2.44	TSHR	Thyroid-stimulating hormone receptor activity	1.93
MTRNR2L10	Neuroprotective and antiapoptotic factor	2.36	KRT6A	Structural constituent of cytoskeleton	1.83
GPR88	G protein-coupled receptor activity	-2.26	ZNF407	May be involved in transcriptional regulation	-1.82
MARCKS	Cell motility, phagocytosis, membrane trafficking and mitogenesis	-1.97	NEB	Structural constituent of muscle and actin binding	1.80
UTRN	Protein kinase binding, calcium ion binding	-1.78	HLA-A	Peptide antigen binding and receptor binding	1.72
COL7A1	Serine-type endopeptidase inhibitor activity	1.75	CTAG2	Undefined	-1.66
MT2A	Binding of heavy metals	1.61	TMEM176B	Undefined	1.59
IQGAP2	Regulate cell morphology and motility	-1.59	KRT17	MHC class II protein binding and structural constituent of cytoskeleton	1.49

Abbreviations: MHC = major histocompatibility complex; PDX = patient-derived xenograft; UTR = untranslated region.

^aFalse discovery rate was <0.05 for all genes.

^bGene function was derived from www.genecards.org.

Knockdown of SLCO1B3 significantly decreased cellular uptake of taxanes. To determine whether other drug transporters could have contributed to the decreased concentrations of docetaxel and cabazitaxel observed in PC346C-DOC tumours, an siRNA screen of putative taxane drug transporters was performed. The only significant (50%) reduction in uptake of docetaxel ($P=0.01$) and cabazitaxel ($P=0.0003$) was observed when PC346C cells were

transfected with the SLCO1B3 siRNA pool (Figure 5 and Supplementary Figure 2).

Higher sensitivity to taxanes in SLCO1B3-overexpressing cells.

To further substantiate the role of SLCO1B3 in taxane sensitivity, cell viability was measured after docetaxel and cabazitaxel exposure in SLCO1B3-negative PC339C-GFP and SLCO1B3-overexpressing PC339C cells (see Supplementary Figure 3 for SLCO1B3 expression levels). The SLCO1B3-overexpressing PC339 cells were more sensitive to taxane treatment than the control-transfected PC339C-GFP cells. A similar correlation between SLCO1B3 and taxane sensitivity was seen in SLCO1B3-transfected, originally SLCO1B3-negative, castration-resistant PC346C-DCC-G subline (Figure 6).

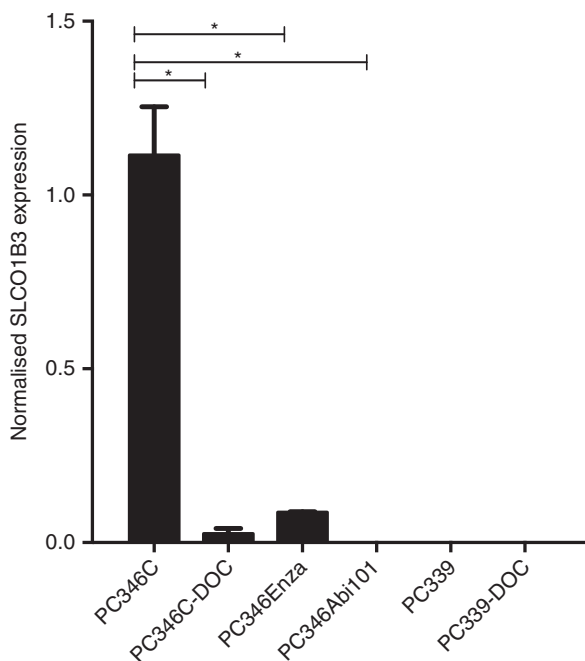


Figure 2. The expression of SLCO1B3 is downregulated in PC346C-DOC, PC346Enza and PC346Abi101. Expression of SLCO1B3 was measured in PC346, PC346C-DOC, PC346Enza and PC346Abi101 tumours or cell lines using real-time PCR. Both PC339 and PC339-DOC lack SLCO1B3 expression. The SLCO1B3 mRNA expression was normalised to HPRT and PBGD. An average \pm s.e.m. of $n=3-6$ tumours is shown. $*P<0.05$.

DISCUSSION

Predictive biomarkers for resistance to taxane chemotherapy are an unmet medical need in the management of mCRPC. Insights into the mechanisms of taxane resistance are crucial to identify and develop reliable predictive biomarkers and potential therapeutic targets. Here we show that downregulation of the influx transporter SLCO1B3 is associated with taxane resistance in a PDX model of prostate cancer, pointing towards a role for SLCO1B3 in taxane sensitivity through regulation of drug uptake, determining intratumoural concentrations and consequently efficacy. As such, SLCO1B3 may be a potential biomarker of docetaxel resistance that warrants further clinical validation.

The generation of two unique docetaxel-resistant PDX models, PC346C-DOC and PC339-DOC, allowed to screen for somatic mutations underlying the resistant phenotype that could have been acquired during the development of docetaxel resistance. Interestingly, we did not find any treatment-induced somatic variants in the transcriptome of either PDX. This finding and the difference in time to development of docetaxel resistance of these models, as well as their contrasting response to cabazitaxel, suggest that PC339-DOC and PC346C-DOC express different resistance mechanisms that are likely to be involved in chemoresistance.

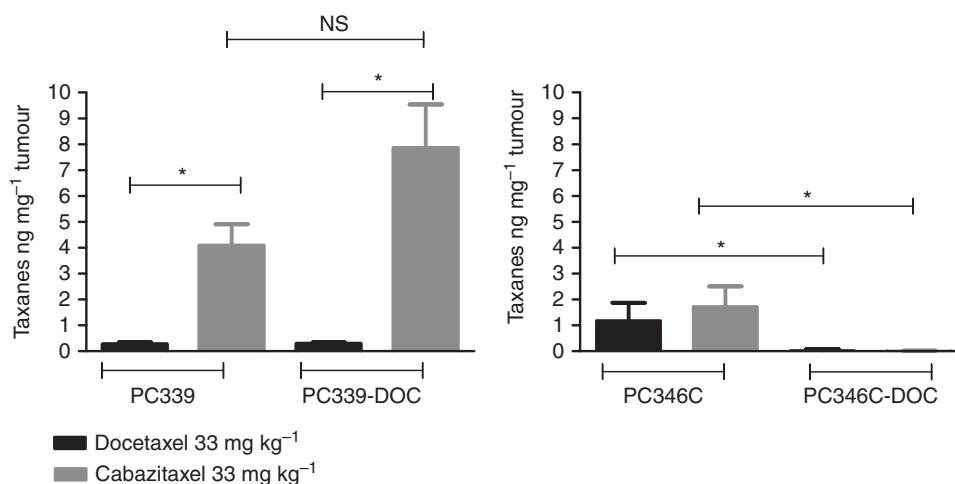


Figure 3. Intratumoural concentrations of docetaxel and cabazitaxel in parental vs docetaxel-resistant PDXs. Intratumoural concentrations were measured in docetaxel-naive and docetaxel-resistant PDXs at 7 days after a single dose with either docetaxel or cabazitaxel. Intratumoural concentrations of both docetaxel and cabazitaxel were significantly reduced in PC346C-DOC compared with PC346C. $*P<0.05$, NS = nonsignificant.

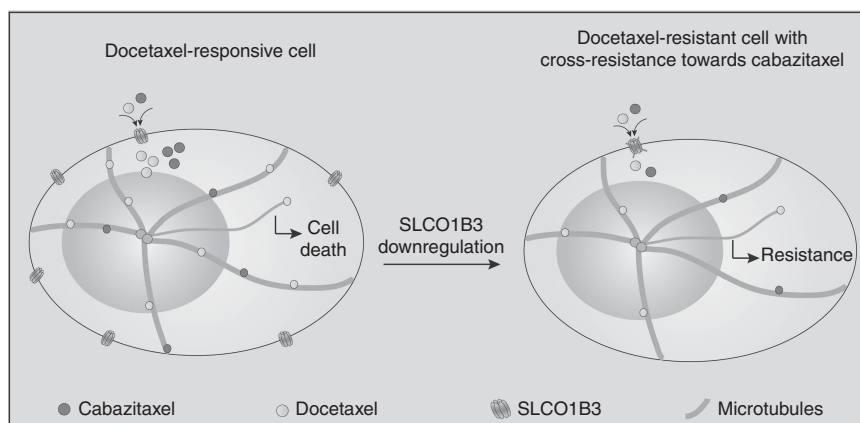


Figure 4. Mechanism of SLCO1B3-mediated resistance to docetaxel. Docetaxel-responsive cells express SLCO1B3. The SLCO1B3 is a known influx transporter of docetaxel, and transports docetaxel into the cell. Cabazitaxel may also be a potential substrate of SLCO1B3, but this hypothesis needs further experimental validation. We previously showed that intratumoural concentrations of cabazitaxel were generally higher in docetaxel-naïve tumours compared with docetaxel. As docetaxel or cabazitaxel enter the cell, they inhibit microtubule dynamics, leading to a cell cycle arrest in the G2/M phase and eventually to apoptosis. In docetaxel-resistant cells, SLCO1B3 expression is downregulated. We found that intratumoural concentrations of both docetaxel and cabazitaxel were decreased in docetaxel-resistant PC346C-DOC xenograft tumours, compared with the parental PC346C xenograft tumours. Experiments in which SLCO1B3 was silenced in PC346C cells showed decreased uptake of docetaxel and cabazitaxel, confirming that SLCO1B3 is at least partly involved in modulating intracellular concentrations of docetaxel and cabazitaxel. Decreased intratumoural concentrations leads to decreased response to therapy as was previously shown (De Morree *et al*, 2016).

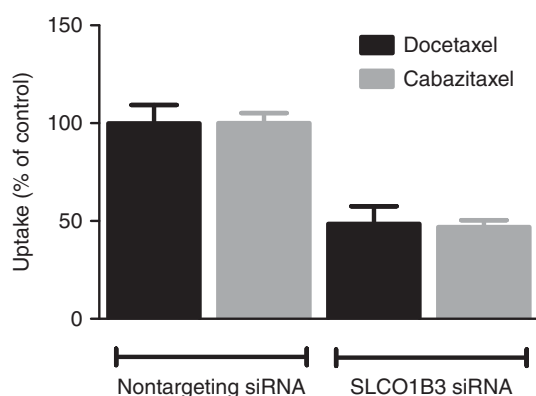


Figure 5. Silencing of SLCO1B3 leads to decreased uptake of docetaxel and cabazitaxel. Uptake and retention of [14 C]-docetaxel and [14 C]-cabazitaxel was measured in PC346C cells after silencing SLCO1B3. The levels of cabazitaxel and docetaxel taken up and retained in the cells were compared with the uptake of taxanes in cells transfected with CTRL siRNA. An average \pm s.d. is shown of $n = 3$ measurements. * $P < 0.05$ compared with the uptake in the control.

Differentially expressed genes that correlated to docetaxel resistance identified SLCO1B3 as an important gene associated with docetaxel resistance in PC346C-DOC. An extensive siRNA screen further revealed that the role of other ABC transporters and transporters from the SLC families, which were previously linked to taxane sensitivity, was less pronounced compared with SLCO1B3 in this model. The lack of SLCO1B3 expression in naïve PC339 and resistant PC339-DOC may explain the relative low sensitivity to docetaxel in this intrinsically resistant PDX, underscoring the relevance of SLCO1B3 in docetaxel sensitivity. This is further substantiated by the observation that overexpressing SLCO1B3 in PC339C cells indeed resulted in increased sensitivity to docetaxel. Interestingly, and in contrast to PC346C and PC346C-DOC, PC339 and PC339-DOC were highly sensitive for cabazitaxel that was further augmented when overexpressing SLCO1B3. These data suggest that cabazitaxel influx and

intratumoural levels also benefit from SLCO1B3 expression. This observation contrasts with previous reports indicating that cabazitaxel uptake may rely more on transmembrane diffusion than carrier-mediated translocation across the plasma membrane because of its higher lipophilicity as compared with docetaxel (Azarenko *et al*, 2014; Vrignaud *et al*, 2014). Clearly, further experiments are required to define whether cabazitaxel is a substrate of SLCO1B3 and its importance for intracellular cabazitaxel levels.

The SLCO1B3 has previously been shown to be expressed in at least 50% of prostate cancer specimens with increased expression in mCRPC compared with primary prostate cancer (Pressler *et al*, 2011; Wright *et al*, 2011). Furthermore, SLCO1B3 expression has been linked to the hormonal status of prostate cancer and to the response to androgen deprivation therapies, as it is also an influx transporter of testosterone (Hamada *et al*, 2008). Of note, in our enzalutamide- and abiraterone-resistant cell lines (van Soest *et al*, 2013, 2015) that represent cross-resistance with docetaxel, SLCO1B3 expression was lost, suggesting that pretreatment with hormonal agents, like enzalutamide and abiraterone, may affect taxane sensitivity via reduction of SLCO1B3 expression. Therefore, SLCO1B3 may not only mediate the cellular uptake of docetaxel, but may also alter the androgen responsiveness of the cell. This potential mechanism of cross-resistance may have implications for potential treatment sequences in the management of prostate cancer. Such possible relationship between SLCO1B3 and androgen status of the patient, inflicted either by conventional ADT or by the use of novel AR-targeted agents, may be particularly relevant in light of recent data, showing a robustly greater survival benefit by docetaxel when given in addition to ADT in patients with metastatic hormone-sensitive prostate cancer (mHSPC) as compared when given at the time of castration resistance (James *et al*, 2015; Sweeney *et al*, 2015). The value of SLCO1B3 expression as a potential candidate biomarker for taxane response in mCRPC and mHSPC patients requires a prospective clinical study in which tumour lesions are biopsied pre and post taxane treatment. As an alternative, circulating tumour cells may be considered as a liquid biopsy to evaluate SLCO1B3 expression over time and determine its predictive value.

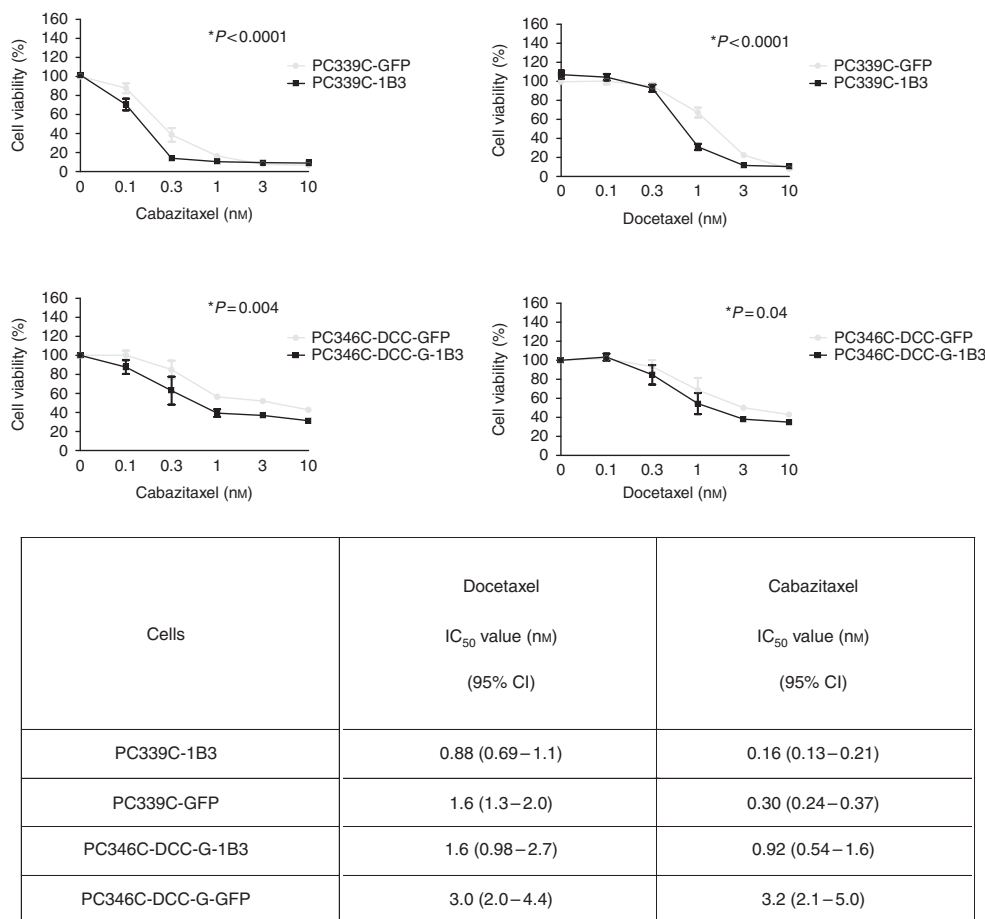


Figure 6. Sensitivity to docetaxel and cabazitaxel is increased in SLCO1B3-overexpressing prostate cancer cells. Two independent prostate cancer cell lines were transfected with a lentiviral expression construct containing SLCO1B3 or turbo-GFP (GFP) as control. Cells were cultured for 10 days in the presence of 0–10 nM docetaxel or cabazitaxel. Average \pm s.e.m. is shown of $n=3$ independent experiments.

CONCLUSIONS

We have shown that SLCO1B3 expression is associated with taxane resistance. In addition, SLCO1B3 may also have a role in cross-resistance with hormonal agents like enzalutamide and abiraterone. Clinical studies are needed to further investigate the potential role of SLCO1B3 as biomarker in patients with mCRPC treated with taxane chemotherapy.

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CONFLICT OF INTEREST

RJ van Soest has received honoraria from Sanofi. Ron HJ Mathijssen has received research funding from Sanofi and Astellas. Ronald de Wit has received consultancy and speaker honoraria from Sanofi, Janssen and Millennium, and research funding from Sanofi. Wytse M van Weerden has received research funding from Sanofi, Janssen and Millennium. The remaining authors declare no conflict of interest.

REFERENCES

- Azarenko O, Smiyun G, Mah J, Wilson L, Jordan MA (2014) Antiproliferative mechanism of action of the novel taxane cabazitaxel as compared with the parent compound docetaxel in MCF7 breast cancer cells. *Mol Cancer Ther* 13(8): 2092–2103.
- Corbett TH, White K, Polin L, Kushner J, Paluch J, Shih C, Grossman CS (2003) Discovery and preclinical antitumor efficacy evaluations of LY32262 and LY33169. *Invest New Drugs* 21(1): 33–45.
- de Bono JS, Oudard S, Ozguroglu M, Hansen S, Machiels JP, Kocak I, Gravis G, Bodrogi I, Mackenzie MJ, Shen L, Roessner M, Gupta S, Sartor AO. TROPIC Investigators (2010) Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: a randomised open-label trial. *Lancet* 376(9747): 1147–1154.
- de Bruijn P, de Graan AJ, Nieuweboer A, Mathijssen RH, Lam MH, de Wit R, Wiemer EA, Loos WJ (2012) Quantification of cabazitaxel in human plasma by liquid chromatography/triple-quadrupole mass spectrometry: a practical solution for non-specific binding. *J Pharm Biomed Anal* 59: 117–122.
- De Morree ES, Van Soest RJ, Aghai A, De Ridder CM, De Bruijn P, Ghobadi Moghaddam-Helmantel IM, Burger H, Mathijssen RH, Wiemer EAC, De Wit R, Van Weerden WM (2016) Understanding taxanes in prostate cancer: importance of intratumoral drug accumulation. *Prostate* 76(10): 927–936.
- Engels FK, Mathot RA, Loos WJ, van Schaik RH, Verweij J (2006) Influence of high-dose ketoconazole on the pharmacokinetics of docetaxel. *Cancer Biol Ther* 5(7): 833–839.
- Hamada A, Sissung T, Price DK, Danesi R, Chau CH, Sharifi N, Venzon D, Maeda K, Nagao K, Sparreboom A, Mitsuya H, Dahut WL, Figg WD (2008) Effect of SLCO1B3 haplotype on testosterone transport and clinical outcome in caucasian patients with androgen-independent prostatic cancer. *Clin Cancer Res* 14(11): 3312–3318.

- James ND, Sydes MR, Clarke NW, Mason MD, Dearnaley DP, Spears MR, Ritchie AW, Parker CC, Russell JM, Attard G, de Bono J, Cross W, Jones RJ, Thalmann G, Amos C, Matheson D, Millman R, Alzouebi M, Beesley S, Birtle AJ, Brock S, Cathomas R, Chakraborti P, Chowdhury S, Cook A, Elliott T, Gale J, Gibbs S, Graham JD, Hetherington J, Hughes R, Laing R, McKinna F, McLaren DB, O'Sullivan JM, Parikh O, Peedell C, Protheroe A, Robinson AJ, Srihari N, Srinivasan R, Staffurth J, Sundar S, Tolan S, Tsang D, Wagstaff J, Parmar MK (2015) Addition of docetaxel, zoledronic acid, or both to first-line long-term hormone therapy in prostate cancer (STAMPEDE): survival results from an adaptive, multiarm, multistage, platform randomised controlled trial. *Lancet* **387**(10024): 1163–1177.
- Kopetz S, Lemos R, Powis G (2012) The promise of patient-derived xenografts: the best laid plans of mice and men. *Clin Cancer Res* **18**(19): 5160–5162.
- Lloyd HH (1975) Estimation of tumor cell kill from Gompertz growth curves. *Cancer Chemother Rep* **59**(2 Pt 1): 267–277.
- Petrylak DP, Tangen CM, Hussain MH, Lara Jr. PN, Jones JA, Taplin ME, Burch PA, Berry D, Moynour C, Kohli M, Benson MC, Small EJ, Raghavan D, Crawford ED (2004) Docetaxel and estramustine compared with mitoxantrone and prednisone for advanced refractory prostate cancer. *N Engl J Med* **351**(15): 1513–1520.
- Pressler H, Sissung TM, Venzon D, Price DK, Figg WD (2011) Expression of OATP family members in hormone-related cancers: potential markers of progression. *PLoS One* **6**(5): e20372.
- Rane JK, Pellacani D, Maitland NJ (2012) Advanced prostate cancer—a case for adjuvant differentiation therapy. *Nat Rev Urol* **9**(10): 595–602.
- Schabel Jr FM, Griswold Jr DP, Laster Jr WR, Corbett TH, Lloyd HH (1977) Quantitative evaluation of anticancer agent activity in experimental animals. *Pharmacol Ther* **1**(4): 411–435.
- Sedelaar MJP, Dalrymple SS, Isaacs JT (2013) Of mice and men—warning intact versus castrated adult male mice as xenograft host are equivalent to hypogonadal versus abiraterone treated aging human males, respectively. *Prostate* **73**(12): 1316–1325.
- Sweeney CJ, Chen YH, Carducci M, Liu G, Jarrard DF, Eisenberger M, Wong YN, Hahn N, Kohli M, Cooney MM, Dreicer R, Vogelzang NJ, Picus J, Shevrin D, Hussain M, Garcia JA, DiPaola RS (2015) Chemohormonal therapy in metastatic hormone-sensitive prostate cancer. *N Engl J Med* **373**(8): 737–746.
- Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, Chi KN, Oudard S, Theodore C, James ND, Turesson I, Rosenthal MA, Eisenberger MA. TAX 327 Investigators (2004) Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. *N Engl J Med* **351**(15): 1502–1512.
- van Soest RJ, de Morree ES, Kweldam CF, de Ridder CM, Wiemer EA, Mathijssen RH, de Wit R, van Weerden WM (2015) Targeting the androgen receptor confers in vivo cross-resistance between enzalutamide and docetaxel, but not cabazitaxel, in castration-resistant prostate cancer. *Eur Urol* **67**(6): 981–985.
- van Soest RJ, van Royen ME, de Morree ES, Moll JM, Teubel W, Wiemer EA, Mathijssen RH, de Wit R, van Weerden WM (2013) Cross-resistance between taxanes and new hormonal agents abiraterone and enzalutamide may affect drug sequence choices in metastatic castration-resistant prostate cancer. *Eur J Cancer* **49**(18): 3821–3830.
- van Weerden WM, Bangma C, de Wit R (2009) Human xenograft models as useful tools to assess the potential of novel therapeutics in prostate cancer. *Br J Cancer* **100**(1): 13–18.
- Frignaud P, Semiond D, Benning V, Beys E, Bouchard H, Gupta S (2014) Preclinical profile of cabazitaxel. *Drug Des Devel Ther* **8**: 1851–1867.
- Wright JL, Kwon EM, Ostrander EA, Montgomery RB, Lin DW, Vessella R, Stanford JL, Mostaghel EA (2011) Expression of SLCO transport genes in castration-resistant prostate cancer and impact of genetic variation in SLCO1B3 and SLCO2B1 on prostate cancer outcomes. *Cancer Epidemiol Biomarkers Prev* **20**(4): 619–627.
- Zimmerman EI, Gibson AA, Hu S, Vasilyeva A, Orwick SJ, Du G, Mascara GP, Ong SS, Chen T, Vogel P, Inaba H, Maitland ML, Sparreboom A, Baker SD (2016) Multikinase inhibitors induce cutaneous toxicity through OAT6-mediated uptake and MAP3K7-driven cell death. *Cancer Res* **76**(1): 117–126.

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