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Review

Improving Taxane-Based Chemotherapy in Castration-Resistant Prostate Cancer

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Currently, the clinical utility of taxane-based drug formulations in castration-resistant prostate cancer (CRPC) is severely limited by acquired chemotherapy resistance, dose-limiting toxicities, and nonresponders. Therefore, approaches to improve taxane-based chemotherapy are desperately required. In this review, we highlight the strategies that aim to overcome these limitations, such as bypassing therapy resistance, targeted drug delivery, and adequate prediction of therapy response. The involvement of the apoptotic pathway, ABC transporters, the glucocorticoid receptor (GR) axis, androgen receptor (AR) splicing, epithelial plasticity, and cancer stem cells in mediating taxane-resistance are outlined. Furthermore, passive and active targeted nanomedicinal drug delivery strategies and the use of circulating tumor cells in predicting docetaxel responses are discussed. Finally, recent advances towards clinical translation of these approaches in CRPC are reviewed.

Trends

Preclinical evidence establishes the therapeutic potential of reverting resistance mechanisms in docetaxel-resistant prostate cancer cells.

Targeted drug delivery of taxanes has the potential to enhance the antitumor efficacy, enhance tolerability, and permit the administration of intensified dosages.

Changes in levels of circulating tumor cells are indicative of responses to docetaxel treatment and can be further characterized to provide robust predictive markers for personalized treatment.

Taxane-Based Chemotherapy in CRPC

Prostate cancer is one of the most common malignancies, with more than 1.1 million cases worldwide, and, although death rates have declined over the past two decades, it is still a major cause of death, with over 300 000 deaths worldwide in 2012 [1,2]. Prostate cancer is a progressive disease with several stages that all warrant distinct treatment (Box 1). In this review, we focus on the challenges and recent developments in the treatment of the most advanced stage of the disease: **CRPC** (see Glossary).

CRPC is the most serious disease stage in prostate tumors, in which tumors progress despite androgen-deprivation therapy. Taxane-based chemotherapeutic drugs are currently the most common treatment of CRPC [3]. In 2004, docetaxel (Taxotere, marketed by Sanofi-Aventis) plus prednisone was approved as a first-line treatment for CRPC after displaying potent clinical activity in two landmark Phase III clinical trials (increased median survival versus control arm: 18.9 versus 16.5 months and 17.5 versus 15.6 months) [4,5]. More recently, a second-generation taxane cabazitaxel (Jevtana, marketed by Sanofi-Aventis) plus prednisone was demonstrated to exhibit superior antitumor activity over mitoxantrone plus prednisone in patients with CRPC progressing on docetaxel (increased median survival versus control arm: 15.1 versus 12.7 months) [6] and, therefore, was positioned as a second-line treatment. Despite the prolonged survival resulting from taxane chemotherapy, CRPC is still poorly managed, as illustrated by the fact that most patients with CRPC die within 3 years of diagnosis [4]. Hence, strategies to improve current taxane-based chemotherapeutics are urgently needed.

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Box 1. The Clinical Course of Prostate Cancer

Prostate cancer is a disease that is defined by multiple disease stages. In early organ-confined prostate cancer, therapeutic options include prostatectomy and radiotherapy, which is curative in 70–80% of patients. In 20–30% of patients, prostate cancer will relapse after 5–10 years, commonly at a metastatic site. Given that prostate cancer is fundamentally androgen receptor (AR) driven, therapies at this disease stage target the AR axis and are collectively referred to as 'androgen-deprivation therapy' (e.g., AR antagonists, LHRH agonists, and antagonists). Although this is initially effective, tumors will inevitably lose responsiveness to this therapy, starting a disease stage referred to as CRPC. Here, taxanes are routinely used; docetaxel as first-line treatment, and cabazitaxel as one of the second-line treatment options. Although all current treatment options for CRPC are life prolonging, most patients will eventually die from their disease.

Recently, data from the phase III CHAARTED trial were published; this trial looked at whether the addition of upfront chemotherapy (docetaxel) to androgen-deprivation therapy (ADT) improved overall survival in patients with hormone-sensitive metastatic prostate cancer. Interestingly, this trial showed that docetaxel treatment (combined with ADT) of patients with hormone-sensitive metastatic prostate cancer increased overall survival by 13.6 months versus ADT alone, after a median-follow up of 28.9 months [7]. Therefore, the beneficial effects of docetaxel may not be restricted to the treatment of patients with CRPC.

Despite the extensive use of docetaxel as the first-line treatment for CRPC, major pitfalls still undermine its clinical feasibility. First, prostate tumors may display initial resistance (decreased levels of biomarker prostate-specific antigen is observed in only 45% of patients with CRPC upon docetaxel treatment [4]) or tumors eventually acquire resistance to docetaxel treatment. Second, treatment with taxanes commonly introduces serious adverse effects, such as anemia, neutropenia, and neuropathy [4,5]. Third, the treatment response of individual patients is variable, resulting in the overtreatment of patients and, therefore, improved prediction of docetaxel response in individual patients with CRPC would strongly facilitate proper treatment choices (i.e., personalized medicine).

Here, we discuss recent developments and novel strategies to optimize taxane use in CRPC, including: (i) increasing the sensitivity for taxane-based chemotherapy (i.e., overcoming chemotherapy-resistant disease); (ii) exploiting nanomedicinal drug delivery systems (to enhance activity and prevent adverse effects); and (iii) predicting taxane-responsiveness to select the suitable patient population for docetaxel treatment.

Regaining Sensitivity to Taxane-Based Drugs in Docetaxel-Refractory CRPC

Taxanes are diterpenoid molecules derived from the *Taxus* genus and include the drugs paclitaxel, docetaxel, and cabazitaxel. Paclitaxel is clinically used in the treatment of many malignancies, although the semisynthetic analogs docetaxel and cabazitaxel are preferred as treatment for prostate cancer [8]. Taxanes exert their antitumor activity via several modes of action. First and foremost, taxanes prevent microtubule disassembly by the binding of beta-tubulin. Microtubules are involved in numerous cellular processes, including mitosis, cell shape maintenance, cellular transport, and cell signalling, and disturbance of these processes can lead to G2/M cell cycle arrest and the induction of apoptosis (Figure 1A) [8,9]. In addition to this, some of the antitumor actions of taxanes can be attributed to their effect on the **AR** axis (i.e., lowered AR expression [10], inhibition of AR nuclear translocation [11], and FOXO1-mediated repression of AR transcriptional activity) (Figure 1B) [12]. Effects on AR are dependent on the taxane used; for example, AR activity is inhibited by docetaxel and paclitaxel [12] but not by cabazitaxel [13]. However, the concentrations of docetaxel that are required for inhibition of AR activity seem to vary between publications. Finally, taxanes have also been reported to inhibit antiapoptotic Bcl-2 expression, which thereby favors apoptotic cell death through the relief of BAX-mediated cytochrome c release (Figure 1C) [14]. Given that the expression of the AR, cell cycle proteins, and apoptotic proteins oscillates over time, appropriate timing of treatment is key (Box 2).

Glossary

Androgen receptor (AR): a receptor for androgens such as testosterone and dihydrotestosterone. Prostate cancer is fundamentally AR driven.
Cancer stem cells (CSC): an aggressive subset of cells that are involved in many tumorigenic processes, including survival, invasion, metastasis, and resistance to therapy.
Castration-resistant prostate cancer (CRPC): an advanced disease stage in prostate cancer in which the tumor becomes resistant to androgen-deprivation therapy.
Circulating tumor cells (CTC): cells that have been shed from the tumor and circulate in the bloodstream; such cells can be isolated and characterized to monitor and predict treatment response.
Epithelial–mesenchymal transition (EMT): a process that involves a phenotypical switch from epithelial cells to migratory mesenchymal cells; often involved in metastasis and associated with resistance to chemotherapy.
Enhanced permeability and retention (EPR) effect: a principle that underlies targeted nanomedicinal drug delivery; dependent on the enhanced vascular permeability and poor lymphatic drainage of tumors.
Glucocorticoid receptor (GR): shown to be involved in resistance to AR-targeting drugs and docetaxel.
Glucocorticoids (GC): a class of corticosteroids that bind the GR. The GCs prednisone and dexamethasone are routinely given to patients with advanced prostate cancer.
P-glycoprotein (P-gp): a drug efflux pump associated with therapy resistance. Docetaxel and paclitaxel are substrates for P-gp, while cabazitaxel is not.
Prostate-specific antigen (PSA): a routinely used serum biomarker to monitor prostate cancer progression and response to therapy.
Prostate-specific membrane antigen (PSMA): a membrane antigen often overexpressed in prostate cancer; can be used to facilitate active targeting.

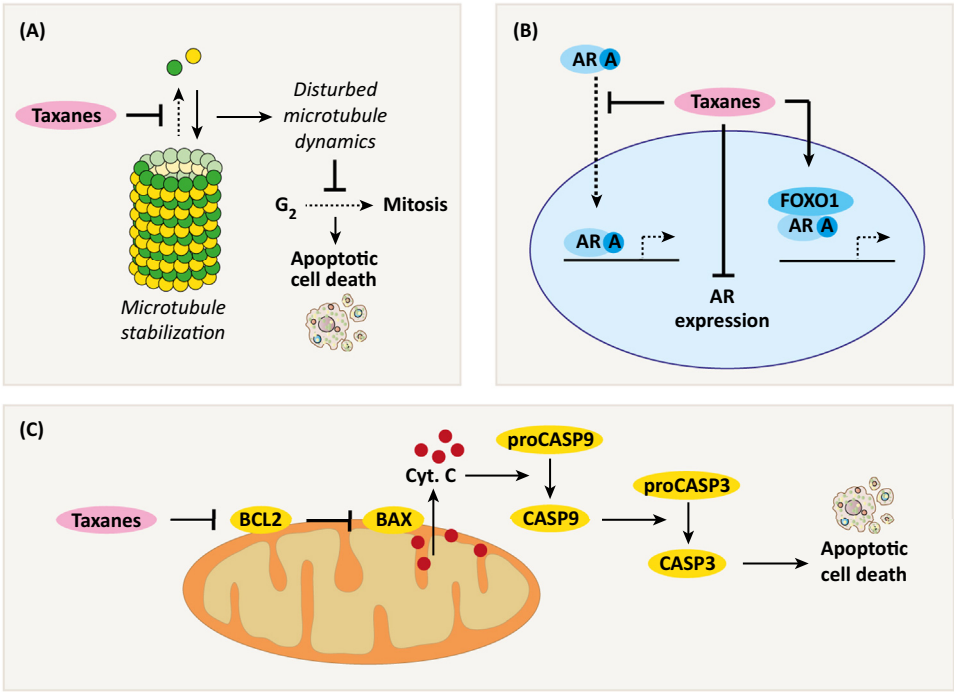

Figure 1. Mechanisms of Action of the Antitumor Activity of Taxanes. Taxanes have been described to exert their antitumor efficacy via distinct modes of action. (A) Taxanes bind to microtubules and thereby prevent their disassembly, resulting in G2/M cell cycle arrest and apoptosis [8,9]. (B) Alternatively, taxanes are able to inhibit androgen receptor (AR) transcriptional activity by constraining AR expression [10], blocking AR nuclear translocation [11], and facilitating FOXO1-mediated repression of AR transcriptional activity [12]. (C) Finally, taxanes may inhibit the expression of antiapoptotic Bcl-2, favoring apoptotic cell death through the relief of BAX-mediated cytochrome c (cyt. C) release [14]. Abbreviation: CASP, caspase.

Approximately half of patients with CRPC initially respond to docetaxel treatment, but, unfortunately, tumors inevitably lose their sensitivity to docetaxel treatment. Therefore, many studies were initiated to identify factors that influence docetaxel sensitivity and facilitate docetaxel resistance (e.g., by proteomic analysis of docetaxel-sensitive and docetaxel-resistant prostate cancer cells [15]). Identified mechanisms involved in docetaxel resistance include alterations in

Box 2. The Proper Timing of Taxane Treatment

Circadian (i.e., 24-h) rhythms regulate a range of cellular functions. Indeed, clock-controlled genes have been shown to influence several cancer-related pathways (e.g., cell cycle, DNA repair, and apoptosis) as well as proteins involved in drug metabolism (e.g., transport, bioactivation, and elimination) [85]. Moreover, the expression of drug targets and proteins involved in chemotherapy resistance may oscillate throughout the day. Based on this, circadian rhythmicity may largely define antitumor response and adverse effects. Therefore, the proper timing of chemotherapeutic treatment is key and is also referred to as 'chronotherapeutics'.

Importantly, the expression and activity of one of the targets of docetaxel, the AR, is regulated by period circadian protein homolog 1 (PER1) [86]. Hence, timing of treatment may benefit the antitumor activity of docetaxel. In addition, misalignment of circadian rhythms in the tumor tissue from those in the rest of the body, which is often the case in prostate cancer, can also be used to improve docetaxel tolerability. Thus, at certain times of the day, activity of docetaxel targets may be high in tumor tissue but relatively low in healthy tissue. Furthermore, proteins that prevent toxicity in healthy tissues (e.g. P-gp) oscillate over time [87]. Optimal docetaxel tolerance was identified to occur 7–11 h after light onset, which coincides with the time of most potent antitumor activity [88]. Based on these findings, the proper timing of treatment may result in a substantial increase in the therapeutic index of docetaxel in patients with advanced prostate cancer. It is important to note that the circadian rhythms of tumors and healthy tissues may differ between individual patients, highlighting the need for personalized timing of treatment.

the docetaxel target beta-tubulin [15], differential expression of apoptosis modulators [16], altered activity of drug influx and efflux pumps [17,18], enhanced GR expression [19], differential expression of AR splice variants [20–23], and the selection of, or enrichment for, resistant subpopulations of cells [24,25]. Here, we discuss the involvement and potential therapeutic application of these main mechanisms (Table 1).

Recovering Docetaxel Sensitivity through the Reversal of Antiapoptotic Processes

Apoptosis is the tightly controlled process of programmed cell death that is characterized by distinct morphological changes, including membrane blebbing, cell shrinkage, and nuclear fragmentation [18,19]. Apoptosis can be triggered by either the intrinsic or the extrinsic apoptotic pathway, ultimately leading to the cleavage and activation of a group of effector enzymes, the so-called 'caspases'. Apoptosis can be induced by a range of apoptotic stimuli, including taxanes, and the balance between pro- and antiapoptotic factors generally determines the cellular fate of tumor cells (i.e., survival versus programmed cell death). Consequently, the downregulation of proapoptotic proteins or upregulation of antiapoptotic proteins is a dominant strategy of tumor cells to avoid treatment-induced cell death, thus leading to resistance to chemotherapeutic drugs.

A key step in the intrinsic apoptotic pathway is the release of cytochrome c from the mitochondria to the cytoplasm, which leads to the formation of the apoptosome that triggers caspase cleavage. The altered expression of several factors influencing mitochondrial release of cytochrome c was described in docetaxel-resistant prostate cancer cells. For instance, expression of proapoptotic Bax, a main component of the mitochondrial pore involved in cytochrome c release, was downregulated in docetaxel-resistant cells [18]. Conversely, antiapoptotic proteins Bcl-2 and Bcl-xL [18], known to neutralize the apoptosis-inducing activities of BAX, were found to be overexpressed in docetaxel-resistant cells [19]. The expression of IκBα, a recently established antiapoptotic protein, was enhanced in docetaxel-resistant prostate cancer cells [18] and protein knockdown significantly sensitized prostate cancer cells to docetaxel treatment [26]. IκBα was shown to strengthen the antiapoptotic interaction between VDAC and hexokinase II at the outer mitochondrial membrane [26], thereby preventing VDAC-mediated cytochrome c release and, thus, heightening the threshold for apoptosis induction by docetaxel. After cytochrome c release through the intrinsic apoptotic pathway or after caspase cleavage as a consequence of extrinsic apoptotic pathway activity, a group of antiapoptotic proteins known

Table 1. Therapeutic Strategies to Overcome Docetaxel Resistance in Prostate Cancer

Approach	Result	Stage of Development	Refs
Antagonizing activity of antiapoptotic proteins	Strong resensitization of docetaxel-resistant cells upon Bcl-2/Bcl-xL antagonism; synergistic antitumor efficacy of Mcl-1 antagonist and docetaxel *in vivo*; synergistic antitumor efficacy of survivin antagonism and docetaxel *in vivo*	Preclinical	[27,29,30]
Blocking ABC transporter activity	Robust resensitization to docetaxel in docetaxel-resistant cells *in vitro* upon P-gp inhibition	Preclinical	[34,35]
Blocking GR axis	Complete resensitization to docetaxel in docetaxel-resistant cells *in vitro* upon GR inhibition	Preclinical	[19]
Targeting mesenchymal cells	Partial resensitization to docetaxel in docetaxel-resistant cells *in vitro* upon ZEB1 antagonism	Preclinical	[50]
Targeting CSCs	Complete blockage of xenograft growth upon docetaxel treatment in combination with NOTCH and Hedgehog inhibition; delayed xenograft growth upon treatment with docetaxel and NOTCH inhibition	Preclinical	[55,58]

as inhibitors of apoptosis (e.g., XIAP, cIAP, and survivin) is able to neutralize caspase activity and, thus, halt apoptotic cell death. The expression and activity of inhibitors of apoptosis may also determine the apoptotic response to taxane-based chemotherapy.

Given that many antiapoptotic proteins are causally involved in prostate cancer docetaxel resistance, it is crucial to determine whether these potential targets can be therapeutically restrained to enhance or regain sensitivity to docetaxel. Few preclinical studies have addressed this objective either *in vitro* or *in vivo*. Small molecules that disrupt the antiapoptotic interaction of Bcl-2/Bcl-xL with BAX/BAK (ABT-263 and ABT-737) were shown to enhance the antitumor efficacy of docetaxel in sensitive prostate cancer cell lines [27,28] and restored docetaxel sensitivity in docetaxel-resistant cells [19,27]. In addition, therapeutic targeting of other anti-apoptotic proteins through selective inhibition of Mcl-1 with sabutoclax [29] or downregulation of survivin with rapamycin [30], resulted in enhanced docetaxel sensitivity in prostate cancer cells. These studies encourage further exploration with antagonists of antiapoptotic proteins in models of prostate cancer docetaxel resistance. Interestingly, a Phase II clinical trial evaluated the effectiveness of docetaxel in combination with LY2181308, an antisense oligonucleotide against the antiapoptotic protein survivin, but failed to demonstrate enhanced antitumor activity [31]. Interestingly, docetaxel-resistant cells may be targeted even if a molecule of interest is not a cause of resistance. Recently, PIAS1 was found to be overexpressed in local and metastatic prostate cancer and its expression was further elevated in tumors after docetaxel treatment as well as in docetaxel-resistant cells [32]. PIAS1 knockdown led to the increased expression of tumor suppressor p21 and declined expression of antiapoptotic protein Mcl1, which caused diminished cell proliferation and tumor growth *in vitro* and *in vivo*. Therefore, it appears that PIAS1 is crucial for docetaxel-resistant prostate cancer cell survival and may be a promising new target for the treatment of primary, metastatic, and docetaxel-resistant prostate cancer.

Bypassing ABC Transporter-Mediated Docetaxel Resistance

To exert potent antitumor efficacy, it is vital to obtain appropriate intracellular drug concentrations in tumor cells. Intracellular docetaxel is dependent on the ratio of drug influx and efflux pumps (the ABC transporters) and their expression was shown to correlate with sensitivity to chemotherapeutic drugs [17]. The most widely studied ABC transporter in the context of CRPC is **P-glycoprotein** (P-gp; also known as ABC-B1 and MDR1), which displays a high affinity for docetaxel and, therefore, can rapidly and efficiently reduce intracellular docetaxel levels to prevent their cytotoxic activities. Clinically, exosomal P-gp levels were found increased in patients with docetaxel-resistant compared with patients with therapy-naïve prostate cancer [33]. Inhibition or silencing of not only P-gp [34,35], but also of the efflux pumps MRP1 and LRP [35], enhanced the antitumor efficacy of docetaxel in primary prostate cancer and in prostate cancer cell lines. Interestingly, AR-targeting drugs that are clinically used in prostate cancer treatment (i.e., abiraterone acetate and enzalutamide) also display inhibitory actions on the P-gp-mediated efflux of taxanes and are thereby able to (re-)sensitize prostate cancer cells for docetaxel treatment [36], endorsing the combination treatment of AR-targeting agents and docetaxel in P-gp-overexpressing prostate cancer cells.

To circumvent ABC transporter-mediated efflux, cabazitaxel has been developed, which is a drug that displays decreased affinity for P-gp [37] and ABC-C4 [38]. This renders cells unable to efficiently facilitate the efflux of this chemotherapeutic agent. Consequently, cabazitaxel demonstrates potent antitumor activity in cells with intrinsic or acquired docetaxel resistance [39] and in patients with docetaxel-refractory CRPC [7]. In addition, it efficiently inhibits the growth of prostate cancer cells resistant to AR-targeting agents [40] and displays antitumor activity in patients with CRPC treated with AR-targeting agents following docetaxel treatment [13,41]. These findings suggest that cross-resistance between commonly used therapeutic agents and cabazitaxel is only limited.

Reversing Docetaxel Resistance through Inhibition of the GR Axis

Glucocorticoids (GC) are steroidal hormones that bind to the **GR** and are regularly used in combination with docetaxel in the treatment of CRPC [42]. The rationales for GC use are plentiful. First, GCs diminish the secretion of adrenocorticotropic hormone by the pituitary, leading to decreased release of (protumorigenic) adrenal androgens. Second, GCs are commonly used as antiemetics during chemotherapy to prevent nausea and vomiting. Third, GCs have strong anti-inflammatory activities that may help reduce pain from distant metastases. Fourth, GCs silence tumor-promoting inflammation and angiogenesis, and, finally, GCs can be directly cytotoxic to prostate cancer cells [43]. Clearly, GC treatment has a range of benefits for patients with CRPC, although recent studies have also indicated adverse effects. Enhanced GR activity (by either GC exposure or stress induction) [44,45] or overexpression [19] are both associated with resistance to taxane-based drugs. Active GR signaling has been shown to induce an upregulation of antiapoptotic proteins [46], thereby preventing apoptosis and, thus, facilitating resistance to docetaxel. Hence, GC use in advanced prostate cancer should approached with caution, although, in this respect, it is reassuring that a recent meta-analysis showed similar overall survival in patients with CRPC treated with docetaxel plus prednisone compared with docetaxel without prednisone [47]. This suggests that the potential tumor-promoting effects of GC (induction of therapy resistance) do not outweigh the antitumor and antiemetic effects in CRPC.

Targeting of Epithelial Plasticity and Cancer Stem Cells in Docetaxel Resistance

Epithelial plasticity, in particular the **epithelial–mesenchymal transition** (EMT), is a dynamic process in which sessile epithelial cells switch to motile mesenchyme-like cells. Although this reversible phenotypic process is a major determinant in the metastatic behavior of tumor cells, it was also recently described to modulate cellular responses the chemotherapeutic drugs [24]. Treatment with docetaxel induces the expression of EMT inducers, such as TWIST1, SNAI1, and SNAI2, which stimulates the acquisition of a mesenchymal phenotype [48] and, in this way, docetaxel may in fact stimulate the metastatic spread of prostate cancer cells. Prostate cancer cells with acquired resistance to docetaxel were shown to diminish expression of epithelial markers (i.e., CDH1, CTNNB1, and E-cadherin), while mesenchymal traits (i.e., vimentin and ZEB1) were promoted [48,49]. ZEB1 knockdown was shown to restore the initial epithelial phenotype [48,50] and was accompanied by recovered docetaxel sensitivity [48]. In addition, therapeutic targeting of ZEB1 with the inhibitor mocetinostat potentiated docetaxel treatment of CRPC cells [50]. One of the hypotheses for the lack of long-term curative effects of current chemotherapeutic treatments for CRPC may lie in the predominant targeting of more differentiated, highly proliferative prostate cancer cells, while leaving the 'root of prostate cancer', the **cancer stem-like cells** or CSCs, largely unaffected. In a variety of solid cancers, including prostate cancer, cells that survive chemotherapy and radiation therapy showed an increased number of cancer cells with stem-like characteristics and features of an EMT [48,49,51,52]. In line with these data, Wnt and Notch signaling were shown to confer resistance of prostate CSCs to radiation [53] and docetaxel [54,55]. More recently, ALDH1A1 expression was also found to be correlated with resistance to radiation therapy and EMT in prostate cancer [56,57].

Recently, the importance of CSCs was established in many processes throughout prostate tumorigenesis [25]. In addition to their role in survival, growth, invasion, and metastasis, the involvement of CSCs in mediating chemotherapy and radiation resistance is increasingly being recognized. Treatment with docetaxel was shown to specifically select for a subpopulation of cells that exhibit a CSC-like phenotype and display enhanced resistance to docetaxel [55]. As a result, cell lines with acquired docetaxel resistance are strongly enriched for the prostate CSC marker $CD44^{HIGH}$ [48,49,55] and display enhanced activity of CSC-associated pathways (e.g., NOTCH [53,55,58,59] and Hedgehog [55]). Knockdown of factors that drive prostate CSC (i.e. NOTCH1 [59], BMI-1 [60], EpCAM [61], and TR4 [62]) strongly sensitizes prostate cancer cell lines for docetaxel treatment. Interestingly, therapeutic targeting of NOTCH with PF-03084014

or dibenzazepine and antagonizing Hedgehog signaling with cyclopamine, as well as combined NOTCH and hedgehog inhibition, strongly decreased the CSC subpopulation and thereby largely restored sensitivity to docetaxel treatment both *in vitro* and *in vivo* [55,58].

Targeted Drug Delivery

Over the past few decades, extensive research has focused on targeted drug delivery systems with the purpose of improving current anticancer drugs. Targeted drug delivery comprises the use of nanomedicinal systems that are designed to preferentially and efficiently deliver a drug payload to the site of disease (i.e., the tumor microenvironment), with the aim of increasing the therapeutic index. The use of targeted drug delivery systems also avoids the use of excipients to solubilize taxanes (e.g., Cremophor in Taxol and Tween-80 in Taxotere), thereby effectively preventing excipient-associated toxicity [63].

In prostate cancer studies, multiple nanomedicinal systems have been used, and these encompass drug-containing nanoparticles based on liposomes, polymeric micelles, and proteins. While some formulations merely provide solubilization (e.g., Abraxane and Cellax), other nanocarrier systems share the property of prolonged circulation kinetics (due to reduced clearance from the circulation) and increased tumor accumulation. The latter is predominantly mediated by the increased permeability of the tumor vasculature and most passive targeting systems are dependent on this so-called '**enhanced permeability and retention**' (EPR) effect [64]. Recently, distinct nanomedicinal strategies were pursued to investigate their utility for targeted delivery of taxanes in prostate cancer models (Table 2). For these approaches, a clear distinction should be made between nontargeting and tumor-targeting nanomedicines.

The first class of (nontargeting) formulations includes docetaxel-carboxymethylcellulose nanoparticles (Cellax) [65,66] and albumin-bound paclitaxel (Abraxane or nab-paclitaxel) [67]. These

Table 2. Overview of Nanomedicinal Strategies Used to Improve Taxane Treatment in Prostate Cancer

Formulation	Xenograft Model and Dose[a]	Principal Findings	Stage of Development	Refs
Albumin-bound paclitaxel (Abraxane)	SC: 30 mg/kg/day	Improved tolerability and increased antitumor efficacy *in vivo*, PSA responses (95%) in neoadjuvant treatment setting	Phase II	[67,69]
Docetaxel-carboxymethylcellulose nanoparticle (Cellax)	SC and intrabone: 170 mg/kg single dose	Enhanced antitumor efficacy and reduced weight loss *in vivo*	Preclinical	[65,66]
Micellar paclitaxel	SC: 10–20 mg/kg	Enhanced antitumor efficacy *in vivo*	Preclinical	[71,72]
Micellar docetaxel	SC: 15 mg/kg every 2 days for three doses	Enhanced antitumor efficacy and reduced weight loss *in vivo*	Preclinical	[70]
Liposomal docetaxel	–	Tolerability at 85–90 mg/m^2 in patients with solid tumors; increased docetaxel exposure upon liposomal encapsulation	Phase I	[73,74]
PSMA-targeted paclitaxel micelles	SC: 15 mg/kg every 2–3 days for four doses	Enhanced uptake and cytotoxicity in 22Rv1 cells, enhanced antitumor efficacy *in vivo*	Preclinical	[76]
	–	Enhanced uptake and cytotoxicity in LNCaP cells *in vitro*	Preclinical	[75]
PSMA-targeted docetaxel nanoparticles	SC: 5 mg/kg/every 4 days for four doses	Enhanced antitumor efficacy and reduced weight loss *in vivo*	Phase I	[77]

[a]Abbreviation: SC, subcutaneous.

formulations mainly provide solubilization and enhanced tolerability (e.g., 170 mg/kg for Cellax compared with 25 mg/kg for conventional docetaxel), but do not display tumor-targeting properties. The enhanced tolerability does allow administration of intensified doses, which could subsequently result in enhanced antitumor efficacy [65–67]. Abraxane is already clinically approved for the treatment of breast cancer (after superior efficacy and a favorable safety profile were demonstrated in a Phase III trial) [68] and, in prostate cancer, the administration of Abraxane as a neoadjuvant treatment (before prostatectomy) resulted in frequent declines in the serum levels of biomarker **prostate-specific antigen** (PSA) (95%) [69]. Despite this, the clinical development of Abraxane in prostate cancer was halted after Phase II clinical trials.

The second class of formulations exhibits tumor-targeting properties, and these can be further divided into passive and active targeting nanomedicines. The rationale behind passive targeting approaches is a combination of increased tumor localization (site-specific delivery) [70–72] and decreased adverse effects (site-avoidance delivery) [70]. Micellar formulations of docetaxel [70] and paclitaxel [71,72] yielded enhanced therapeutic efficacy in preclinical prostate cancer models, but were not further explored in a clinical setting. Liposome-encapsulated docetaxel was assessed in a clinical (Phase I) study in patients with advanced solid tumors [73,74]. These clinical studies reported favourable pharmacokinetics of liposomal docetaxel, acceptable tolerability (less edema and cumulative neuropathy), and hints of clinical activity. Phase II clinical studies are needed to determine the potential benefit of liposomal docetaxel in patients with advanced prostate cancer.

In addition to the above-mentioned strategies, a range of studies used 'active targeting' approaches, by coupling a targeting moiety to the outer surface of the nanoparticles to facilitate tumor localization and uptake. The **prostate-specific membrane antigen** (PSMA) provides a suitable target in prostate cancer, because it is often overexpressed in these tumors. PSMA-targeted systems display enhanced cellular uptake by PSMA-positive prostate cancer cells *in vitro* [75,76] and enhanced *in vivo* antitumor efficacy in prostate cancer xenografts [76,77]. In clinical studies, PSMA-targeted docetaxel-loaded nanoparticles exhibited improved pharmacokinetics in human subjects; approximately 100-fold higher plasma concentration of docetaxel at 12–24 h post-injection in the PSMA formulation compared with free docetaxel [77]. A Phase II clinical trial with this formulation in metastatic CRPC with the aim of determining safety and efficacy is currently ongoing (NCT01812746).

Personalized Medicine Based on Adequate Prediction of Taxane Antitumor Activity and Toxicity in Individual Patients

Approximately 55% of patients with CRPC are unresponsive to docetaxel treatment [4] and, despite the risks associated with taxane treatment, docetaxel is still routinely given to almost all patients with CRPC. This clearly stresses the need for robust predictive markers for docetaxel response to stratify patients who will benefit from this chemotherapeutic agent, and to provide an alternative treatment modality for (predicted) nonresponders. Over the past few years, research has focused on **circulating tumor cells** (CTCs) and the presence of CTCs was shown to predict overall survival in patients with metastatic prostate cancer [78]. CTCs are cells that have shed from the tumor and circulate in the bloodstream. To date, several assays and devices have been developed to detect, analyze, and isolate CTCs from the blood, providing a method to monitor therapy response in prostate cancer [79]. The level of CTCs after three courses of docetaxel treatment was shown to predict overall survival of patients with CRPC. Patients with <5 CTCs/7.5 ml blood had an overall survival of 25.0 months on docetaxel treatment compared with 10.5 months overall survival in patients with >5 CTCs/7.5 ml blood [80]. The predictive value of pretreatment CTC levels as a measure for docetaxel response has not yet been addressed.

Prostate cancer cells can be further characterized (e.g., for the expression of AR splice variants) and this information may be used to optimize treatment selection. Recently, AR splice variants

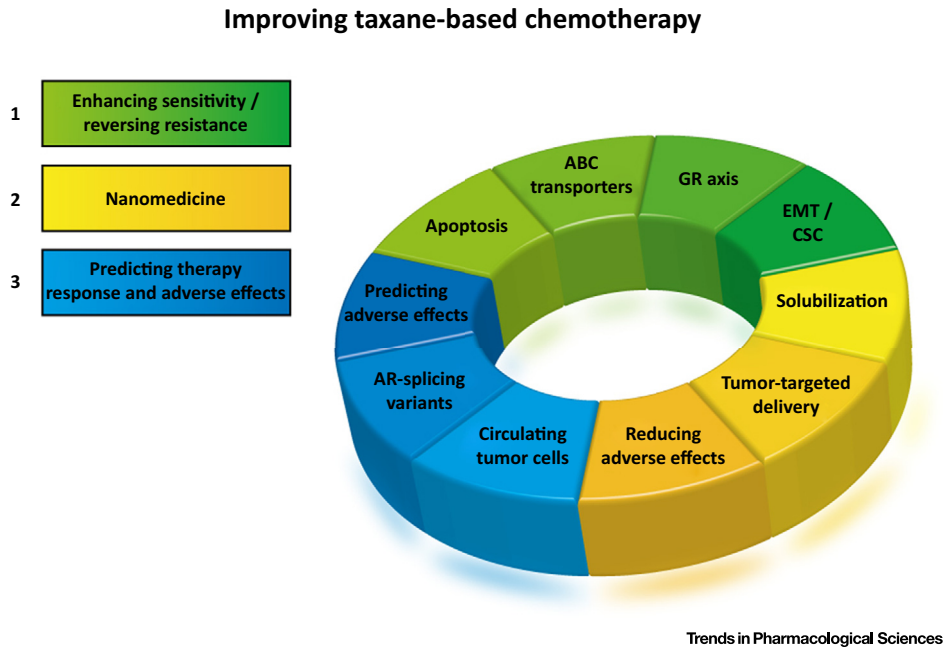

Figure 2. Strategies to Improve Taxane-Based Chemotherapy. The different strategies to improve taxane-based chemotherapy encompass: (1) increasing the sensitivity and/or reversal of resistance; (2) the use of nanomedicine; and (3) the prediction of therapy response and toxicity. Abbreviations: AR, androgen receptor; CSC, cancer stem cells; EMT, epithelium–mesenchymal transition; GR, glucocorticoid receptor.

Outstanding Questions

Recent preclinical studies indicate great therapeutic potential for the reversal of taxane-resistance mechanisms. Can these therapeutic strategies also be applied in clinical practice?

Nanomedicine was shown to have the potential to enhance antitumor efficacy and reduce adverse effects of taxanes. Do preclinical model systems adequately represent the actual human situation (i.e., enhanced permeability and retention effects, interstitial fluid pressure, and microenvironment)?

To stimulate clinical translation of nanomedicine, it is vital to carefully address the toxicity of both excipient and drug-loaded vehicles. Does the excipient carrier on its own induce toxicity?

Does nanomedicinal encapsulation of taxanes change the toxicity profile of the drug by inducing altered tissue distribution, thereby potentially giving rise to unexpected toxicities?

were identified that display constitutive active AR signaling in a ligand-independent matter [81]. Importantly, prostate tumors expressing the AR-V7 splice variant appear to be unresponsive to AR-targeting drugs [82]. Taxanes have also been described to (partially) exert their antitumor activity via interference with AR signaling, and preclinical studies suggest that expression of AR-V7 diminishes the antitumor activity of docetaxel [20,21]. It was found that the clinically relevant AR splice variant ARv7 differentially associates with microtubules and the dynein motor protein, thereby resulting in decreased taxane sensitivity *in vitro* and *in vivo*. Therefore, it may be that AR variants in CRPC cells utilize distinct pathways of nuclear import that affect the antitumor efficacy of taxanes, suggesting a mechanistic rationale to customize treatments for patients with CRPC, which might improve outcomes [20].

However, the exact role of AR variants such as ARv7 in taxane resistance has remained elusive since clinical studies revealed similar response rates upon taxane treatment in patients with AR-V7-positive and AR-V7-negative CTCs [22,23]. Given that taxanes still result in clinical responses in patients with AR-V7-positive CTCs, AR-V7 screening may be a valuable tool for treatment selection (Figure 2).

Concluding Remarks

Although the introduction of taxanes in the clinic for the treatment of CRCP has led to advanced overall survival, many challenges remain to improve its clinical utility (see Outstanding Questions). A persisting obstacle is the ability of tumors to acquire resistance, but the exposure to docetaxel may also introduce additional risks, because it yields more aggressive (highly metastatic) cells. Despite the identification of targetable mechanisms of resistance, these efforts were arrested in a preclinical stage. To further evolve these strategies, future efforts should focus on toxicology and pharmacokinetic studies in animals, thereby paving the way for further clinical investigation in patients with docetaxel-refractory CRPC.

Nanomedicine research in prostate cancer routinely addresses the critical balance between antitumor efficacy and adverse effects (see Outstanding Questions). Typically, a reduction in adverse effects was observed as a result of nanomedicinal formulation of taxanes, permitting intensified treatment. In combination with an enhanced antitumor activity (due to increased maximum tolerated dose allowing the administration of higher doses), this can lead to an increased therapeutic index obtained by nanomedicinal encapsulation of taxanes. To date, few taxane nanomedicine formulations have been introduced in the clinical setting. However, the clinical evidence obtained strongly hints at therapeutic superiority over the conventional taxane formulations, thus encouraging further efforts to develop nanomedical drug delivery systems for the treatment of patients with advanced prostate cancer.

It is crucial to stratify patients who are eligible for novel and existing therapeutic agents and protocols (see Outstanding Questions). This is especially important because cross-resistance between chemotherapeutic drugs and AR-targeting drugs have been described. Therefore, first-line treatment may undermine subsequent second-line treatment and this calls for careful consideration of the sequence of treatments in CRPC [83,84]. The identification of reliable, easy-to-use predictive markers will facilitate personalized treatment approaches (i.e., administration of docetaxel if predicted to be effective; and enzalutamide, abiraterone acetate, or cabazitaxel as alternative treatment options).

In conclusion, a broad range of strategies has been described that may lead to improvement of taxane-based chemotherapy in CRPC. Further identification of proteins involved in taxane resistance, the development of efficient and well-tolerated tumor-targeted nanomedicines, and the establishment of appropriate biomarkers are expected to become of great benefit to patients with CRPC.

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