Overcoming docetaxel resistance in prostate cancer: a perspective review

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Abstract: The treatment of metastatic castrate-resistant prostate cancer has been historically challenging, with few therapeutic successes. Docetaxel was the first cytotoxic therapy associated with a survival benefit in castrate-resistant prostate cancer. Toxicity is typical of other cytotoxic agents, with myelosuppression being the dose-limiting toxicity and neurotoxicity also a notable side effect for some patients. Unfortunately, a significant proportion of men with castrate-resistant prostate cancer will not respond to docetaxel-based therapy and all patients will ultimately develop resistance. Because it is an effective therapy, docetaxel is likely to remain an important part of the treatment arsenal against metastatic prostate cancer for the foreseeable future, despite its toxicities and limitations. Overcoming docetaxel resistance has been a challenge since docetaxel was first established as front-line therapy for metastatic castrate-resistant prostate cancer. Recent studies have shown that several new drugs, including cabazitaxel and abiraterone, are effective after docetaxel failure, dramatically changing the therapeutic landscape for these patients. In addition, a greater understanding of the mechanisms underlying docetaxel resistance has led to several new treatment approaches which hold promise for the future. This review will discuss recent therapeutic advances in metastatic castrate-resistant prostate cancer as well as ongoing clinical trials.

Keywords: abiraterone, Alpharadin, cabazitaxel, docetaxel, MDV3100, prostate cancer, TAK-700, taxane resistance, tubulin

Background

Prostate cancer remains the most common non-cutaneous malignancy and the second leading cause of cancer death in American men [Jemal *et al.* 2010]. While many men present with localized and potentially curable disease, the large numbers of deaths from prostate cancer are driven by the development of metastatic disease. Unfortunately, there are no curative options for metastatic prostate cancer and treatment of metastatic disease remains a significant public health burden. The initial treatment of choice for metastatic prostate cancer is androgen deprivation. While prostate cancer typically responds to the initiation of hormonal therapy, resistance is inevitable, leading to a state termed castrate-resistant prostate cancer (CRPC). Since its approval by the US Food and Drug Administration (FDA) in 2004, docetaxel has been a mainstay of therapy for patients with metastatic CRPC (mCRPC). However, resistance to docetaxel is a significant clinical problem given that half of patients do not respond to therapy [Petrylak *et al.* 2004; Tannock *et al.* 2004]. Moreover, even patients who initially respond to therapy will ultimately develop resistance. Improving treatment outcomes for patients with docetaxel resistance is a high priority because of the limited number of treatment options historically available to this group of patients. This review will discuss recent advances and future directions in the treatment of docetaxel-resistant prostate cancer.

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Therapeutic advances in castrate-resistant prostate cancer: historical overview

Until recently, there were few therapeutic options available for patients with mCRPC. Historically, prostate cancer was considered relatively refractory to cytotoxic therapy, with disappointing results

reported in multiple phase II studies. Early reviews of these clinical trials found that the objective response rate for the majority of cytotoxic agents studied was less than 10% [Eisenberger *et al.* 1985; Yagoda and Petrylak, 1993]. At that time, the only cytotoxic drug approved for CRPC was estramustine, an estradiol/nitrogen mustard complex which was associated with an objective response rate of approximately 30% when used as a single agent [Eisenberger *et al.* 1985]. Although estramustine was designed as an alkylating agent, it also exerts cytotoxic effects by binding tubulin and affecting microtubule dynamics [Dahllof *et al.* 1993; Laing *et al.* 1997; Panda *et al.* 1997; Stearns and Tew, 1985; Tew *et al.* 1983]. Usage of this agent has since waned because of its association with thromboembolic events [Lubiniecki *et al.* 2004] and the advent of more effective therapy.

After the FDA approval of estramustine in 1981, no new drugs showed sufficient activity to merit an indication in prostate cancer until the approval of mitoxantrone in 1996. Mitoxantrone is an anthracenedione structurally related to anthracyclines, such as doxorubicin. Its mechanism of action is attributed primarily to stabilization of type II topoisomerase–DNA complexes and generation of DNA strand breaks [Faulds *et al.* 1991; Nitiss, 2009]. The approval of mitoxantrone was based on a notable phase III trial conducted by Tannock and colleagues and reported in 1996. A total of 161 patients with symptomatic mCRPC were randomized to receive mitoxantrone 12 mg/m^2 plus 10 mg prednisone daily or prednisone alone [Tannock *et al.* 1996]. The key finding in the study was the effectiveness of mitoxantrone and prednisone to palliate cancer-related pain compared with prednisone alone. Palliation of pain symptoms was more likely (29% *versus* 12%, $p = 0.01$), and the duration of palliation was significantly longer in the mitoxantrone arm (43 weeks *versus* 18 weeks, $p < 0.0001$). These effects on palliative endpoints were confirmed in a subsequent study performed by the Cancer and Leukemia Group B [Kantoff *et al.* 1999] in which 242 patients with CRPC received mitoxantrone (14 mg/m^2) plus hydrocortisone (40 mg daily) or hydrocortisone alone. Of note, no survival benefit was seen in either of these studies. In a follow-up phase III study conducted in a population of asymptomatic patients, mitoxantrone similarly delayed progression without demonstrating a survival benefit [Berry *et al.* 2002].

Given this landscape, the finding that docetaxel improved survival for patients with metastatic prostate cancer was a major advance. Two landmark phase III trials established the role of docetaxel as a first-line agent in mCRPC [Petrylak *et al.* 2004; Tannock *et al.* 2004]. In the TAX-327 trial, Tannock and colleagues compared two docetaxel-based treatment arms to standard therapy with mitoxantrone and prednisone. A total of 1006 patients were randomized to treatment with weekly docetaxel (30 mg/m^2), docetaxel every 3 weeks (75 mg/m^2), or mitoxantrone (12 mg/m^2). All patients received prednisone 5 mg twice daily. Although both docetaxel arms were associated with better prostate-specific antigen (PSA) and pain response compared with mitoxantrone, a survival benefit was only seen for docetaxel given every 3 weeks [median overall survival (OS) 18.9 months *versus* 16.5 months for mitoxantrone arm, $p = 0.009$]. Results were confirmed when results were updated in 2008 (median OS 19.2 months for docetaxel every 3 weeks *versus* 16.3 months for mitoxantrone arm, $p = 0.004$) [Berthold *et al.* 2008].

A Southwest Oncology Group trial, S9916, reported similar findings comparing a docetaxel/estramustine combination therapy arm to mitoxantrone/ prednisone [Petrylak *et al.* 2004]. This study randomized 770 men to one of two treatment arms: docetaxel 60 mg/m^2 every three weeks plus estramustine 280 mg three times daily on days 1–5 of a 21 day cycle; or mitoxantrone 12 mg/m^2 every 3 weeks plus prednisone 5 mg twice daily. An OS benefit was seen in the docetaxel/estramustine arm (median OS 17.5 months *versus* 15.6 months in the mitoxantrone arm, $p = 0.02$). A subsequent trial demonstrated that estramustine did not contribute significantly to the effectiveness of docetaxel in this setting [Machiels *et al.* 2008], confirming the widespread acceptance of docetaxel 75 mg/m^2 every 3 weeks as the standard frontline therapy for patients with CRPC. On the basis of these two studies, TAX 327 and S9916, both demonstrating a survival benefit of docetaxel over mitoxantrone, docetaxel was approved for patients with CRPC in 2004.

Although docetaxel clearly deserves a place in the treatment of patients with CRPC, further examination of the data presented in TAX 327 and S9916 underscores why the treatment of patients with docetaxel resistance remains a pressing

problem [Petrylak *et al.* 2004; Tannock *et al.* 2004]. Although docetaxel was the first cytotoxic agent associated with a survival benefit in CRPC, the reported differences in median OS could still be considered modest (1.9–2.9 months). TAX 327 and S9916 reported on response rates using PSA criteria, assessment of pain, and objective response for patients with measurable disease. The rate of PSA response to docetaxel (proportion of patients with at least 50% decline in baseline PSA) was reported as 45% in TAX 327 and 50% in S9916. An improvement in pain was seen in about one-third of patients, while objective responses to docetaxel-based treatment were seen in only 12–17% of evaluable patients. From these response rates, it is clear that a significant proportion of patients do not respond to docetaxel-based therapy, despite the OS benefit seen in the entire population. In addition, responses to docetaxel are not durable; progression-free survival on docetaxel treatment approaches 0% by 3 years [Petrylak *et al.* 2004]. In other words, clinical resistance to docetaxel eventually emerges, even in patients who initially respond to therapy.

Recent clinical developments in overcoming docetaxel resistance

Docetaxel exerts its cytotoxic activity through the stabilization of microtubules [Cortes and Pazdur, 1995]. Microtubules are filamentous polymers composed of α- and β-tubulin heterodimers. Microtubules are critical for cell division, and it is thought that this role is the primary target of docetaxel action. During mitosis, microtubule polymers rapidly polymerize and depolymerize to orchestrate an orderly separation of chromosomes. Docetaxel binds β-tubulin, stabilizing microtubule structures and poisoning the mitotic spindle apparatus. Cells susceptible to docetaxel-induced cytotoxicity undergo mitotic arrest, and ultimately, apoptosis. Mechanisms of resistance to docetaxel are not completely understood but a significant body of literature has emerged addressing this issue. Known mechanisms of docetaxel resistance include limiting intracellular drug concentration, antagonizing the drug-stabilizing effect on microtubules, and antagonizing or circumventing the cytotoxic effect of taxanes through alternative growth pathways or apoptotic escape [Galletti *et al.* 2007; Seruga *et al.* 2011]. Although not discussed in detail here, these mechanisms are described to highlight how a greater understanding of resistance mechanisms can lead to success in the clinical setting.

One well described chemotherapy-resistance mechanism is mediated through multidrug transporters, which are able to act as efflux pumps that reduce the intracellular concentrations of chemotherapeutic agents. As such, they are associated with a multidrug resistance (MDR) phenotype, resistant to many physically and functionally unrelated chemotherapy agents. Multidrug transporters are membrane proteins belonging to the adenosine triphosphate binding cassette (ABC) family of transporters. There are currently 49 known human ABC transporters, which are divided into eight families [Vasiliou *et al.* 2009]. The best known of these transporters are P-glycoprotein (P-gp), encoded by the *mdr1*/*ABCB1* gene, MDR protein 1 encoded by *MRP1*/*ABCC1*, and breast cancer resistance protein (BCRP) encoded by *mxr*/*ABCG2*. With respect to the clinical relevance of P-gp in prostate cancer, it is known that P-gp is weakly expressed in normal prostate [Fojo *et al.* 1987; Kawai *et al.* 2000] but expression is increased in tumor epithelium and associated with tumor stage and grade [Bhangal *et al.* 2000]. P-gp expression was present in 35% of treatment-naive prostatectomy specimens [Homma *et al.* 2007; Sullivan *et al.* 1998]. P-gp was also expressed by primary prostate cancer cell cultures and associated with resistance to chemotherapy [Sanchez *et al.* 2009]. Recently, *mdr1*/*ABCB1* genetic variation was shown to associate with clinical outcome and toxicity in patients with prostate cancer receiving docetaxel [Sissung *et al.* 2008].

MRP1 and BCRP are associated with the MDR phenotype in cancer cells. Compared with P-gp, MRP1 is more commonly expressed in prostate cancer specimens and cell lines [David-Beabes *et al.* 2000; van Brussel *et al.* 1999, 2001]. In DU145 and PC3 cell lines, MRP1 but not P-gp expression was associated with an induced MDR phenotype [Zalcberg *et al.* 2000]. Expression of MRP1 also correlates with advanced prostate cancer and Gleason score [Bhangal *et al.* 2000; Sullivan *et al.* 1998; van Brussel *et al.* 2001]. BCRP/ABCG2 has also been shown to have a possible role in prostate cancer, with presence of the C421A polymorphism correlated with worsened survival in patients with prostate cancer [Gardner *et al.* 2008]. Phosphorylation of BCRP/ABCG2 by serine/threonine kinase Pim-1 was shown to mediate docetaxel resistance in prostate cancer cell lines [Xie *et al.* 2008]. BCRP/ABCG2 is also recognized as a universal stem cell marker, which is also present in putative prostate stem cells [Ding *et al.* 2010; Mathew *et al.* 2009]. Given

Table 1. Summary of strategies to overcome docetaxel resistance in metastatic castration-resistant prostate cancer.

Drugs		*N*	Clinical benefit	Reference
Bypassing efflux through multidrug transporters				
Cabazitaxel 25 mg/m^2 every 21 days + prednisone 10 mg daily	Phase III	755	Median OS of 15.1 months for cabazitaxel *versus* 12.7 months for MP (p<0.0001) Median PFS of 2.8 months for cabazitaxel *versus* 1.4 months for MP (p<0.0001) PSA RR 39.2% for cabazitaxel *versus* 17.8% for MP (p = 0.0002)	[de Bono *et al.* 2010]
Tubulin targeting				
Ixabepilone 35 mg/m^2 every 21 days	Phase II	86	Median OS 10.4 months for ixabepilone *versus* 9.8 months for MP PSA RR of 17% for ixabepilone *versus* 20% for MP	[Rosenberg *et al.* 2007]
Androgen receptor signaling				
Abiraterone 1000 mg orally every day + prednisone 5 mg orally twice a day	Phase III	1195	Randomized 2:1 to placebo + prednisone 5 mg orally twice a day Median OS 14.8 months for abiraterone *versus* 10.9 months for placebo (p<0.001) Median PFS 5.6 months for abiraterone *versus* 3.6 months for placebo (p<0.001) PSA RR 29% for abiraterone *versus* 6% for placebo (p < 0.001)	[de Bono *et al.* 2011]
TAK-700	Phase III	1083	Randomized, double-blind, placebo-controlled phase III study TAK-700 + prednisone *versus* placebo + prednisone Primary endpoint of study is OS	NCT01193257
MDV3100 160 mg daily	Phase III	1199	Patients randomized 2:1 to MDV3100 160 mg/day *versus* placebo. Median OS 18.4 months for MDV3100 *versus* 13.6 months for placebo (p < 0.0001)	[Scher *et al.* 2012]
Alternative growth pathway signaling				
XL184	Phase III	246	Randomized, double-blind, comparing XL184 and MP Primary endpoint of study is palliation of pain	NCT01522443
Radiopharmaceuticals				
Alpharadin 50 kBq/kg every 4 weeks	Phase III	922	Patients randomized 2:1 to radium-223 or placebo every 4 weeks × 6 58.4% of patients had received prior docetaxel Median OS 14.0 months *versus* 11.2 months (p = 0.00185)	[Parker *et al.* 2011]
Apoptosis targeting				
Custirsen	Phase III	800	Randomized phase III study in chemotherapy-naïve mCRPC Two arms: docetaxel/prednisone or docetaxel/prednisone/custirsen	NCT01188187
	Phase III	292	Randomized, double-blind, placebo-controlled phase III study for patients who have progression after first-line docetaxel, comparing custirsen or placebo in combination with a taxane	NCT01083615

OS, overall survival; MP, mitoxantrone plus prednisone; mCRPC, metastatic castrate-resistant prostate cancer; PFS, progression-free survival; PSA RR, % of patients with prostate-specific antigen declines of ≥50%.

the known role of BCRP/ABCG2 in conferring a MDR phenotype, it has been hypothesized that expression of BCRP/ABCG2 may be responsible for the relative chemoresistance of stem cells.

MRP4/MOATB/ABCC4 is highly expressed in prostate tissue [Lee *et al.* 1998, 2000] and may contribute generally to chemotherapy resistance in prostate cancer. In the prostate, ABCC4 RNA expression is induced by androgens and is suppressed by androgen ablation [Cai *et al.* 2007; Ho *et al.* 2008]. This pattern of androgen regulation is reversed in kidney tissue [Maher *et al.* 2006]. Expression driven by the MRP4/ABCC4 promoter does not appear to respond to testosterone or antiandrogens [Ho *et al.* 2008], suggesting an indirect mechanism of androgen regulation. ABCC4 expression has been reported to affect sensitivity to chemotherapeutic agents such as camptothecins, cyclophosphamide, topotecan, methotrexate, and nucleoside analogues [Chen *et al.* 2002; Leggas *et al.* 2004; Tian *et al.* 2005]; however, there are no data to suggest that docetaxel is a substrate of this ABC transporter, or that MRP4 expression contributes to docetaxel resistance.

Preclinical data uncovering the role of multidrug transporters in chemotherapy resistance led to the development of second-generation taxanes which were designed to have low affinity for P-gp. Unlike docetaxel, which is a known P-gp substrate, these drugs would not be susceptible to MDR through this mechanism, a fact which was borne out in preclinical data showing activity in MDR cell lines. One of these second-generation taxanes, cabazitaxel, was recently reported to have an OS benefit in patients with prostate cancer who have progressed on docetaxel [de Bono *et al.* 2010]. In this randomized phase III trial, 755 men with mCRPC with progressive disease in the post-docetaxel setting were treated with prednisone 10 mg/day and either mitoxantrone (12 mg/m^2 every 3 weeks) or cabazitaxel (25 mg/m^2 every 3 weeks). Patients in the cabazitaxel arm had improvements in overall and progression-free survival. The median OS was 15.1 months in the cabazitaxel arm and 12.7 months in the mitoxantrone arm while the hazard ratio for death in the cabazitaxel arm was 0.70 compared with the mitoxantrone arm ($p < 0.0001$). Median progression-free survival was 2.8 months in the cabazitaxel arm compared with 1.4 months in the mitoxantrone group (hazard ratio 0.74, $p < 0.0001$). Toxicity was notable for significantly more grade 3 neutropenia and diarrhea in the cabazitaxel group and a relatively high toxic death rate of almost 5% in the cabazitaxel arm. The majority of these deaths were attributed to neutropenic sepsis, prompting a recommendation for the use of growth factor support in at-risk patients (older than 65 years, poor performance status, previous episodes of febrile neutropenia, extensive prior radiation ports, poor nutritional status, or other serious comorbidities).

On the basis of these findings, cabazitaxel is indicated for the treatment of patients with mCRPC who have previously been treated with docetaxel, and was approved by the FDA in June 2010. Although these positive findings provide proof of principle that circumventing drug efflux through P-gp can be an effective way to overcome docetaxel resistance for some patients, subset analysis may suggest that cabazitaxel may not be as effective in certain populations of patients. Although a trend for OS benefit was seen in all subgroups analyzed, this benefit was least pronounced in patients who had received the smallest previous cumulative dose of docetaxel. Patients who had received a total docetaxel dose of less than 225 mg/m^2 ($n = 59$) had a hazard ratio for death of only 0.96 [95% confidence interval (CI) 0.49–1.86] compared with a hazard ratio of 0.51 (95% CI 0.33–0.79) for patients who had previously received at least 900 mg/m^2 of docetaxel ($n = 134$). Although it is not clear whether early docetaxel discontinuation occurred because of disease progression, poor tolerability, or some other reason, it is interesting to speculate whether some shared factor may contribute to a relative lack of efficacy of cabazitaxel in patients with early docetaxel failure.

The other line of inquiry that has resulted in recent clinical success in the post-docetaxel setting is the targeting of androgen receptor pathways. Patients with prostate cancer will typically be prescribed docetaxel once progression is documented in the setting of castrate levels of testosterone. Even in this setting, data suggest that androgen receptor signaling remains a critical driver of prostate cancer growth. Mechanisms of resistance in this setting include increased enzymatic synthesis of androgens, an increased sensitivity to androgens by overexpression of the receptor, or mutations in the androgen receptor leading to promiscuous or constitutive activity [Attard *et al.* 2009; Holzbeierlein *et al.* 2004; Montgomery *et al.* 2008; Scher and Sawyers,

2005; Stanbrough *et al.* 2006]. This knowledge has led to the development of novel and more effective methods to target the androgen receptor pathway in men with prostate cancer, such as abiraterone acetate, TAK-700 (orteronel) and MDV3100.

Abiraterone irreversibly antagonizes the cytochrome P450 enzyme CYP17A1, a critical enzyme in testosterone synthesis with 17, 20 lyase and 17-hydroxylase activity [Attard *et al.* 2008, 2011]. CYP17 inhibition blocks androgen biosynthesis within the adrenal gland, testes, and the tumor, thus targeting a known prostate cancer resistance mechanism in men with castrate levels of testosterone. After promising results in early trials, oral administration of abiraterone acetate was recently shown to extend OS in a phase III study [de Bono *et al.* 2011]. A total of 1195 patients with mCRPC who had previously received docetaxel were registered to the study. Patients were randomized in a 2:1 ratio to receive abiraterone or placebo. Both arms received 5 mg of prednisone twice daily. With respect to the primary endpoint, median OS was 14.8 months in the abiraterone arm compared with 10.9 months in the control arm ($p < 0.001$ by log-rank analysis). These results were initially reported in 2010, leading to FDA approval in April 2011, and providing another option for patients in the post-docetaxel setting.

In addition to these phase III results with abiraterone, other strategies of targeting the androgen receptor also appear clinically promising. TAK-700 is also an oral CYP17 antagonist but was designed to have selectivity for 17, 20 lyase over 17-hydroxylase inhibition, theoretically reducing levels of testosterone without decreasing cortisol [Yamaoka *et al.* 2010]. While still unpublished, results from a phase I/II clinical trial of TAK-700 show clinical activity in mCRPC. In the most updated report [Agus *et al.* 2011], 96 patients with chemotherapy-naïve mCRPC were treated with TAK-700 at various doses (from 300 mg twice daily to 600 mg twice daily). Treatment cohorts with and without prednisone were studied. PSA response rates (more than 50% decline in PSA from baseline) in the treatment cohorts ranged from 41% to 63% in each cohort, with a PSA response rate of 63% in the 23 patients who received TAK-700 without prednisone. In addition to these results in chemotherapy-naïve patients, TAK-700 is also being evaluated as a strategy to overcome docetaxel resistance. TAK-700 is currently being evaluated in combination with docetaxel in a phase I/II trial [ClinicalTrials.gov identifier: NCT01084655] as well as in a phase III study for patients who have failed taxane-based therapy [ClinicalTrials.gov identifier: NCT 01193257].

MDV3100 is another agent which is being explored to target the androgen receptor pathway in patients whose condition has failed to respond to docetaxel. MDV3100 is a pure androgen receptor antagonist with higher affinity for the androgen receptor than previous antiandrogens, and the ability to inhibit androgen receptor nuclear translocation, DNA binding, and coactivator recruitment [Tran *et al.* 2009]. Clinical results reported with MDV3100 are notable for a 56% PSA response rate (response defined as more than 50% PSA decline) in a phase I/II study involving 140 patients with CRPC [Scher *et al.* 2010]. Thirty-eight of the responders had previously received chemotherapy (PSA response rate of 51% in patients who had received prior chemotherapy), again providing proof of principle that targeting the androgen receptor pathway is an effective approach to overcoming docetaxel resistance. A randomized placebo-controlled phase III study recently reported positive results for MDV3100 in patients previously treated with docetaxel [Scher *et al.* 2012]. A total of 1199 patients were randomized in a 2:1 fashion to MDV3100 (160 mg orally every day) *versus* placebo. Preliminary analysis indicated a survival benefit for MDV3100 compared with placebo (median OS 18.4 months *versus* 13.6 months, $p < 0.0001$).

Radiopharmaceuticals are another approach to treating patients with mCRPC. Alpharadin (radium-223) targets the sclerotic bone metastases typical in prostate cancer through its calcium-mimetic properties. The radiation released from radium-223 is almost exclusively in the form of α particles, which have a higher energy and shorter range compared with β emissions. The higher energy may facilitate greater cytotoxic effect while the shorter path length tends to spare normal bone marrow. Given the favorable effect on OS observed in a randomized phase II study [Nilsson *et al.* 2007], Alpharadin was tested in a phase III study [ClinicalTrials.gov identifier: NCT00699751]. A total of 922 patients with CRPC and symptomatic bone metastases were randomized in a 2:1 fashion to receive either Alpharadin or intravenous saline injection every 4 weeks for six doses. Patients were

eligible regardless of having received docetaxel previously or not, and were stratified on this basis. While data have not been presented formally, the trial was stopped early because of a positive outcome of the primary endpoint of OS at an interim analysis. A preliminary report indicated that the OS benefit in the experimental arm was 14 months compared with 11.2 months at the interim analysis (p = 0.00185) [Parker *et al.* 2011]. Given what is known about radiopharmaceuticals and Alpharadin in particular, a benefit from Alpharadin in the post-docetaxel setting is anticipated, although subgroup analysis evaluating that question has not yet been reported.

Targeting alternative growth pathways is also a promising strategy in overcoming docetaxel resistance. As an example, cabozantinib (XL184), a dual tyrosine kinase inhibitor of MET and vascular endothelial growth factor receptor 2, was recently described to have significant effects on bone pain and resolution of abnormalities on bone scans in the context of a randomized phase II discontinuation study, even in patients who had previously received docetaxel therapy [Hussain *et al.* 2011]. There were 168 patients enrolled on the prostate arm of this study and improvement in bone scans were reported in 86% (56 of 65) of evaluable patients. Enthusiasm for this approach was tempered somewhat by several toxic deaths attributed to cabozantinib toxicity (6 out of 483 patients) [Gordon *et al.* 2011]. Given the positive results in CRPC, a phase III study in CRPC to further evaluate cabozantinib is planned.

Biomarkers to predict docetaxel response

Complementing the development of therapies that overcome docetaxel resistance is the attempt to predict in advance patients who will not respond to docetaxel. At present, clinicians are urged to wait 12 weeks before determining whether a patient with mCRPC is responding to therapy [Scher *et al.* 2008]. Predicting docetaxel response would potentially identify nonresponders early and spare them ineffective and potentially toxic therapy. A greater understanding of the molecular mechanisms of resistance to docetaxel offers the potential to identify genes that mediate docetaxel response which may explain, and ultimately predict, the heterogeneity of response to docetaxel in patients with mCRPC. One potential biomarker is the expression of βIII-tubulin.

In an attempt to understand taxane resistance, β-tubulin, the intracellular target of taxanes, has surfaced as a relevant resistance mechanism [Galletti *et al.* 2007]. *In vitro* exposure to taxanes often selects for resistant clones that have β-tubulin mutations. While mutations in β-tubulin have been shown to confer resistance to tubulin-binding agents *in vitro*, it is unclear what role β-tubulin mutations play in clinically significant taxane resistance [Berrieman *et al.* 2004]. Expression of different β-tubulin isoforms has also been shown to correlate with taxane resistance. There are seven distinct isotypes of β-tubulin present in humans. Although the functional differences between them are not completely delineated, it has been shown that heterodimers with different tubulin isoforms can affect the dynamic properties of microtubules *in vitro*. Elevations of certain β-tubulin isoforms, especially βIII-tubulin, are associated with taxane resistance.

Elevated βIII-tubulin is correlated with poor response to tubulin-targeting agents as well as poor prognosis in a variety of human malignancies, including lung, breast, ovarian, gastric cancer, and unknown primary [Seve and Dumontet, 2008]. While these findings have not been definitively extended to prostate cancer, a recent report provides initial evidence supporting the hypothesis that expression of βIII-tubulin correlates with outcomes in patients receiving docetaxel-based therapy [Ploussard *et al.* 2010]. In this small study, initial biopsy specimens from 37 patients who subsequently underwent docetaxel chemotherapy were evaluated for βIII-tubulin expression using immunohistochemistry. Median OS was significantly shorter for patients with tumors that stained positive for βIII-tubulin compared with those that stained negative (13.5 *versus* 41.6 months; p = 0.019). A lower PSA response rate (35% *versus* 52%, p = 0.337) and a shorter time to progression (4.7 *versus* 9.8 months, p = 0.522 by log-rank analysis) were also observed in patients with positive βIII-tubulin staining, but these findings were not statistically significant.

These findings may have therapeutic implications as well with regard to finding chemotherapeutics with activity in cells expressing high levels of βIII-tubulin. Ixabepilone is a member of the epothilone family of tubulin-binding agents that have cytotoxic activity in taxane-resistant cell lines. Ixabepilone has also been shown to have activity in cells which overexpress βIII-tubulin, presumably by preferential stabilization of

α/βIII-tubulin microtubule heterodimers [Dumontet *et al.* 2009]. In addition, ixabepilone, which is structurally divergent from the taxanes, may not be a substrate for MDR proteins [Lee and Swain, 2005]. Ixabepilone, which is currently FDA approved for breast cancer and is available to patients in the clinic, is associated with a low response rate in the docetaxel-refractory population, but has comparable activity to the previous standard of care, mitoxantrone, in this population [Rosenberg *et al.* 2007].

Levels of inflammatory cytokines are another class of promising biomarkers associated with docetaxel response in patients with mCRPC. Inflammation has clearly been implicated in prostate carcinogenesis [De Marzo *et al.* 2007], although the relationship between inflammation and chemotherapy response is not as well established. Several studies have evaluated the correlation between inflammatory cytokines and outcomes of patients with mCRPC treated with docetaxel. Docetaxel-induced decreases in interleukin-6 (IL-6) [Domingo-Domenech *et al.* 2006; Ignatoski *et al.* 2009] and macrophage inhibitory cytokine 1 (MIC1) [Zhao *et al.* 2009] have been shown to correlate with PSA response. Decreases in MIC1 also correlated with improved OS [Zhao *et al.* 2009]. However, these studies were small (fewer than 50 patients) and did not determine whether changes in inflammatory markers provided additional information to parameters such as PSA response. In addition, baseline levels of MIC1 did not predict whether patients responded to docetaxel-based therapy [Zhao *et al.* 2009]. IL-6 and C-reactive protein (CRP) seem more promising as true predictive markers, as studies have shown that baseline levels of IL-6 and CRP correlate with PSA response [Beer *et al.* 2008; Domingo-Domenech *et al.* 2006]. However, the correlation between baseline IL-6 levels and PSA response was not confirmed in two subsequent studies [Beer *et al.* 2008; Ignatoski *et al.* 2009]. Further studies will be required before these markers can be used clinically.

Future directions in treating patients after docetaxel failure

This review has focused on factors mediating docetaxel resistance in mCRPC with potential clinical utility, highlighting recent progress in treating patients in the post-docetaxel setting. Greater insights into the molecular pathways that underlie prostate cancer growth and docetaxel resistance have fueled this success. As other docetaxel resistance mechanisms are uncovered [reviewed in Seruga *et al.* 2011], therapeutic strategies targeting these resistance mechanisms will near the clinic. For example, defects in the apoptotic pathway can confer resistance to docetaxel-induced cytotoxicity. Clusterin is a stress-induced protein which inhibits apoptosis and confers treatment resistance in prostate cancer cells [Miyake *et al.* 2000b, 2000c, 2003; Zellweger *et al.* 2003]. Antisense knockdown of this protein has been successfully shown to overcome prostate cancer chemoresistance *in vitro* [Miyake *et al.* 2000a; Sowery *et al.* 2008]. A phase II study with custirsen (OGX-011), an antisense oligonucleotide targeting clusterin, showed activity in combination with chemotherapy in patients with progressive disease following first-line docetaxel [Saad *et al.* 2011]. Promising results were also seen in a randomized phase II trial of docetaxel with or without custirsen in chemotherapy-naïve patients [Chi *et al.* 2010]. There were 82 patients randomized in a 1:1 fashion to either standard treatment with docetaxel/prednisone or an experimental arm of docetaxel and prednisone in combination with weekly custirsen. The combination of docetaxel, prednisone and custirsen was associated with an improvement in OS compared with the control arm (median OS 23.8 months *versus* 16.9 months). Progression-free survival, rates of PSA response, and objective responses were similar between the two arms. Two phase III studies looking at the activity of custirsen both in combination with docetaxel and after docetaxel failure [ClinicalTrials.gov identifiers: NCT01083615 and NCT01188187] are currently underway.

Summary

Docetaxel is likely to remain a mainstay of treatment for men with mCRPC for the foreseeable future. As with many chemotherapeutic agents, the impact of this therapy is limited by clinical resistance. Abiraterone and cabazitaxel are two therapies that have recently been approved by the FDA to treat patients after docetaxel failure. Alpharadin and MDV3100 also improve survival and are pending FDA approval. The clinical success of these strategies stems from a greater understanding of docetaxel-resistance mechanisms. Additional strategies are under clinical evaluation but their efficacy is still undetermined.

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Conflicts of interest statement

The author declares no conflict of interest.

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