Castration-resistant Prostate Cancer: From New Pathophysiology to New Treatment Targets

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Abstract

Context: Castration-resistant prostate cancer (CRPC) refers to patients who no longer respond to surgical or medical castration. Standard treatment options are limited.

Objective: To review the concepts and rationale behind targeted agents currently in late-stage clinical testing for patients with CRPC.

Evidence acquisition: Novel targeted therapies in clinical trials were identified from registries. The MEDLINE database was searched for all relevant reports published from 1996 to October 2009. Bibliographies of the retrieved articles and major international meeting abstracts were hand-searched to identify additional studies.

Evidence synthesis: Advances in our understanding of the molecular mechanisms underlying prostate cancer (PCa) progression has translated into a variety of treatment approaches. Agents targeting androgen receptor (AR) activation and local steroidogenesis, angiogenesis, immunotherapy, apoptosis, chaperone proteins, the insulin-like growth factor (IGF) pathway, RANK-ligand, endothelin receptors, and the Src family kinases are entering or have recently completed accrual to phase 3 trials for patients with CRPC.

Conclusions: A number of new agents targeting mechanisms of PCa progression with early promising results are in clinical trials and have the potential to provide novel treatment options for CRPC in the near future.
1. Introduction

Prostate cancer (PCa) remains a significant medical burden in developed countries and a major cause of morbidity and mortality. In men, it is the most commonly diagnosed noncutaneous cancer and the second to third most common cause of cancer death in the western world [1]. Many patients with localised disease have an excellent long-term survival and high cure rates with standard approaches [2]. However, patients with high-risk, locally advanced, and metastatic disease have a poor prognosis; and although hormone therapy (HT) in the form of medical or surgical castration can induce significant long-term remissions, development of castration-resistant disease is inevitable. Castration-resistant prostate cancer (CRPC) has been used synonymously with androgen-independent PCa and hormone-refractory PCa but is the preferred term, as we now know that many men with CRPC respond to additional manipulations that ablate or block PCa growth stimulation by androgens. CRPC is clinically detected by a rise in prostate-specific antigen (PSA), typically defined as three consecutive rises over nadir in the context of castrate levels of serum testosterone and after antiandrogen withdrawal for at least 4 wk and despite secondary hormonal manipulations and/or radiologic progression [3,4].

Systemic therapy for metastatic CRPC is remarkable for the relatively few options that have been developed. After failure of HT, treatments have been approved primarily for symptomatic benefit, such as mitoxantrone chemotherapy [5], radioactive isotopes [6], and the bisphosphonate zoledronic acid [7]. Despite multiple trials of cytotoxic chemotherapy in patients with metastatic CRPC, only docetaxel has been shown to improve overall survival (OS) [8,9], with a median improvement in OS of 2.4 mo over mitoxantrone. In addition, an improvement in patient-reported pain and quality of life scores were also superior in docetaxel-treated patients and has now become a standard of care. Gains in this late-stage patient population may translate to greater benefits when applied to earlier states of PCa in the future to prevent or delay metastases and increase cure rates and OS. Indeed, a recent update of a study randomising patients with castration-sensitive disease to receive either the oral bisphosphonate clodronate or placebo has suggested that the secondary endpoint of OS was improved in those patients who received clodronate [10]. To this end, trials are ongoing with zoledronic acid in patients with metastatic castration-sensitive and high-risk localised disease (Table 1). Trials evaluating docetaxel given in an adjuvant or neoadjuvant fashion to patients with high-risk, clinically localised disease undergoing surgery or radiation therapy (RT) are also underway. Additionally, randomised studies testing the benefit of docetaxel in patients with PSA-recurrent or castration-naive metastatic disease are ongoing (Table 1).

Over the past decade, there has been a significant increase in understanding of the biologic basis for PCa progression, fuelled in part from the development of high-throughput genomic, transcriptomic, and proteomic technologies. The mechanisms of androgen independence can be divided into those that are mediated by the androgen receptor (AR)—for example, through a hypersensitive, promiscuous, or amplified AR—and others that bypass it [11,12]. Mechanisms common to all cancers underlying malignant proliferation, angiogenesis, metastases, and avoidance of immune surveillance are also implicated in PCa progression. From these advances, a large number of potential therapeutic targets have been identified. This article reviews the current concepts behind targeted therapy for CRPC with a focus on novel agents that are currently in late-stage clinical testing for patients with advanced PCa.

2. Androgen receptor signalling

It has long been recognized that after failure of castration therapy, second-line HTs can often be associated with clinical responses, initially presumed on the basis of extragonadal production of androgens [13]. An additional factor underlying such responses may also reside within PCa tissue itself. Several studies have demonstrated amplification and increased expression of AR in xenografts with hormone resistance and in PCa tissues from patients with CRPC [14–16]. In vitro and in vivo, increased AR expression has been shown to be required for transformation of prostate cell lines from a hormone-sensitive to a hormone-refractory phenotype, with the effect being both ligand dependent and genotropic [15]. High AR levels were also associated with a change in the effect of bicalutamide from an AR antagonist to agonist [15], providing a further mechanism for antiandrogen withdrawal effects observed in the clinic. Additionally, inhibition of the AR in hormone-refractory tumour models has been demonstrated to induce regression [17]. PCa cells and human tissues also possess the biochemical machinery to synthesize androgens [18–21], invoking an autocrine/paracrine mechanism for hormone-refractory progression. Finally, ligand-independent mechanisms may occur through AR splice variants lacking the ligand-binding domain that are constitutively active [22]. Thus, a strategy to develop more potent inhibitors of extragonadal androgen production and small-molecule antagonists of the AR has been a rational and active area for drug development. Results of clinical trials of two such agents targeting mechanisms of AR activation have been recently reported: abiraterone acetate (Cougard Biotechnology, Inc.) and MDV3100 (Medivation, Inc.). The early results of these clinical trials are encouraging and confirm clinically that CRPC commonly remains dependent on AR signalling.

Abiraterone acetate is an orally administered inhibitor of the cytochrome P450 enzyme CYP17A1, which has dual function as a 17α-hydroxylase and C17,20-lyase, both of which are necessary for androgen synthesis from cholesterol precursors. In a phase 1 trial that enrolled patients with HT-resistant but chemotherapy naïve disease, treatment at all dose levels was well tolerated [23]. Mechanistic-based toxicities of mineralocorticoid excess were observed (hypertension, hypokalemia, oedema); however, these could be controlled by administration of an aldosterone antagonist (eplerenone) or corticosteroids. PSA declines of
<table>
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<tr>
<th>Disease state</th>
<th>Identifier</th>
<th>Treatment arms</th>
<th>Estimated enrolment</th>
<th>Primary endpoint</th>
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<tr>
<td>Castration sensitive, metastatic</td>
<td>NCT00242567</td>
<td>Early vs delayed ZA (open label)</td>
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<td>Skeleton-related event–free survival</td>
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<td>Arm 1: ZA</td>
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<td>Arm 2: Placebo</td>
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<td>Castration sensitive, metastatic</td>
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<td>AST ± DOC</td>
<td>378</td>
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<td>Arm 2: AST + ZA</td>
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<td>Arm 3: AST + ZA + prednisolone</td>
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<td>Arm 4: AST + celecoxib</td>
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<td>Arm 5: AST + ZA + DOC + prednisolone</td>
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<td>Arm 6: AST + ZA + celecoxib</td>
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<td>200</td>
<td>2-yr PFS</td>
<td>Rete Oncologica Piemontese</td>
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<td>or non-metastatic high risk</td>
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<td>Castration sensitive, post-prostatectomy with rising PSA</td>
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<td>3-yr PSA PFS</td>
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<td>AST and DOC</td>
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<td>OS</td>
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<td>AST with RT ± neoadjuvant DOC</td>
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<td>DFS</td>
<td>NCIC Clinical Trials Group</td>
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<td>Prostatectomy ± adjuvant DOC</td>
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<td>DFS</td>
<td>Department of Veterans Affairs</td>
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<td>OS</td>
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<td>8-yr survival rate</td>
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<td>Localised, intermediate to high risk</td>
<td>NCT00193856 (RADAR)</td>
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<td>Arm A: AS for 6 mo</td>
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<td>Arm B: AS for 6 mo + ZA</td>
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<td>Arm C: AS for 18 mo</td>
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<td>Arm D: AS for 18 mo + ZA</td>
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<td>Localised, intermediate to high risk</td>
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<td>AST with RT ± adjuvant DOC</td>
<td>924</td>
<td>PSA progression rate</td>
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ZA = zoledronic acid; AST = androgen suppression therapy; DOC = docetaxel; OS = overall survival; ECOG = Eastern Cooperative Oncology Group; EAU = European Association of Urology; PFS = progression-free survival; PSA = prostate-specific antigen; RT = radiation therapy; DFS = disease-free survival; NCIC = National Cancer Institute of Canada; AS = androgen suppression.
PCa has been associated with antitumour effects [28]. VEGF of the VEGF/VEGFR pathways in experimental models of metastatic CRPC, increased plasma VEGF—either as a continuous or dichotomous variable—has been correlated with poor prognosis and disease progression [27]. Inhibition may also have an indirect antitumour effect when VEGF-A and causes potent inhibition of VEGFR signalling through VEGF receptors (VEGFR) 1 and 2 to promote angiogenesis. Elevated VEGFR—notably VEGFR-2—has been associated with progression of PCa in the transgenic adenocarcinoma of mouse prostate (TRAMP) model and is also overexpressed in human PCa [26]. In patients with metastatic CRPC, increased plasma VEGF—either as a continuous or dichotomous variable—has been correlated with poor prognosis and disease progression [27]. Several mediators of angiogenesis have been identified, including vascular endothelial growth factor (VEGF), which signals through VEGF receptors (VEGFR) 1 and 2 to promote angiogenesis. Several trials have demonstrated the safety of aflibercept in combination with docetaxel [33]. A phase 3, randomised, double-blind, placebo-controlled trial is underway for patients with metastatic CRPC (ClinicalTrials.gov identifier: NCT00519285). Sunitinib is an orally administered agent that inhibits the receptor tyrosine kinase activity of VEGFR as well other kinases, including platelet-derived growth factor receptor (PDGFR) and KIT. Sunitinib is approved for use in metastatic renal cell cancer, where it has been shown to significantly improve progression-free survival (PFS) [34]. Adverse events are similar to bevacizumab, which is expected given the similar mechanisms of action. Phase 1 trials have demonstrated the safety of aflibercept in combination with docetaxel [33]. A phase 3, randomised, double-blind, placebo-controlled trial is underway for patients with metastatic CRPC (ClinicalTrials.gov identifier: NCT00519285). Aflibercept (VEGF Trap) is a recombinantly produced fusion protein consisting of human VEGFR extracellular domains fused to the Fc portion of human immunoglobulin (Ig) G1 (IgG1). It contains sequences encoding Ig domain 2 from VEGFR1 fused to Ig domain 3 from VEGFR2, which in turn is fused to the hinge region of the human IgG1 Fc domain. Aflibercept is a potent inhibitor of VEGF and VEGF-B and the other VEGF family members that binds to VEGFR-1 and VEGFR-2, including placental growth factor, by binding and inactivating these circulating factors [32]. Adverse events are similar to bevacizumab, which is expected given the similar mechanisms of action. Phase 1 trials have demonstrated the safety of aflibercept in combination with docetaxel [33]. A phase 3, randomised, double-blind, placebo-controlled trial is underway for patients with metastatic CRPC (ClinicalTrials.gov identifier: NCT00519285). Sunitinib is an orally administered agent that inhibits the receptor tyrosine kinase activity of VEGFR as well other kinases, including platelet-derived growth factor receptor (PDGFR) and KIT. Sunitinib is approved for use in metastatic renal cell cancer, where it has been shown to significantly improve progression-free survival (PFS) [34]. Adverse events are similar to bevacizumab, which is expected given the similar mechanisms of action. Phase 1 trials have demonstrated the safety of aflibercept in combination with docetaxel [33]. A phase 3, randomised, double-blind, placebo-controlled trial is underway for patients with metastatic CRPC (ClinicalTrials.gov identifier: NCT00519285). Sunitinib is an orally administered agent that inhibits the receptor tyrosine kinase activity of VEGFR as well other kinases, including platelet-derived growth factor receptor (PDGFR) and KIT. Sunitinib is approved for use in metastatic renal cell cancer, where it has been shown to significantly improve progression-free survival (PFS) [34]. Adverse events are similar to bevacizumab, which is expected given the similar mechanisms of action. Phase 1 trials have demonstrated the safety of aflibercept in combination with docetaxel [33]. A phase 3, randomised, double-blind, placebo-controlled trial is underway for patients with metastatic CRPC (ClinicalTrials.gov identifier: NCT00519285).
4. Immunotherapy

The harnessing of the body’s immune system to illicit an antitumour effect and overcome immunologic tolerance of malignancies has been a long-sought-after oncologic therapeutic strategy. Active specific immunotherapy seeks to induce an immune-mediated antitumour effect by immunisation of a patient with tumour-specific antigens. Antigen-presenting cells such as dendritic cells are integral in the processing and presentation of antigens, via major histocompatibility complex (MHC) class I and class II molecules, to T cells to illicit a specific immune response.

Sipuleucel-T (Dendreon Corp.) is a dendritic cell–based vaccine designed to stimulate T cell immunity against prostatic acid phosphatase (PAP), which is abundantly expressed in benign and malignant prostate epithelium compared to very low levels in nonprostatic tissues. To prepare the vaccine, patients undergo a 1.5–2.0 blood volume mononuclear cell leukapheresis. Antigen-presenting cells (APC) are isolated from the leukapheresis product at a central facility and cultured with a fusion protein that consists of PAP linked to granulocyte-macrophage colony-stimulating factor (GM-CSF), resulting in activation of the APCs and loading and processing of the PAP antigen for presentation to T cells. Phase 1 and 2 trials demonstrated the feasibility of the approach, with no dose-limiting toxicities observed and the main limitation being the number of injections that could be feasibly performed [38]. Evidence of immune response was observed by immunoblot analyses of lysates of the two modified cell lines against patient sera at baseline and at post-treatment. PSA declines were also seen in several patients, although a decrease of >50% from baseline was rare, and those patients receiving higher doses of GVAX appeared to have longer time to progression and survival. Two phase 3 studies in patients with CRPC were conducted with primary endpoints of OS. One study randomised patients to receive GVAX immunotherapy with docetaxel versus docetaxel and prednisone (ClinicalTrials.gov identifier: NCT00133224). Four hundred eight patients were accrued, but the study was stopped by the independent data-monitoring committee in August 2008 after an imbalance in deaths was observed in the immunotherapy arm. The second study, which accrued 626 asymptomatic patients and randomised them to receive GVAX immunotherapy versus docetaxel and prednisone (ClinicalTrials.gov identifier: NCT00089856) was also terminated early after interim analysis had determined that the study had a <30% chance of meeting its primary objective.

A third approach to immunotherapy taken for patients with CRPC has been through blockade of the cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) co-stimulatory molecule expressed on the surface of T cells. After APC presentation of antigen to T cells, activation and proliferation are mediated by recognition of the antigen/MHC complexes by the T cell receptor in conjunction with a co-stimulatory signal delivered through CD80 and CD86 on the APC and CD28 on the T cell. Activated T cells then express CTLA-4, which also binds CD80 and CD86 but mediates an inhibitory signal, providing a negative feedback loop. Thus, blocking CTLA-4 signalling may enhance and maintain the activation and proliferation of tumour-specific T cells [39], although at the potential risk of breaking self-tolerance and inducing autoimmunity as an adverse toxicity. In vivo studies of CTLA-4 blockade have demonstrated successful induction of tumour immunity and rejection [40].

Ipilimumab (Medarex, Inc) is a fully humanized monoclonal antibody against CTLA-4. A pilot trial in patients with CRPC was undertaken with a single dose of ipilimumab at 3 mg/kg [41]. Twelve patients were treated, and two had a 50% PSA decline from baseline. Clinical autoimmunity occurred in one patient (grade 3 rash/pruritis), requiring corticosteroids. A phase 1 trial of ipilimumab and RT was subsequently conducted and recently presented [42]. In preclinical models, RT had been shown to result in tumour antigen release and enhancement of antitumour effects of CTLA-4 blockade; hence, its inclusion in the clinical trial protocol. Doses ≤10 mg/kg were delivered, and 33 patients had been enrolled at the time of presentation. Immunerelated adverse events that were grade 3 and higher (severe or life threatening) included colitis (24%), hepatitis (18%), and rash (3%), which appeared dose related. One patient died of an opportunistic infection after 3 mo of immunosuppression for colitis. Seven patients (21%) had a PSA decline >50% from baseline, and one patient experienced a
complete response in measurable disease. Accrual to expansion cohorts was continuing on this trial, and randomised studies are being considered.

Combination immunotherapy is also being evaluated clinically as a rational approach to enhance immune response to vaccine therapy. As an example, ipilimumab and GVAX have been tested in a phase 1 combination trial, and a preliminary report documented evidence of immune responses observed in the form of tumour-reactive antibodies being identified by serologic analysis and PSA declines seen in patients treated at higher doses of ipilimumab [43].

5. Apoptosis

Resistance to apoptosis, or programmed cell death, is another mechanism attributed to PCa progression and treatment resistance. The B-cell leukaemia/lymphoma 2 gene (BCL-2) is the prototype of a class of oncogenes that contributes to neoplastic progression by enhancing tumour cell survival through inhibition of apoptosis. Initially identified in follicular lymphoma as a result of the characteristic t14,18 translocation, BCL-2 is a mitochondrial membrane protein that functions to heterodimerize with BCL-2–associated X protein (BAX) and other pro-apoptotic regulators, thus preventing release of cytochrome c and subsequent activation of the apoptotic cascade. The selective and competitive dimerization between pairs of these antagonists and agonists of the BCL-2 family of proteins determines how a cell responds to an apoptotic signal. Several lines of evidence have implicated overexpression of BCL-2 with treatment resistance. In PCa, BCL-2 has been found expressed in clinical samples of androgen-dependent and independent disease [44], and experimental and clinical studies report that increased expression of BCL-2 confers or is associated with the development of androgen independence and treatment resistance [45,46]. Thus, BCL-2 is an attractive target to improve the efficacy of treatment of patients with PCa by enhancing chemotherapy-induced apoptosis.

G3139 (Genta, Inc.) is an 18-mer phosphorothioate antisense oligonucleotide complimentary to the first six codons of the initiating sequence of the human BCL-2 mRNA. In preclinical PCa models, G3139 and other BCL-2 antisense oligonucleotides have shown significant activity in inhibiting expression of BCL-2, delaying time to the development of androgen independence and enhancing the effects of chemotherapy by increased apoptosis [46]. Phase 1 trials with prolonged infusions of G3139 as a single agent or in combination with chemotherapy have demonstrated the tolerability of G3139 with tolerable side effects observed, including fatigue, anorexia, hypophosphatemia, and reversible transaminase elevation [47,48]. A randomised, phase 2 trial in 115 patients with CRPC was conducted by the European Organization for Research and Treatment of Cancer (EORTC) and initial results reported [49]. Patients randomised to receive combination therapy received a median of 2 fewer cycles of treatment (six vs eight cycles), and the PSA response rate was numerically lower in the combination therapy arm (37% vs 46%). Further trials for this agent in CRPC are not known to be planned.

One possible explanation for a lack of significant clinical benefit observed with G3139 is that there are several pro-survival BCL-2 family members. The specific targeting of one member may simply be insufficient to overcome apoptotic resistance exerted by the other BCL-2 family members. Preclinical testing supports this hypothesis, with enhanced regressions in cancer models when treated with combined targeting of antiapoptotic BCL-2 family members [50]. AT-101 (Ascenta Therapeutics Inc) is a small-molecule inhibitor of multiple antiapoptotic BCL-2 family members (BCL-2, BCL-XL, BCL-W, MCL-1) derived from gossypol, which is a natural compound from cotton seeds. AT101 has single-agent and combination activity in vitro and in vivo. In a single-agent phase 2 trial with 23 chemotherapy-naive patients with CRPC, two (9%) had PSA declines ≥50% that were confirmed, and five (22%) additional patients had PSA declines ranging from 3% to 54% [51]. Adverse events included diarrhoea, fatigue, nausea, anorexia, and small bowel obstruction necessitating dose reduction. A phase 1/2 trial of AT-101 in combination with standard docetaxel and prednisone with escalating doses of AT-101 has been completed, and a recommended phase 2 dose was identified, with a number of PSA responses observed [52]. A randomised phase 2 trial is currently ongoing in a planned 180 patients with chemotherapy-naive metastatic CRPC evaluating docetaxel with or without AT-101. The trial has a primary endpoint of PFS (ClinicalTrials.gov identifier: NCT00571675).

6. Chaperone proteins

The heat shock protein-90 (HSP90) molecular chaperone complex is essential for AR stability and maturation and thus has been identified as a potential therapeutic target for CRPC. In addition, HSP90 acts as a chaperone to a number of other client proteins associated with malignant progression, including Akt, Raf-1, Her-2, and hypoxia-inducible factor 1α (HIF-1α) [53]. HSP90 is an ATP-dependent chaperone, and a number of specific inhibitors have been developed against its ATPase activity. Small-molecule inhibitors of histone deacetylase (HDAC) can also result in the loss of HSP90 ATP-binding activity through acetylation, with subsequent degradation of AR [54–57]. HDAC inhibitors are also of interest, as the frequently occurring TMPRSS2 fusions with oncogenic ETS factors in PCa [58] may result in epigenetic reprogramming, including upregulation of HDAC 1 and downregulation of its targets [59] and thus susceptibility to HDAC inhibition [60].

17-allylamino-17-demethoxygeldanamycin (17-AAG) is a benzoquinone ansamycin antibiotic with antitumour activity in preclinical models that, along with its parent compound geldanamycin, is considered to act by binding to the HSP90 adenosine triphosphate (ATP) binding site. Phase 1 trials have demonstrated the safety of the agent in humans; however, a recent phase 2 trial in patients with CRPC demonstrated minimal clinical activity [61]. Similarly, HDAC inhibitors in phase 2 trials involving patients with
CRPC have yet to demonstrate any clinically significant activity [62]; however, other HSP90 and HDAC inhibitors continue to be evaluated.

Another chaperone protein of interest is clusterin. Clusterin exists as both an intracellular truncated 55-kDa nuclear form (nCLU) that is pro-apoptotic [63] and a 75–80-kDa secreted heterodimer disulphide-linked glycoprotein (sCLU), which has been shown to be anti-apoptotic [64]. In cancer, clusterin has been largely defined in its role of inhibiting apoptosis. Clusterin is activated after therapeutic stress, functioning as a cytoprotective chaperone similar to an ATP-independent small HSP and is transcriptionally activated by heat shock factor-1 [65]. Clusterin’s ability to inhibit apoptosis has also been shown to act through inhibition of activated BAX, a critical pro-apoptotic BCL-2 family member [64]. Furthermore, overexpression of clusterin leads to activation of the PI-3Kinase/Akt pathway through the megalin cell surface receptor [66]. In xenograft models, clusterin expression increases in response to cell stress induced by a variety of factors, and forced overexpression of clusterin in cancer models confers resistance to radiation, hormone, and chemotherapy, whereas inhibition of clusterin expression enhances apoptotic death from these treatment modalities [67]. In preclinical models of PCa, clusterin has been associated with androgen-independent progression [68]. Clusterin is overexpressed in a variety of human cancers, including prostate, and its expression increases with castration-resistant disease [69].

OGX-011 (OncoGenex Pharmaceuticals Inc) is a phosphorothioate antisense molecule that also incorporates 2-methoxyethyl modification second-generation chemistry, which serves to increase potency and tissue half-life. OGX-011 has been shown to significantly decrease sCLU expression through inhibition of clusterin mRNA translation in vitro and in vivo. Phase 1 trials have established that OGX-011, delivered as a 2-hr intravenous infusion, can inhibit clusterin expression in PCa tissues in humans, and standard doses of chemotherapy can be delivered with OGX-011 at biologically active doses [70,71]. A randomised phase 2 trial of OGX-011 with mitoxantrone or docetaxel in patients with CRPC who had previously progressed on or within 3 mo of completing docetaxel demonstrated tolerability but also interesting antitumour activity [72]. In patients treated with mitoxantrone with OGX-011, a commonly used second-line chemotherapy agent for CRPC, 27% of patients had a PSA decline >50% from baseline, and median OS was 11.4 mo. In those subjects who received OGX-011 with docetaxel, 40% had a >50% decline in PSA from baseline and a median OS of 14.7 months, which is of interest considering that all these patients had previously progressed while receiving or shortly after completing docetaxel therapy. Furthermore, prior studies with cytotoxic agents in the post-docetaxel setting have reported PSA response rates in the 20% range or less and a median OS on the order of 12 mo. Another phase 2 study with OGX-011 randomised 82 patients with chemotherapy-naive metastatic CRPC to receive first-line docetaxel with or without OGX-011 [73]. PSA declines were similar, although there appeared to be fewer patients with progression as best response as well as a longer time to progression in the docetaxel–OGX-011 combination therapy group. A recent press release has reported a median OS of 16.9 mo for the docetaxel group and 27.5 mo for those subjects treated with the docetaxel–OGX-011 combination. A phase 3 trial is being planned comparing docetaxel–OGX-011 versus docetaxel in patients with metastatic CRPC, with a primary endpoint of OS.

7. **Insulin-like growth factor 1 and receptor**

The insulin-like growth factor (IGF) axis is composed of two peptide growth factors (IGF-1 and IGF-2), two transmembrane receptors (IGF-IR and IGF-IIR), six IGF binding proteins (IGFBP-1 to IGFBP-6), and IGFBP proteases. IGFs are synthesized primarily in the liver and have effects on protein and carbohydrate metabolism but also regulate cellular processes of proliferation, differentiation, and apoptosis. These later attributes have resulted in the IGF axis being associated with a critical role in the development of a number of malignancies, including PCa. Higher blood concentrations of IGF-1 and the risk of PC have been correlated in meta-regression analysis, and high plasma IGF-1 and low IGFBP-3 have been associated with more advanced-stage PCa [74,75]. In human primary PCa, IGF-IR, IGF-1, IGF-2, and IGFBP-2 have all been reported to be increasingly expressed over normal prostate tissue and are also increased in advanced and metastatic disease [76]. An increasing body of evidence has linked activation of the IGF axis with androgen-independent progression of PCa [77]. Thus, targeting the IGF axis is an attractive concept for PCa. Preclinical studies have supported this approach using a variety of methods to block IGF signalling [78].

Humanised monoclonal antibodies specific to IGF-IR, selected to have limited agonistic effect or affinity to the homologous insulin receptor, have been particularly effective and have entered clinical testing. Single-agent activity has been observed with IGF-IR blockade in patients with Ewing’s sarcoma, where IGF-1–dependent growth is implicated. Two humanised monoclonal therapeutic antibodies against IGF-IR have entered clinical trials for patients with PCa: CP-751,871 (Pfizer Inc) and IMC-A12 (ImClone Systems Inc). Treatment with both agents has been well tolerated in early clinical studies. A phase 1 combination trial of CP-751,871 with docetaxel demonstrated the tolerability of the regimen, and a randomised, phase 2 trial of docetaxel with or without CP-751,871 has been conducted, with results currently pending (ClinicalTrials.gov identifier: NCT0313781). A single-agent study with IMC-A12 has also been conducted, and initial results are expected in 2009 (ClinicalTrials.gov identifier: NCT00520481).

8. **Bone targeting**

Given the predilection of PCa to metastasize to bone, therapies directed towards the biologic underpinnings of bone progression are a rational treatment direction. Bisphosphonates, which inhibit bone the bone resorbing
activity of osteoclasts by binding to the mineralised bone surface, are already an established treatment for patients with metastatic CRPC, and studies continue to define their use for earlier disease. Additional agents targeting bone metastases–related biologic targets are also under clinical development.

8.1. **RANK ligand**

Receptor activator of nuclear factor κ B (RANK) and its ligand (RANK-L) have been identified as mediators that increase osteoclastogenesis. RANK-L is a member of the TNF superfamily and is expressed by osteoblasts. RANK-L binding to RANK is both necessary and sufficient to stimulate osteoclast cell differentiation and proliferation as well as inhibit osteoclast apoptosis. The effects of RANK are blocked by the secretory glycoprotein osteoprotegerin (OPG), which is a decoy receptor for RANK-L and thus functions to hinder the ability of RANK-L to stimulate bone resorption. The interplay between OPG, RANK-L, and RANK is critical in the pathogenesis of bone metastases [79] and implicated in advanced PCa [80,81].

Denosumab (Amgen Inc.) is a fully humanised monoclonal antibody specifically directed against RANK-L that can be administered via subcutaneous injection. Denosumab has proven activity in reducing bone resorption and increasing bone density compared to placebo or bisphosphonates in patients with physiologic and treatment-related bone loss [82,83]. Treatment with denosumab is well tolerated, with little difference in related adverse event rates between denosumab–treated patients and those treated with placebo; in the trials that have been reported to date, there have been no occurrences of osteonecrosis of the jaw—an infrequent but significant toxicity associated with bisphosphonate usage that can occur in ≤5–10% of patients but appears dependent on the length of exposure [84]. A randomised, double-blind, placebo-controlled trial of denosumab versus zoledronic acid in patients with bone-metastatic CRPC has completed accrual. The primary endpoint of the trial is incidence of skeleton-related events, and final results are expected to be reported after 2010 (ClinicalTrials.gov identifier: NCT00321620).

8.2. **Endothelin-1**

Endothelins (ET) are 21-amino acid peptides that were first described as potent vasoconstrictors but later identified as being elevated in men with metastatic PCa and a mediator of the osteoblastic response of bone to metastatic disease [85]. Three forms of ET have been described (ET-1, ET-2, and ET-3) that bind to two receptors: ET receptor A (ET-A) and ET receptor B (ET-B). ET-1 preferentially binds to ET-A where, in addition to mediating a vasoconstriction response, ET-A signalling has been associated with proliferation, anti-apoptotic effects, and pain. ET-B functions as a decoy receptor and clearance mechanism for ET-1, thus mitigating its effects. ET-1 production by metastatic prostate cells in bone has been shown to be stimulated by osteoblasts, which are in turn stimulated by ET-1 to proliferate, stimulating new bone formation and osteoblastic metastases, contributing to a vicious cycle of progression [86].

Atrasentan (Abbott Laboratories) is an orally bioavailable competitive inhibitor of ET-1, binding with 1800-fold selectivity to the ET-A receptor compared to ET-B. Treatment with atrasentan is well tolerated, and side-effects appear mechanism related to vasodilation and include peripheral oedema, rhinitis, and headache. There have been rare incidences of heart failure, and hypotension and hypotension were dose limiting in phase 1 trials. In a randomised, double-blind, phase 2 study, 288 patients with metastatic CRPC received atrasentan either at a lower dose (2.5 mg), higher dose (10 mg), or placebo [87]. A nonsignificant increase in time to progression was observed in the intention-to-treat population for the 10-mg dosing group (183 vs 137 d). This achieved statistical significance when only per-protocol evaluable patients were considered (196 vs 129 d, P = 0.021, n = 244). Changes in bone deposition and turnover markers were also assessed in this trial and were lower in the atrasentan–treated patients. A multinational, double-blind, placebo-controlled trial was subsequently undertaken [88]. Eight hundred nine patients were randomised to either placebo or atrasentan at 10 mg daily dosing. There was evidence of biologic effect with atrasentan, with a delay in the increase in bone alkaline phosphates as a marker of bone deposition; however, time to progression—the primary endpoint—was not significantly different, with most patients progressing at the first disease assessment at 12 wk into the study. As progression was overwhelmingly defined on radiographic changes as opposed to clinical/symptomatic progression, it is unclear whether the study design allowed for a true assessment of the clinical effects of atrasentan, as the changes in imaging may have been reflecting progression that had occurred between baseline scanning and start of study drug or just after start of study drug. A second phase 3 trial in patients with nonmetastatic CRPC, with progression as the primary endpoint, was also carried out. Similarly, biologic effects were observed, with a decrease in bone alkaline phosphatase and PSA increases; however, time to progression was not different statistically [89].

Development of atrasentan continues in combination with docetaxel. A phase 1–2 trial has been carried out in patients with CRPC, with a maximum tolerated dose of docetaxel identified as 70–75 mg/m² and with atrasentan given at 10 mg daily [90]. Drug-related grade 3–4 toxicities included a fairly high febrile neutropenia rate of 16–25%, and confirmed PSA responses were 23%—lower than what would be expected from prior studies—as was the median OS of 17.6 mo. A phase 3 trial involving 930 patients with metastatic CRPC and a primary endpoint of OS is being conducted by the Southwest Oncology Group (SWOG) comparing docetaxel and prednisone versus docetaxel, prednisone, and atrasentan (ClinicalTrials.gov identifier: NCT00134056).

ZD4054 (AstraZeneca) is a more specific inhibitor of ET-A, with no demonstrated binding to ET-B and thus a potentially more active agent, as the mitigating effects of ET-B on ET-1/ET-A signalling remain unhampered. A randomised, phase 2 trial was carried out comparing
patients who had received ZD4054 at either a 10-mg daily dose, 15-mg dose, or placebo [91]. No difference was observed in the primary endpoint of progression; however, an OS difference emerged in favour of the ZD4054 10-mg and 15-mg treatment groups (24.5 and 23.5 mo, respectively, vs 17.3 mo for placebo). Three phase 3 trials are now enrolling comparing ZD4054 to placebo in patients with CRPC and no clinical metastases (ClinicalTrials.gov identifier: NCT00626548), with bone metastases but with minimal or no symptoms (ClinicalTrials.gov identifier: NCT00554229), and in patients receiving docetaxel chemotherapy (ClinicalTrials.gov identifier: NCT00617669).

8.3. Src family kinases

Src is the prototypical member of the Src family of nonreceptor tyrosine kinases. Src is involved in signal transduction downstream of multiple cell surface receptors, including EGFR, PDGFR, VEGFR, and integrins among others, and thus implicated in multiple cellular processes, including differentiation, proliferation, adhesion, and migration. Src-related kinases, and their upstream cell surface receptors have been associated with PCA progression, and frequently overexpressed. Src signalling is important for normal functioning of osteoclasts and bone resorption as well as osteoblast proliferation and bone deposition, and they are further implicated in bone metastases progression [92].

Dasatinib (Bristol-Myers Squibb Co) was clinically developed for its activity against the BCR-ABL tyrosine kinase associated with chronic myelogenous leukaemia. Dasatinib was subsequently shown to have significant activity against Src family kinases, resulting in direct antitumour effects in preclinical models of PCa, suppressing cell adhesion, migration, and invasion [93]. Phase 2 trials using dasatinib in a twice-daily [94] and once-daily [95] dosing regimen have been carried out in patients with PCa. Only one patient in each of the trials had a PSA response of $>50\%$ decline; however, lesser declines were noted, and decreases in bone turnover markers (serum bone alkaline phosphatase and urinary N-telopeptide) were also observed, providing proof of principal activity data. A randomised phase 3 trial of docetaxel with or without dasatinib is now being conducted, with a primary endpoint of OS and planned accrual of $>1300$ patients (ClinicalTrials.gov identifier: NCT00744497). AZD-0530 (AstraZeneca) is another orally administered Src kinase inhibitor in clinical trials. A phase 2 study is being conducted randomising patients with bone metastases from breast or prostate cancer to receive either AZD-0530 or zoledronic acid (ClinicalTrials.gov identifier: NCT00558272). The primary objective of this study is to compare changes in markers of bone turnover.

9. Conclusions

Over the past two decades, there has been an increased understanding of the biologic underpinnings of cancer in general and PCa specifically relating to androgen-independent progression, immune tolerance, factors affecting cell proliferation and survival, and mediators of metastases. Coupled with rational drug design and more sophisticated trial endpoints, a large number of therapeutic agents have entered into clinical trials over the past decade that promise to significantly change our management of patients with CRPC in the near future. Any benefits that emerge in the late-stage castration-resistant state with these agents would provide a strong rationale for bringing them forward as combination strategies and to earlier disease settings.

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References


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