Pathways of chemotherapy resistance in castration-resistant prostate cancer

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Abstract

Chemotherapy remains the major treatment option for castration-resistant prostate cancer (CRPC) and limited cytotoxic options are available. Inherent chemotherapy resistance occurs in half of all patients and inevitably develops even in those who initially respond. Docetaxel has been the mainstay of therapy for 6 years, providing a small survival benefit at the cost of significant toxicity. Cabazitaxel is a promising second-line agent; however, it is no less toxic, whereas mitoxantrone provides only symptomatic benefit. Multiple cellular pathways involving apoptosis, inflammation, angiogenesis, signalling intermediaries, drug efflux pumps and tubulin are implicated in the development of chemoresistance. A thorough understanding of these pathways is needed to identify biomarkers that predict chemotherapy resistance with the aim to avoid unwarranted toxicities in patients who will not benefit from treatment. Until recently, the search for predictive biomarkers has been disappointing; however, the recent discovery of macrophage inhibitory cytokine 1 as a marker of chemoresistance may herald a new era of biomarker discovery in CRPC. Understanding the interface between this complex array of chemoresistance pathways rather than their study in isolation will be required to effectively predict response and target the late stages of advanced disease. The pre-clinical evidence for these resistance pathways and their progress through clinical trials as therapeutic targets is reviewed in this study.

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Introduction

Prostate cancer causes substantial morbidity and mortality worldwide (Ferlay et al. 2004) and is the second leading cause of cancer death in men in developed countries (AIHW & AACR 2008, Jemal et al. 2009). Metastatic prostate cancer initially responds to anti-androgen therapy; however, it eventually becomes resistant to hormonal manipulation. Chemotherapy remains the only treatment option in the setting of castration-resistant prostate cancer (CRPC) providing modest survival and palliative benefits. Only half of all patients will respond to docetaxel, a mitotic spindle poison that is the current mainstay of chemotherapy. Docetaxel improves median survival by 2 months at the cost of significant toxicity, particularly in this elderly patient population (Petrylak et al. 2004, Tannock et al. 2004). Mitoxantrone, a DNA intercalator, is less toxic but delivers only palliative benefits (Tannock et al. 1996, Kantoff et al. 1999). Inevitably, resistance to first-line chemotherapy will develop and the disease then becomes difficult to control. Although newer chemotherapeutics such as satraplatin and cabazitaxel have demonstrated activity as second-line agents, survival benefits remain modest with median overall survival just beyond 1 year (Sternberg et al. 2009b, De Bono et al. 2010c).

A thorough understanding of chemoresistance pathways and how they interact would facilitate two important outcomes. Identifying patients who will not benefit from chemotherapy prior to their exposure will avoid unnecessary toxicity and allow them to move on to alternative treatment options. Targets for further drug development may also arise. In this era of personalised cancer therapy, significant treatment
advances have occurred through a better understanding of cytotoxic resistance and the heterogeneity among patients with the same disease. This concept has already proved successful across other cancer streams and is just the beginning of a new paradigm in cancer treatment. Indeed, many of the pathways implicated in prostate cancer chemoresistance may well be applicable to other cancer types.

There is an urgent need to identify markers and mechanisms of drug resistance to personalise treatment and improve survival. In this study, we have reviewed the current data regarding mechanisms of drug resistance in CRPC.

Apoptotic pathways

Clusterin

Clusterin is a glycoprotein whose mRNA is almost ubiquitously expressed in animal tissue. It occurs in two forms: an intracellular truncated nuclear form and a secreted heterodimeric disulphide linked form (sCLU). The nuclear form promotes apoptosis by translocating from the cytoplasm to the nucleus after cytotoxic events (Leskov et al. 2003), whereas the secreted form acts extracellularly to inhibit apoptosis by binding toxic molecules and targeting them for endocytic degradation (Wilson & Easterbrook-Smith 2000). In response to heat shock, sCLU is transcriptionally activated by heat-shock factor-1 and acts as a chaperone and stabilise protein conformations at times of cell stress, similar to the small heat-shock proteins (HSPs; Wilson & Easterbrook-Smith 2000). sCLU also binds a variety of other molecules including Bax, which is normally activated by chemotherapeutic drugs leading to caspase activation and apoptosis, thereby preventing cell death (Zhang et al. 2005a). In prostate cancer cell lines, overexpression of clusterin increases resistance to apoptosis following treatment with chemotherapeutic agents such as taxanes and camptothecin. Furthermore, cytotoxic treatment of cells induces expression of clusterin, suggesting that this is a cytoprotective mechanism (Miyake et al. 2000d, Mizutani et al. 2006, Patterson et al. 2006). Clusterin expression may be regulated by the transcription factor Stat1. In hormone-resistant DU145 cells, Stat1 expression increases with docetaxel treatment, mirroring clusterin expression in these cells. In the docetaxel-resistant line, DU145-DR, inhibition of clusterin expression enhances docetaxel sensitivity (Patterson et al. 2006). In vivo, LNCaP xenografts that overexpress clusterin are less responsive to paclitaxel than controls (Miyake et al. 2000d).

Clinically, sCLU expression is increased in radical prostatectomy specimens from patients who received neoadjuvant androgen ablation and docetaxel compared with those who had no preoperative therapy ($P < 0.001$; Sowery et al. 2008).

Therapeutic anti-sense oligonucleotides (ASO) against clusterin have now been developed. Treatment of prostate cancer cell lines with clusterin ASO alone has no effect; however, it dramatically enhances the cytotoxic effect of chemotherapeutic agents, such as mitoxantrone and taxanes, when used in combination. A reduction in the IC₅₀ of these agents by over 50% is seen even in previously chemoresistant cell lines (Miyake et al. 2000b,d,e, Sowery et al. 2008). These results have been replicated in prostate cancer xenografts with greater reductions in tumour volume and circulating PSA levels when chemotherapy was combined with clusterin ASO compared with chemotherapy alone (Miyake et al. 2000b,d,e, Springate et al. 2005, Sowery et al. 2008). In phase I trials, a clusterin ASO (OGX011) was well tolerated both as a single agent and in combination with weekly docetaxel (Chi et al. 2005a,b; Table 1). In a randomised phase II trial of 82 chemonaı¨ve patients with CRPC where patients received docetaxel and OGX011/placebo, there was little difference in the primary endpoint, PSA response rate. However, there was a strong trend towards a substantial overall survival difference (27.5 vs 16.9 months; $P=0.07$; Chi et al. 2009b). In docetaxel-resistant patients, a phase II study where all patients received OGX011 and were randomised to receive docetaxel or mitoxantrone better PSA response rates were seen in the docetaxel arm (40 vs 27%) and overall survival mirrored this (14.7 vs 11.4 months; Saad et al. 2008). Phase II data of OGX011 in combination with docetaxel appears promising in both chemonaı¨ve and chemoresistant CRPC patients. Phase III studies are now in progress to verify these results.

Heat-shock proteins

HSPs are intracellular molecular chaperones that stabilise damaged proteins following a stressful insult such as heat-shock, hypoxia or cytotoxic therapy, thus inhibiting cell death. HSPs are classified according to their size in kilodaltons (e.g. HSP70 is 70 kDa in size). HSP27 prevents protein precipitation and sequesters their size in kilodaltons (e.g. HSP70 is 70 kDa in size). In docetaxel-resistant patients, a phase II study where all patients received OGX011 and were randomised to receive docetaxel or mitoxantrone better PSA response rates were seen in the docetaxel arm (40 vs 27%) and overall survival mirrored this (14.7 vs 11.4 months; Saad et al. 2008). Phase II data of OGX011 in combination with docetaxel appears promising in both chemonaı¨ve and chemoresistant CRPC patients. Phase III studies are now in progress to verify these results.
Expression by ASO and siRNA significantly enhances paclitaxel-induced apoptosis (Rocchi et al. 2004). A second-generation ASO OGX427 targeting HSP27 has entered phase I clinical trials.

HSP90 chaperones multiple proteins, including many signalling molecules that are important in oncogenesis (e.g. Akt, Raf1, Her2). This interaction prevents protein degradation by the ubiquitin proteosome pathway. HSP90 is overexpressed in prostate cancer tissue compared with normal prostate epithelium and may have a role in the evolution of prostate cancer to hormone resistance (Banerji 2009). 17-AAG, an analogue of geldanamycin, specifically inhibits HSP90 by blocking its ATP binding site. In hormone-resistant prostate cancer cells, 17-AAG prevents the ligand-independent nuclear translocation and activation of androgen receptors suggesting that HSP90 has a role in the development of androgen-independent disease (Saporita et al. 2007). In phase I clinical trials, 17-AAG was well tolerated in combination with docetaxel and paclitaxel (Solit et al. 2005, Musquire et al. 2007, Ramalingam et al. 2008; Table 1). A phase II study with 19 CRPC patients revealed that IPI504 (a water soluble form of 17-AAG) has no single-agent activity (Oh et al. 2009).

HSP70 and 72 have also been implicated in resistance to chemotherapy (Gabai et al. 2005, Ren et al. 2008) in prostate cancer cell lines; however, no therapeutic compounds directed against them are currently in trial. Therapy directed against HSPs has not been successful in early-phase trials; however, the full potential of these targets has not yet been thoroughly explored.

### B-cell leukaemia/lymphoma 2

The protein products of the B-cell leukaemia/lymphoma 2 (Bcl2) gene family regulate apoptosis. Some members are pro-apoptotic (Bax, Bcl-XS, Bad), whereas others inhibit cell death (Bcl2, Bcl-xL, Mcl1). Bcl2, the prototypical member, exerts its anti-apoptotic effects by heterodimerising with pro-apoptotic members of the Bcl2 family (e.g. Bax) leading to their inhibition. This prevents apoptosis by inhibiting mitochondrial cytochrome c release and subsequent activation of the caspase cascade. Bcl2 up-regulation is implicated in the progression to androgen independence in prostate cancer (McDonnell et al. 1992, Raffo et al. 1995). Following treatment with paclitaxel, Bcl2 is phosphorylated in PC3 and LNCaP cells, inhibiting its anti-apoptotic action by preventing heterodimerisation with other members of the Bcl2 family and enhancing cell death (Haldar et al. 1996, Miyake et al. 2000a). Interestingly, DU145 cells, which do not express Bcl2, are inherently resistant to paclitaxel (Haldar et al. 1996). This suggests that the apoptotic action of taxane chemotherapy may rely, in part, on an interaction with Bcl2 rather than being mediated solely by microtubule stabilisation. Yoshino et al. (2006) further supported this theory with the finding that higher Bcl2 expression in prostate cancer tissue at...
baseline was an independent predictor for survival following taxane chemotherapy \((P<0.01)\). Bcl-xL, another anti-apoptotic member, increases after androgen blockade, remains high in androgen-independent prostate cancer and correlates with increasing grade and stage. Forced overexpression of Bcl-xL \textit{in vitro} leads to chemotherapy resistance while downregulation enhances chemosensitivity \cite{Lebedeva2000}. High Mcl1 expression is seen in prostate cancer cell lines, high Gleason grade disease and bone metastases \cite{Krajewska1996, Zhang2010}. \textit{In vitro}, Mcl1 overexpression has been implicated in resistance to cytokine-induced apoptosis and may also be involved in the anti-apoptotic action of interleukin 6 (IL6; Cavarretta \textit{et al.} 2007, Dash \textit{et al.} 2010).

Therapeutic ASOs directed against bcl2 family proteins have been developed and tested in the clinic. In pre-clinical studies, Bcl2 ASO is synergistic with taxane chemotherapy in both cell lines and xenografts \cite{Miyake2000c, Leonetti2007}. The combination of two ASOs directed against Bcl2 and Bcl-xL with paclitaxel appeared to be especially effective \cite{Miyake2000c}. Bispecific ASOs directed against both Bcl2 and Bcl-xL induce caspase activation and apoptosis in prostate cancer cell lines when used alone. Sensitivity to taxanes and mitoxantrone \textit{in vitro} is also enhanced by these bispecific agents \cite{Yamanaka2005, 2006}. Oblimersen (G3139) is an ASO directed against the initiating sequence of Bcl2. Phase I trials of oblimersen demonstrated minimal toxicities both as a single agent and in combination with docetaxel or mitoxantrone \cite{Chi2001, Morris2002, 2005, Marshall2004, Tolcher2004; Table 1}. Disappointing results were seen in an EORTC first-line randomised phase II trial of 115 patients with CRPC given docetaxel with oblimersen or placebo. The end points were not met with a PSA response rate of 37\% in the combination arm compared with 46\% in the standard treatment arm \cite{Sternberg2009a}.

Multitargeted inhibitors of several Bcl family members are in clinical trials. A phase II study of docetaxel, prednisone and AT101, a small molecule inhibitor of Bcl2, Bcl-xL, Bcl-w and Mcl1, in 36 chemonaive patients with CRPC demonstrated a PSA response rate of 67\% \cite{MacVicar2009}. The same regimen used in a phase II trial with 34 patients with docetaxel refractory CRPC resulted in a PSA response rate of 18\% with an objective response rate of 24\% \cite{Poiesz2009}. A randomised phase II trial of docetaxel with AT101 or placebo in chemonaive patients with metastatic CRPC is in process (clinical-trials.gov ID: NCT00571675). There have been mixed results in early-phase clinical studies of Bcl inhibitors. Multitargeted agents have promising activity that needs to be verified in a randomised setting.

### P53/murine double minute 2 protein

P53 is an extensively studied tumour suppressor protein that is activated during cell stress, including chemotherapy treatment or following DNA damage. Activated p53 regulates the transcription of genes to cause cell cycle arrest, DNA repair and occasionally apoptosis \cite{Lane1992}. Murine double minute 2 protein (MDM2) acts as an oncogenic protein by targeting p53 for proteasomal degradation \cite{Haupt1997, Honda1997}, whereas MDM2 levels are increased by p53 in an autoregulatory loop \cite{Bond2005}. \textit{In vitro} studies in prostate cancer cell lines suggest that MDM2 also acts via p53-independent pathways by modulating various cellular proteins including p21, Bax, pRb and Bcl2 \cite{Zhang2003}. MDM2 is amplified in many human cancers including prostate cancer and is a marker of advanced disease \cite{Momand1998}. In PC3, DU145 and LNCaP cells, ASO directed against MDM2 (AS-MDM2) cause dose-dependent apoptosis when used alone and increase chemosensitivity when used with paclitaxel and camptothecin. In PC3 and DU145 xenografts, AS-MDM2 exhibits anti-tumour activity alone and enhances sensitivity to paclitaxel and irinotecan chemotherapy \cite{Wang2003, Zhang2003}. Therapy directed against MDM2 has not yet entered clinical trials.

### Inhibitors of apoptosis proteins

Inhibitors of apoptosis proteins (IAPs) are a family of five proteins (XIAP, survivin, HIAP1 and 2 and neuronal apoptosis inhibitory protein) that promote cell survival \cite{Duckett1996, Liston1996, Chiou2003}. They inhibit the caspase cascade and apoptosis by directly binding to caspases and cytochrome c. This occurs in response to a wide variety of apoptotic signals including chemotherapy treatment. IAPs also have a role in regulating cell cycle progression and modulating receptor-mediated signal transduction \cite{Deveraux1998}. They are induced by the transcription factor nuclear factor-kappa B (NF-\kappa B) and via a feedback loop the HIAPs up-regulate NF-\kappa B activity \cite{LaCasse1998}.

XIAP and survivin are the most well-understood IAPs with regard to carcinogenesis and chemoresistance. \textit{In vitro}, overexpression of XIAP leads to paclitaxel resistance by preventing cleavage of procaspase 3 to caspase 3 and ultimately apoptosis.
(Nomura et al. 2003). An inhibitor of XIAP improves response to cisplatin by increasing caspase 3 activity in prostate cancer cell lines normally resistant to platinum therapy (DU145; Amantana et al. 2004). Survivin inhibition across several prostate cancer cell lines (LNCaP, DU145, PC3, C42B) increases sensitivity to docetaxel and etoposide (Hayashi et al. 2005, Rahman et al. 2009), whereas survivin overexpression increases paclitaxel resistance both in vitro and in vivo (Zhang et al. 2005b). In DU145 and PC3 xenografts, survivin inhibition either via an adenoviral anti-sense DNA vector or via a small molecule inhibitor leads to significant tumour regression alone and enhances the response to docetaxel and etoposide (Hayashi et al. 2005, Nakahara et al. 2007).

XIAP and survivin inhibitors have entered early-phase clinical trials (Table 1). An anti-sense inhibitor of XIAP (AEG35156) was well tolerated in a phase I trial in patients with refractory malignancies (Dean et al. 2009). Two phase I trials of this compound in combination with docetaxel in advanced solid tumours are ongoing (clinicaltrials.gov ID: NCT00372736 and NCT00514267). A survivin ASO (LY2181308) was also well tolerated in 24 patients with refractory tumours and no grade 3 or 4 toxicities were seen (Talbot et al. 2008). A randomised phase II trial of first-line therapy with docetaxel and LY2181308 or placebo in CRPC is in progress (NCT00642018). YM155, a small molecule inhibitor of survivin, was well tolerated in two phase I trials (Tolcher et al. 2008, Satoh et al. 2009) and preliminary activity was suggested in patients with docetaxel refractory CRPC (Tolcher et al. 2008). A phase I/II study in advanced CRPC of docetaxel in combination with YM155 is ongoing (NCT00514267). Agents targeting IAPs have recently entered the early stages of clinical testing and evidence of therapeutic activity is not yet available.

**Inflammation**

**Nuclear factor-kappa B**

There is mounting evidence that the inflammatory response plays an integral role at all stages of prostate carcinogenesis from prostate intraepithelial neoplasia (PIN; De Marzo et al. 2007) to the development of hormonal therapy and chemotherapy resistance. NF-κB is a transcription factor central to the inflammatory response. In the cytoplasm, it is bound to inhibitory proteins (IκBs) that render it inactive; however, on stimulation of the IκB kinase (IKK) complex, these inhibitory proteins are proteolysed leaving NF-κB free to translocate to the nucleus and activate a wide spectrum of genes for interleukins growth factors, stress response elements (including cyclooxygenase-2) and anti-apoptotic proteins (Paule et al. 2007). Increased expression of NF-κB is seen in the progression from normal prostatic epithelium to high-grade PIN to invasive prostate cancer (Sweeney et al. 2004). NF-κB is activated by tumour necrosis factor α (TNF-α; Singh & Aggarwal 1995) promoting cell survival in androgen-independent cell lines. Conversely, in androgen-dependent LNCaP cells, TNF-α causes apoptosis with no NF-κB interaction (Zhao et al. 1992, Chopra et al. 2004, Srinivasan et al. 2010). Many cytotoxic agents including taxanes, cisplatin, 5-fluorouracil and doxorubicin also induce NF-κB (Chuang et al. 2002, Li et al. 2005, Shaikh et al. 2008). It is constitutively activated in androgen-independent prostate cancer cell lines, PC3 and DU145, and is associated with elevated IL6 production in conditioned media. In contrast, androgen-dependent LNCaP cells do not exhibit increased NF-κB activity or IL6 secretion. Indirect inhibition of NF-κB with an IKK complex inhibitor enhances docetaxel sensitivity in PC3 and DU145 cells, while having no effect on LNCaP cells (Domingo-Domenech et al. 2006). Inhibitors of NF-κB derived from natural compounds, such as curcumin, genistein and docosahexaenoic acid, display synergistic effects with cytotoxic agents (taxanes, cisplatin and 5-fluorouracil) in prostate cancer cell lines (Hour et al. 2002, Li et al. 2005, Shaikh et al. 2008). No specific NF-κB inhibitors have yet entered prostate cancer clinical trials.

**Interleukin 6**

IL6 is an important downstream cytokine implicated in many aspects of prostate cancer progression. Androgen-independent prostate cancer cell lines (PC3 and DU145) constitutively produce IL6, which acts as an autocrine growth factor and is at least in part responsible for the transition of cells from an androgen-dependent to androgen-independent state (Hobisch et al. 1998, Chung et al. 1999, Chen et al. 2000, Wallner et al. 2006). In vitro, inhibiting IL6 enhances chemosensitivity, whereas exogenous IL6 inhibits cytotoxic drug-induced apoptosis (Borsellino et al. 1995, Pu et al. 2004). These effects may be mediated via the Bcl and Stat signalling pathways (Pu et al. 2004).

In clinical cohorts of CRPC patients receiving docetaxel chemotherapy, elevated baseline of serum IL6 levels inversely correlate with response (P=0.039), time to progression (P=0.023) and prostate cancer-specific and overall survival
were rendered less sensitive to androgen blockade and docetaxel. The resistance to docetaxel was reversed by concomitant treatment with NF-κB and src inhibitors suggesting that IL8 activation of these pathways is involved in chemoresistance (Araki et al. 2007). PC3 and DU145 cells also exhibit increased NF-κB activity when exposed to exogenous IL8. NF-κB activity is up-regulated by treatment with oxaliplatin leading to increased transcription of IL8 and CXCR2 genes. Pre-treatment with a CXCR2 antagonist (AZ10397767) reduces oxaliplatin-induced NF-κB activation and enhances chemosensitivity (Wilson et al. 2008). IL8 may also be implicated in the relative chemoresistance of hypoxic cancer cells. When PC3 cells are rendered hypoxic, expression of IL8 and CXCR 1 and 2 is enhanced. Following treatment with etoposide, hypoxic PC3 cells are less sensitive than normoxic cells; however, siRNA inhibition of IL8 normalises the response of hypoxic cells to chemotherapy suggesting that IL8 plays a central role in this differential response (Maxwell et al. 2007).

Macrophage inhibitory cytokine 1

Macrophage inhibitory cytokine 1 (MIC1), also known as growth differentiation factor 15, is a member of the transforming growth factor β superfamily. MIC1 appears to have anti-tumour activity as it plays a role in cell cycle arrest, induction of apoptosis and anti-angiogenesis. High levels are only normally found in the placenta; however, expression increases in disease states such as acute injury, inflammation and malignancy. In particular, high levels are found in metastatic prostate, colon and breast cancers (Bauskin et al. 2006). In early low-grade prostate cancer, the unprocessed form of MIC1 found in stromal stores inversely correlates with the risk of future relapse (Bauskin et al. 2005), whereas serum MIC1 levels in combination with PSA improve on the specificity of PSA alone in screening for prostate cancer (Brown et al. 2006). In PC3 and DU145 cells, exposure to docetaxel and mitoxantrone leads to MIC1 overexpression (Huang et al. 2007). On proteomic analysis comparing docetaxel-sensitive (PC3) and docetaxel-resistant

Table 2  Reported clinical studies targeting inflammatory pathways in CRPC

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Inhibitors</th>
<th>Phase</th>
<th>Population</th>
<th>Intervention</th>
<th>Outcome</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL6</td>
<td>CNTO 328</td>
<td>I</td>
<td>Chemonaïve CRPC</td>
<td>CNTO 328 + DTX</td>
<td>Tolerated</td>
<td>Hudes et al. (2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II</td>
<td>DTX-resistant CRPC</td>
<td>CNTO 328</td>
<td>RR 4%</td>
<td>Pinski et al. (2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II</td>
<td>DTX-resistant CRPC</td>
<td>MTX ± CNTO 328 (rand)</td>
<td>Terminated early</td>
<td>De Bono et al. (2010a)</td>
</tr>
</tbody>
</table>

IL6, interleukin 6; DTX, docetaxel; CRPC, castration-resistant prostate cancer; MTX, mitoxantrone; RR, response rate; rand, randomised.
(PC3-Rx) cells, there was pronounced overexpression of MIC1 in PC3-Rx cells (Zhao et al. 2009). PC3 cells exposed to recombinant MIC1 exhibited increased docetaxel resistance, whereas MIC1 inhibition in PC3-Rx cells via siRNA restored docetaxel sensitivity (Zhao et al. 2009). In a cohort of 38 men with CRPC receiving docetaxel or mitoxantrone chemotherapy, an increase in circulating MIC1 after the first cycle of chemotherapy was inversely correlated with PSA response \((P = 0.006)\) and overall survival \((P = 0.002;\) Zhao et al. 2009). The identification of this promising biomarker may herald the beginning of our ability to personalise therapy and detect chemotherapy resistance earlier in the treatment course.

**Vasculature**

Dysregulated angiogenesis associated with malignancy may contribute to chemotherapy resistance. Abnormal vascular organisation, altered stromal composition and increased permeability of blood vessels result in increased interstitial fluid pressure and ultimately limited drug permeability (Heldin et al. 2004). Transient hypoxia and nutrient deprivation of cancer cells may also result in reduced cell proliferation and impaired chemotherapy sensitivity (Bellone et al. 2008). By normalising tumour vasculature, delivery of oxygen and cytotoxic drugs to tumour tissue may be improved (Jain 2005). There are a variety of drugs that act to modulate angiogenesis, including targeted agents and more conventional chemotherapeutics.

Agents targeted against VEGF include bevacizumab, sunitinib, sorafenib and aflibercept. The VEGF family comprises at least seven members (namely A, B, C, D, E, F and placental growth factor) with VEGFA playing the most important role in angiogenesis. It binds to two tyrosine kinase receptors (VEGFR1 and 2; Epstein 2007). In 197 patients on a phase III study in CRPC, high pre-treatment plasma VEGF levels correlated with reduced overall survival (George et al. 2001). In vitro, docetaxel has inherent anti-angiogenic properties that are negated when endothelial cells are stimulated by VEGF and bFGF. When a human breast adenocarcinoma xenograft (MCF7) is treated with docetaxel and a VEGF inhibitor, this protective effect is overcome, resulting in synergistic activity (Sweeney et al. 2001). The murine equivalent of bevacizumab, a humanised monoclonal antibody directed against VEGF (Presta et al. 1997), inhibits tumour growth and metastases in CRPC xenografts when used as a single agent (Borgstrom et al. 1998, Melnyk et al. 1999) and is synergistic with paclitaxel (Fox et al. 2002).

Clinically, bevacizumab has no significant activity in prostate cancer as a single agent (Reese et al. 2001; Table 3); however, it appears promising in combination with cytotoxic agents. In 20 patients on a

**Table 3** Reported clinical studies targeting vascular pathways in CRPC

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Inhibitors</th>
<th>Phase</th>
<th>Population</th>
<th>Intervention</th>
<th>Outcome</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF</td>
<td>Bevacizumab</td>
<td>I</td>
<td>Chemonaïve CRPC</td>
<td>Bv + Ev + DTX</td>
<td>Tolerated</td>
<td>Gross et al. (2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II</td>
<td>CRPC</td>
<td>Bv</td>
<td>No activity</td>
<td>Reese et al. (2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II</td>
<td>DTX-resistant CRPC</td>
<td>Bv + DTX</td>
<td>RR 55%</td>
<td>Di Lorenzo et al. (2008)</td>
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<tr>
<td></td>
<td></td>
<td>II</td>
<td>Chemonaïve CRPC</td>
<td>Bv + DTX + E</td>
<td>RR 65%</td>
<td>Picus et al. (2003)</td>
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<tr>
<td></td>
<td></td>
<td>II</td>
<td>Chemonaïve CRPC</td>
<td>Bv + DTX + Th</td>
<td>RR 88%</td>
<td>Ning et al. (2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III</td>
<td>Chemonaïve CRPC</td>
<td>DTX ± Bv (rand)</td>
<td>No OS benefit</td>
<td>Kelly et al. (2010)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Su</td>
<td>RR 6%</td>
<td>Dror Michaelson et al. (2009)</td>
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<tr>
<td></td>
<td>Sunitinib</td>
<td>II</td>
<td>CRPC</td>
<td>Su</td>
<td>RR 6%</td>
<td>Castellano et al. (2010)</td>
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<td></td>
<td>DTX-resistant CRPC</td>
<td>SS</td>
<td>RR 24%</td>
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<td></td>
<td>Su + MTX or DTX</td>
<td>Tolerated</td>
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<td></td>
<td>DTX</td>
<td>RR 46%</td>
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<td>So</td>
<td>Discordant response</td>
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<td></td>
<td>Af+ DTX</td>
<td>Tolerated</td>
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<td>Th (LD versus HD)</td>
<td>RR 18% (LD) versus 0% (HD)</td>
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<td></td>
<td></td>
<td>DTX ± Th (rand)</td>
<td>RR 53 vs 35%</td>
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<td></td>
<td>Lenalidomide</td>
<td>I</td>
<td>Chemonaïve CRPC</td>
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<td></td>
<td></td>
<td></td>
<td>Solid malignancies incl</td>
<td>CRPC</td>
</tr>
</tbody>
</table>

MTX, mitoxantrone; DTX, docetaxel; Ev, everolimus; rand, randomised; CRPC, castration-resistant prostate cancer; RR, response rate; Bv, bevacizumab; E, estramustine; OS, overall survival; Su, sunitinib; So, sorafenib; Af, aflibercept; Th, thalidomide; LD, low dose; HD, high dose; Ln, lenalidomide.
phase II trial with chemotherapy refractory CRPC, docetaxel and bevacizumab resulted in a PSA response rate of 55% (including four patients who did not respond initially to docetaxel alone; Di Lorenzo et al. 2008). Two first-line phase II trials revealed PSA response rates over 80% with bevacizumab and docetaxel combined with thalidomide or estramustine (Picus et al. 2003, Ning et al. 2008). Unfortunately, a large randomised phase III trial (CALGB 90401) of first-line docetaxel and bevacizumab or placebo in 1050 CRPC patients revealed no overall survival benefit and was associated with greater morbidity and mortality (Kelly et al. 2010).

Sunitinib is a small molecule receptor tyrosine kinase inhibitor that has many targets, including VEGFR and platelet-derived growth factor receptor (PDGFR). Synergistic activity was seen in combination with docetaxel in CRPC xenografts (Cumashi et al. 2008, Guerin et al. 2008), whereas in early clinical studies, single-agent sunitinib exhibited some activity (Dror Michaelson et al. 2009, Castellano et al. 2010). Phase I and II trials combining sunitinib and docetaxel in CRPC are ongoing (clinicaltrials.gov ID: NCT00137436, NCT00879619) whereas a phase II trial of maintenance sunitinib following response to docetaxel is in progress (NCT00550810). A randomised phase III placebo-controlled trial of sunitinib alone in docetaxel refractory CRPC is also in the recruitment phase (NCT00676650).

Sorafenib is another small molecule receptor tyrosine kinase inhibitor that targets VEGF, PDGFR and Raf. As a single agent, sorafenib inhibits growth of both androgen-sensitive (LNCaP) and androgen-resistant (PC3) cells in vitro. Further studies on chemotherapy-resistant cells are in progress (Culig et al. 2009). Clinically, a phase II study of first-line sorafenib and docetaxel in metastatic CRPC revealed a PSA response rate comparable to docetaxel alone in the combination therapy arm (Figg et al. 2001a). A randomised phase II study of first-line weekly docetaxel and thalidomide or placebo in 75 CRPC patients demonstrated favourable PSA response rates of 53% in the combined arm versus 37% with docetaxel alone. The overall survival was also improved with combined therapy (25.9 vs 14.7 months). Patients required prophylactic low molecular weight heparin due to an increased incidence of thromboembolism in the combination therapy arm (Figg et al. 2001a, 2005).

Compared to thalidomide, lenalidomide has a better safety profile and is a more potent angiogenic inhibitor (Tohnya et al. 2006). It was well tolerated both alone and in combination with docetaxel in phase I trials (Tohnya et al. 2006, Moss et al. 2007). Phase II trials in CRPC of lenalidomide in combination with taxanes and/or bevacizumab are currently recruiting (clinicaltrials.gov ID: NCT00933426, NCT00942578). A randomised phase III trial of first-line docetaxel and lenalidomide or placebo in CRPC is also in the recruitment phase (clinicaltrials.gov ID: NCT00988208).

**Signalling intermediaries**

**Stat**

The signal transducers and activator of transcription (Stat) proteins are a family of seven cytoplasmic transcription factors (Stat 1, 2, 3, 4, 5A, B and 6) that dimerise and translocate to the nucleus on activation. They regulate gene expression to influence differentiation, proliferation, apoptosis and angiogenesis. In stress-induced responses, they are activated via cytokine signalling to modulate pro- and anti-apoptotic

**Aflibercept (VEGF trap)** is a soluble decoy receptor that binds to circulating VEGF via the human VEGFR extracellular domains fused to the Fc portion of human IgG1. This was well tolerated in combination with docetaxel in a phase I trial in chemotherapy refractory-advanced solid tumours (Isambert et al. 2008). A randomised phase III trial of first-line docetaxel and aflibercept or placebo in CRPC has completed enrolment (VENICE study, clinicaltrials.gov ID NCT00519285).

Biological therapies targeted at VEGF have demonstrated promising results in phase I and II clinical trials. Phase III data with first-line bevacizumab and docetaxel was disappointing; however, we await results of randomised trials with other anti-VEGF agents.

Thalidomide and its analogue lenalidomide are cytotoxic drugs with anti-angiogenic properties. While the anti-angiogenic mechanism is still uncertain, a reduction in VEGF and bFGF may play a role (Aragon-Ching & Dahut 2008). A phase II CRPC clinical trial of high- versus low-dose thalidomide revealed very poor tolerance of the high-dose and an 18% PSA response rate in the low-dose arm (Figg et al. 2001b). A randomised phase II study of first-line weekly docetaxel and thalidomide or placebo in 75 CRPC patients demonstrated favourable PSA response rates of 53% in the combined arm versus 37% with docetaxel alone. The overall survival was also improved with combined therapy (25.9 vs 14.7 months). Patients required prophylactic low molecular weight heparin due to an increased incidence of thromboembolism in the combination therapy arm (Figg et al. 2001a, 2005).

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genes (Stephanou & Latchman 2003). Stat1 was initially thought to act as a tumour suppressor as Stat1-deficient mice developed early aggressive tumours and Stat1-deficient cancer cells were more resistant to chemotherapy (Stephanou & Latchman 2003). However, in prostate cancer cell lines, Stat1 appears to be associated with docetaxel resistance. Stat1 is overexpressed in docetaxel-resistant DU145-DR and PC3-DR cell lines. Following docetaxel treatment of sensitive DU145 cells, Stat1 expression increases over time, mirroring the reduction in apoptosis. SiRNA targeting Stat1 rendered these cells more sensitive to docetaxel-induced apoptosis. As discussed earlier, these actions appeared to be linked to clusterin with clusterin expression decreasing following treatment with Stat1 siRNA in DU145-DR cells (Patterson et al. 2006). Stat3 also appears to be involved in docetaxel resistance in prostate cancer cell lines. This action may be due to induction of PIM1 kinase, a serine threonine kinase that promotes cell survival. Following treatment with docetaxel, PIM1 kinase is overexpressed in DU145 cells and xenografts leading to reduced sensitivity to docetaxel. It has been postulated that Stat3 phosphorylation, which is promoted by docetaxel treatment, leads to overexpression of PIM1 kinase, increased NF-κB activity and ultimately drug resistance (Zemskova et al. 2008). Interestingly, Stat3 is also activated by IL6 (Stephanou & Latchman 2003), linking this further with the inflammatory process. Stat inhibition has not yet progressed into clinical studies.

**Insulin-like growth factors**

The insulin-like growth factor (IGF) axis includes circulating peptide growth factors (IGF1 and 2), transmembrane receptors (IGF1R and IIR) and six IGF binding proteins (IGFBP1–6). It affects carbohydrate and protein metabolism and regulates cellular proliferation, apoptosis and differentiation (Chi et al. 2009a). Circulating IGF and IGFBP levels correlate with stage and grade of prostate cancer (Figueroa et al. 1998, Chan et al. 2002), while IGFBP overexpression is associated with progression to androgen independence (Miyake et al. 2000b,e). Humanised monoclonal antibodies directed against the IGF1 receptor have entered the clinical arena in an attempt to alter chemotherapy resistance. A randomised phase II study testing docetaxel and one such Ab, CP751,871/placebo, is currently recruiting, including chemotherapy naïve and docetaxel refractory arms (clinicaltrials.gov ID; NCT00313781). Two phase II trials of IMC-A12 are underway. A single-arm study will test IMC-A12 alone in chemotherapy naïve CRPC patients (NCT00520481) and a second study includes docetaxel-resistant CRPC patients randomised to mitoxantrone and IMC-A12 or IMC1121B, an anti-VEGFR2 fully human monoclonal antibody (NCT00683475). Early-phase testing of IGF receptor inhibitors has just commenced with no data available to date.

**Phosphoinositide 3’-kinase/Akt/mammalian target of rapamycin pathway**

Dysregulation of intracellular pathways involving the kinases phosphoinositide 3’-kinase (PI3K), Akt and mammalian target of rapamycin (mTOR) leads to enhanced cell survival, cell cycle progression, neoplastic transformation and chemotherapy resistance. In normal circumstances, the tumour suppressor protein, PTEN, negatively regulates this pathway; however, this function is lost in up to 80% of prostate cancers leading to constitutive activation of Akt. Akt prevents apoptosis by phosphorylating and inhibiting pro-apoptotic factors such as BAD, pro-caspase 9, FKHR and Bim. It also inhibits release of the caspase cascade inducer, cytochrome c. PI3K activation may promote the development of chemoresistance by up-regulating the expression of multidrug resistance protein 1 (MRP1), a drug efflux pump. Both PI3K and Akt indirectly activate mTOR causing overexpression of various proto-oncogenes and growth factors, including c-myc, cyclin D1 and VEGF (Lee et al. 2008, Meric-Bernstam & Gonzalez-Angulo 2009).

*In vitro*, PTEN expressing DU145 cells are more sensitive to doxorubicin and paclitaxel chemotherapy than PC3 cells, which do not express PTEN. However, the chemosensitivity of PC3 cells is restored when the pathway is inhibited, either by PTEN transfection or by direct mTOR inhibition with rapamycin (Grunwald et al. 2002, Lee et al. 2004). Long-term androgen ablated cells, LNCaP-abl, are resistant to chemotherapy, but following treatment with a PI3K inhibitor, LY294002, chemo-sensitivity is restored, further highlighting the potential of this pathway as a target for treatment (Pfeil et al. 2004). Everolimus, an mTOR inhibitor, has exhibited some clinical activity in combination with bevacizumab and docetaxel as first-line treatment in CRPC (Gross et al. 2009; Table 3). A phase II trial of first-line docetaxel and everolimus in CRPC is ongoing (clinicaltrials.gov ID; NCT00459186). Another mTOR inhibitor, ridaforolimus, has completed phase II testing in the docetaxel-resistant setting, with results pending (clinicaltrials.gov ID; NCT00110188). Activity of mTOR inhibitors in CRPC is yet to be established.
Platelet-derived growth factor

PDGF receptors are overexpressed in prostate cancers compared with benign prostatic epithelium making them a potential target for enhancing chemosensitivity. However, the level of overexpression may be modest with one study showing PDGFR expression in only 16% of advanced CRPC tumour tissue (Hofer et al. 2004). Imatinib is a PDGFR tyrosine kinase inhibitor that has been assessed in both pre-clinical and clinical studies in CRPC. When multidrug-resistant prostate cancer cells (PC–3MM2–MDR) were treated in vitro with imatinib and paclitaxel, resistance to both drugs was seen. However, in PC–3MM2–MDR mouse tibial xenografts, treatment with imatinib alone and in combination with paclitaxel led to reduced bone tumour incidence, reduced tumour weight, reduced bone lysis, less lymph node metastases and decreased mean vessel density. This suggested that the target for imatinib was the tumour-associated endothelial cells rather than the prostate tumour cells themselves (Kim et al. 2006). Cell growth of PC3, DU145 and LNCaP cells is inhibited with imatinib; however, antagonistic effects were seen when combined with docetaxel, particularly in PC3 cells (Kubler et al. 2004). Clinically, the combination of cytotoxic therapy with imatinib has not been promising (Table 4). Two single-arm phase II trials combining docetaxel and imatinib revealed only modest response rates and significant toxicity (Gillison et al. 2009, Gomez-Pinillos et al. 2009). A larger randomised, double-blind phase II trial with 104 CRPC patients with bone metastases and no prior taxane exposure tested docetaxel and imatinib/placebo. The primary end point, time to progression, was worse in the experimental arm (4.4 vs 5.3 months). Again, toxicity was significant, particularly gastrointestinal (Mathew et al. 2006). Interestingly, an associated biomarker study revealed that in the standard treatment arm, reduced PDGFR phosphorylation in peripheral blood mononuclear cells was associated with poorer outcomes including decreased progression-free and overall survival ($P = 0.04$). It was suggested that this may be a marker or mechanism for docetaxel resistance; however, further studies have not yet been published (Mathew et al. 2008).

Multidrug resistance proteins

MDRPs, including P-glycoprotein (P-gp; encoded by the $MDRI$ gene) and MRP1, are ATP binding cassette (ABC) transporters in cell membranes of the biliary tract, intestinal epithelium, the blood–brain barrier and tumours (Bradshaw & Arceci 1998). They act as drug efflux pumps with a wide range of substrates, including docetaxel and mitoxantrone (van Zuylen et al. 2000). In prostate cancer cell lines, P-gp appears to be variably related to chemotherapy resistance. Chemoresistant PC3 cells do not overexpress P-gp, whereas resistant DU145 cells exhibit both overexpression of the protein and restored chemosensitivity with P-gp inhibition (Makarovskyi et al. 2002, Takeda et al. 2007). Furthermore, exposure to mitoxantrone and

<table>
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<tr>
<th>Pathway</th>
<th>Agents</th>
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<td>Multidrug resistance</td>
<td>laniquidar</td>
<td>I</td>
<td>Solid malignancies</td>
<td>La + DTX</td>
<td>Tolerated</td>
<td>Van Zuylen et al. (2002)</td>
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<td>RR 30%</td>
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<td>DTX-resistant CRPC</td>
<td>Ca or MTX (rand)</td>
<td>OS 15.1 vs 12.7 months</td>
<td>De Bono et al. (2010b)</td>
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<td>Tubulin</td>
<td>epothilones</td>
<td>I/II</td>
<td>DTX-resistant CRPC</td>
<td>Ix + MTX</td>
<td>RR 31%</td>
<td>Rosenberg et al. (2009)</td>
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<td>Ix</td>
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<td>DTX-resistant CRPC</td>
<td>Ix</td>
<td>RR 22%</td>
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<td>Ix</td>
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<td>II</td>
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<td></td>
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<td>Pa</td>
<td>RR 45%</td>
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<td>imatinib</td>
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<td>Im + DTX</td>
<td>RR 41%</td>
<td>Gillison et al. (2009)</td>
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<td>Im + DTX</td>
<td>RR 47%</td>
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<td>II</td>
<td>Chemonaive CRPC</td>
<td>DTX ± Im</td>
<td>No difference</td>
<td>Mathew et al. (2006)</td>
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MTX, mitoxantrone; DTX, docetaxel; CRPC, castration-resistant prostate cancer; RR, response rate; OS, overall survival; La, laniquidar; Zo, zosuquidar; Bi, biricodar; Ca, cabazitaxel; Ix, ixabepilone; Pa, patupilone; PDGF, platelet derived growth factor; Im, imatinib.
docetaxel results in increased multidrug-resistant protein expression in chemo-sensitive prostate cancer cell lines (Sanchez et al. 2009). Although MDR1 is well understood, MRP1 is also emerging as an important element in prostate cancer chemoresistance (Zalcberg et al. 2000, Lee et al. 2008). Up-regulated MRP1 expression is found in chemoresistant DU0.03 and PC0.03 cells with no up-regulation in P-gp (Zalcberg et al. 2000). Both p53 and PIM1 kinase appear to be involved in modulating ABC transporter expression and chemoresistance, providing potential future targets for treatment (Sullivan et al. 2000, Xie et al. 2008). In a cohort of 73 CRPC patients receiving docetaxel ± thalidomide, genotyping for the ABCB1 transporter was performed. Particular variants of this gene were significantly correlated with overall survival ($P=0.0048$) and toxicity (including neuropathy and neutropenia) suggesting that specific ABC transporter genotypes may predict outcome with chemotherapy (Sissung et al. 2008).

Therapeutic manipulation of MDR proteins was initially attempted with co-administration of classical MDR modulators (such as verapamil, cyclosporine A and valsodapar) and chemotherapeutic agents. Unfortunately, these drugs were disappointing due to their toxicity and unpredictable effects on pharmacokinetics. Since then, third-generation modulators that cause minimal pharmacokinetic interference have been developed. P-gp inhibitors, including laniquidar, biricodar and elacridar, are well tolerated in combination with chemotherapy for CRPC in phase I trials, however exhibited minimal clinical activity on phase II analysis (van Zuylen et al. 2002, Lokiec et al. 2003, Rago et al. 2003, Fracasso et al. 2004; Table 4).

Cabazitaxel is a novel taxane, which avoids cellular extrusion due to poor affinity for P-gp. In vitro, potent cytotoxic effects were achieved in a variety of cell lines, including those with docetaxel resistance due to P-gp overexpression. These results were mirrored in vivo, including in a CRPC xenograft model (DU145; Mita et al. 2009). In a recent phase III study, 755 men with docetaxel-resistant CRPC were randomised to receive cabazitaxel or mitoxantrone. Overall survival was improved in the experimental arm (15.1 vs 12.7 months; $P<0.0001$; De Bono et al. 2010b) making cabazitaxel a promising second-line chemotherapy option.

**Tubulin**

Growing pre-clinical evidence suggests that resistance to taxane chemotherapy in CRPC may be attributed to changes in β-tubulin isotypes, the primary target of these drugs. There are at least seven isotypes of β-tubulin with the predominant type in most normal tissues being isotype I (Luduena 1998). Paclitaxel resistance is associated with a switch from class I to class III β-tubulin in multiple cancer cell lines (Kamath et al. 2005). In CRPC cell lines, an increase in isotypes III and IV correlates with docetaxel and paclitaxel resistance (Ranganathan et al. 1998, Makarovskiy et al. 2002). Oestrogen treatment of hormone-dependent LNCaP cells suppressed expression of β tubulin IVa and led to improved docetaxel sensitivity in xenografts (Montgomery et al. 2005). In a phase II clinical trial with 29 CRPC patients, first-line diethylstilboestrol and docetaxel led to a 75% PSA response rate (Montgomery et al. 2006).

Novel agents targeting tubulin have recently been developed. Epothilones are cytotoxic macrolides, which, like taxanes, prevent tubulin depolymerisation causing mitotic arrest and apoptosis. While epothilones bind tubulin at the same site as taxanes, they have a more potent effect on polymerisation. This results in greater inhibition of tumour growth in prostate cancer xenografts than paclitaxel (Newman et al. 2001). Epothilones are effective in some taxane-resistant settings. In tumours overexpressing P-gp and in many with taxane-resistant tubulin mutations, epothilones remain active (Altmann 2005, Larkin & Kaye 2006). In docetaxel-refractory CRPC, ixabepilone and patupilone, as single agents, exhibit modest phase II clinical activity with PSA response rates around 20% (Rosenberg et al. 2007, Wilding et al. 2008, Hussain et al. 2009; Table 4). In combination with mitoxantrone in docetaxel-resistant CRPC, ixabepilone resulted in a PSA response rate of 31% (Rosenberg et al. 2009). A more promising phase II study of patupilone alone in 83 patients with docetaxel-resistant CRPC revealed a PSA response rate of 45% (Beardsley et al. 2009). While these agents are active in the taxane refractory setting, they have not yet entered phase III testing.

**Conclusion**

Prostate cancer represents a large burden of disease in our community and there are still limited therapeutic options available in the advanced castration-resistant setting. Docetaxel is the only cytotoxic agent that consistently improves survival; however, around half of patients will never respond to treatment, while all will eventually develop resistant disease. A greater understanding of resistance pathways is needed to both predict resistance early in the course of treatment and to ultimately manipulate this resistance and improve
outcomes. While multiple aspects of resistance have been explored, few have been successfully manipulated in the clinical setting. Many agents are still in the early phases of clinical testing and with the large number of ongoing trials progress is anticipated. Until recently, identification of reliable resistance markers has been unproductive; however, recognition of MIC1 as an early marker of treatment response may be an early element in a new generation of biomarker discovery. At present, recognition of resistance occurs after at least 6 weeks of cytotoxic therapy (Tannock et al. 2004), by which time cumulative toxicity is significant. It is likely that resistance pathways differ among individual prostate cancers and that a set of multiple resistance markers encompassing various mechanisms of resistance will be required. Similarly, to combat resistance, effectively targeting multiple pathways simultaneously will almost certainly be necessary. Exploration of interactions among resistance pathways rather than their study in isolation may facilitate these goals.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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