Current and Emerging Therapies for Bone Metastatic Castration-Resistant Prostate Cancer

Jeremy S. Frieling, David Basanta, PhD, and Conor C. Lynch, PhD

**Background:** A paucity of therapeutic options is available to treat men with metastatic castration-resistant prostate cancer (mCRPC). However, recent developments in our understanding of the disease have resulted in several new therapies that show promise in improving overall survival rates in this patient population.

**Methods:** Agents approved for use in the United States and those undergoing clinical trials for the treatment of mCRPC are reviewed. Recent contributions to the understanding of prostate biology and bone metastasis are discussed as well as how the underlying mechanisms may represent opportunities for therapeutic intervention. New challenges to delivering effective mCRPC treatment will also be examined.

**Results:** New and emerging treatments that target androgen synthesis and utilization or the microenvironment may improve overall survival rates for men diagnosed with mCRPC. Determining how factors derived from the primary tumor can promote the development of premetastatic niches and how prostate cancer cells parasitize niches in the bone microenvironment, thus remaining dormant and protected from systemic therapy, could yield new therapies to treat mCRPC. Challenges such as intratumoral heterogeneity and patient selection can potentially be circumvented via computational biology approaches.

**Conclusions:** The emergence of novel treatments for mCRPC, combined with improved patient stratification and optimized therapy sequencing, suggests that significant gains may be made in terms of overall survival rates for men diagnosed with this form of cancer.

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**Introduction**

Prostate cancer is the second most common cancer in American men with approximately 233,000 newly diagnosed cases in 2014.\(^1\) With an aging population, the incidence of prostate cancer is likely to continue to increase. Patients whose disease is detected at an early stage benefit from a range of treatment strategies, including radiotherapy and prostatectomy, with survival rates near 100%.\(^2\) However, the clinical reality is that many men present with advanced stages of the disease. Currently, the main treatment option for men with advanced cancer is hormone therapy. Historic contributions from Huggins and Hodges\(^3\) in 1941 revealed that removing androgens could inhibit the progression of prostate cancer. These early observations paved the way for the development of androgen-deprivation therapy — either surgically or chemically — which has remained the standard treatment for men with advanced disease for the last 70 years. Despite the initial response to androgen deprivation for most men, the disease typically progresses to a castration-resistant state within 18 to 24 months.\(^4\)

Castration-resistant prostate cancer (CRPC) is defined by disease progression that, despite chemical castration, is often indicated by rising levels of prostate-specific antigen (PSA).\(^5\) The development of resistance to hormonal intervention and why the disease progresses is not fully understood, although some mechanisms have been demonstrated, with the majority focusing on the continued androgen receptor (AR) activity in addition to TMPRSS2/ERG fusion, PTEN, Nkx3.1, and EGR1. As the disease progresses, the CRPC ultimately metastasizes (mCRPC). Patients with mCRPC have a poor prognosis and a predicted survival rate of fewer than 2 years from the initial time of progression, comprising a large portion of the 30,000 prostate cancer-related deaths per year.\(^6,7\) Currently, mCRPC is an incurable disease and represents a major clinical hurdle.

Prostate cancer preferentially metastasizes to bone.\(^8\) As the disease transitions from castration sen-
sensitive to castration resistant, the incidence of bone metastasis increases, with more than 90% of patients with mCRPC developing bone metastases. Patients with mCRPC who are symptomatic are at a high risk for skeletal-related events (SREs), including spontaneous fracture and spinal cord compression, that are a source of significant pain and decreased quality of life. Pain from the metastases is a major component of the disease and is an important aspect to be considered regarding a patient’s treatment regimen. Depending on the level of pain, medications ranging from ibuprofen to morphine are prescribed. Because prostate to bone metastases are primarily bone-forming sclerotic lesions, bone scanning using technetium-99m is often preferred for diagnosis due to the incorporation of the radionuclide tracer into regions of new bone formation by osteoblasts. Magnetic resonance imaging (MRI) and positron emission tomography (PET)/computed tomography (CT) are also used for detection. A trial comparing 18F-sodium fluoride PET/CT, 18F-fluorodeoxyglucose PET/CT, MRI, and technetium-99m identified strengths for each modality. However, the ability to detect occult or micrometastases less than 5 mm remains a current limitation for each imaging technique.

**Approved Therapeutic Options**
Currently, mCRPC remains incurable, and many treatment options are palliative in nature. However, the treatment landscape of mCRPC is expanding both in broad-spectrum and targeted therapies that are likely to positively impact overall survival rates within the next decade. This expansion began with docetaxel, which, in 2004, was the first therapy to provide improved survival rates to patients with mCRPC. However, many patients develop resistance. To combat this issue, 5 new agents have received approval by the US Food and Drug Administration (FDA) to treat...
mCRPC since 2010 (abiraterone acetate, enzalutamide, cabazitaxel, radium-223, and sipuleucel-T). Some of these agents may be administered in combination with steroids, such as prednisone, which has been shown to decrease testosterone levels and reduce tumor growth as well as counteract adverse events (eg, nausea, allergic reactions, inflammation, pain). Recently FDA-approved agents that target the cancer and host compartments are discussed below and are also illustrated in Fig 1.

### Targeting Metastatic Castration-Resistant Prostate Cancer Cells

One of the defining measures of mCRPC is resistance to androgen deprivation. The mechanism of castration resistance is not fully understood but inroads have been made. For example, prostate cancer cells circumvent castration by overexpressing and increasing the sensitivity of the AR to residual androgens, acquiring AR gene mutations that lead to functional gain or promiscuous ligand interactions, splice variants resulting in constitutive AR activation, and post-translational modifications affecting the stability, localization, and activity of the receptor. Alternative methods utilized by prostate cancer cells to synthesize dihydrotestosterone (DHT) have also been shown to circumvent androgen deprivation methods. Efforts to target DHT synthesis have resulted in FDA-approved androgen deprivation therapy (ADT) options. Abiraterone acetate is one such option that works by inhibiting the activity of the CYP17A1 enzyme, thereby preventing androgen synthesis. Abiraterone has improved the overall survival and radiographic progression-free survival rates of men with mCRPC. Another therapeutic strategy for preventing androgen utilization by mCRPC cells is to directly target the AR with reagents such as flutamide, nilutamide, and bicalutamide. Enzalutamide was recently approved for the treatment of mCRPC in a postdocetaxel setting without the administration of corticosteroids. Enzalutamide has a superior affinity to the AR compared with other AR antagonists and works by preventing nuclear translocation of the receptor, DNA binding, and recruitment of coactivators of the AR to increase overall survival rates and delay the onset of SREs. Results of a phase 3 trial demonstrated enzalutamide activity in patients naive to chemotherapy, and FDA approval of enzalutamide as a first-line therapeutic option for mCRPC may be on the horizon.

A list of approved therapies for the treatment of mCRPC appears in Table 1. Cabazitaxel is a more recent derivative of the taxoids that has shown increases in overall survival rates, improvements in progression-free survival rates, and improved PSA response rates in men with mCRPC. Cabazitaxel-associated toxicities were minor, leading to the FDA approval of the therapy for the treatment of patients with mCRPC after treatment with docetaxel.

### Targeting the Microenvironment

Given the heterogeneity of mCRPCs and the likelihood of ADT/chemotherapy resistance, targeting the genetically stable host microenvironment supporting the mCRPC represents an attractive treatment approach. Immune evasion is a hallmark of cancer progression, and the goal of sipuleucel-T is to make mCRPC more visible to cytotoxic T cells. Sipuleucel-T is an autologous immunotherapy approved for the treatment of asymptomatic or minimally symptomatic mCRPC. Sipuleucel-T harnesses the properties of the patient’s immune system by collecting peripheral blood mononuclear cells and activating them ex vivo by exposing them to a fusion protein consisting of prostatic acid phosphatase (PAP; commonly expressed by prostate cancer cells) and granulocyte-macrophage colony-stimulating factor. Patients receive 3 separate infusions of the activated cells at 2-week intervals to generate PAP-expressing dendritic cells that activate T cells to recognize and eliminate PAP-expressing prostate cancer cells.

Most mCRPCs arise in the bone matrix where they induce extensive bone remodeling by stimulating osteoblasts and osteoclasts. The process promotes the growth of the mCRPCs via the solubilization of bone matrix–sequestered growth factors, causing

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### Table 1. Approved Therapies for the Treatment of Metastatic Castration-Resistant Prostate Cancer

<table>
<thead>
<tr>
<th>Drug</th>
<th>Target</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abiraterone acetate</td>
<td>CYP17A1</td>
<td>Reduces circulating testosterone levels</td>
</tr>
<tr>
<td>Cabazitaxel</td>
<td>Microtubules</td>
<td>Microtubule stabilization, disrupts cell cycle</td>
</tr>
<tr>
<td>Denosumab</td>
<td>RANKL</td>
<td>Decreases bone resorption</td>
</tr>
<tr>
<td>Docetaxel</td>
<td>Microtubules</td>
<td>Microtubule stabilization, disrupts cell cycle</td>
</tr>
<tr>
<td>Enzalutamide</td>
<td>AR</td>
<td>AR antagonism, prevents signaling</td>
</tr>
<tr>
<td>Radium-223</td>
<td>Bone</td>
<td>Localized radiation</td>
</tr>
<tr>
<td>Sipuleucel-T</td>
<td>Ex vivo activation of PBMCs via GM-CSF and PAP</td>
<td>T-cell activation</td>
</tr>
<tr>
<td>Zoledronic acid</td>
<td>Osteoclasts</td>
<td>Decreases bone resorption</td>
</tr>
</tbody>
</table>

AR = androgen receptor, GM-CSF = granulocyte-macrophage colony-stimulating factor, PAP = prostatic acid phosphatase, PBMC = peripheral blood mononuclear cell, RANKL = receptor activator of nuclear κB ligand.
pain and SREs (eg, pathological fractures). Therefore, preventing the interaction of cancer and bone has been a major focus of treatment for several decades. Bisphosphonates, such as zoledronic acid, are reagents that can “stick” to bones undergoing remodeling; upon resorption by osteoclasts, they can induce apoptosis and limit the amount of cancer-induced bone disease. In the clinical setting, zoledronic acid has demonstrated a benefit for patients with mCRPC by delaying the time to SRE incidence. However, no increase in overall survival rates has been demonstrated. Receptor activator of nuclear κB ligand (RANKL) is a molecule critical for the maturation and activation of bone-resorbing osteoclasts. Denosumab is a fully humanized monoclonal antibody that prevents RANKL interaction with the RANK receptor. For patients with bone mCRPC, a significant delay has been demonstrated in the time to first SRE compared with zoledronic acid. Evidence suggests that denosumab may have direct effects on tumor burden, particularly tumor cells expressing RANK. Furthermore, preclinical in vivo animal studies have highlighted the efficacy of docetaxel/denosumab treatment in increasing median survival rates, suggesting that combination approaches with denosumab could enhance the overall survival rates of men with mCRPC.

At the time of publication, the most recent agent to receive FDA approval for mCRPC is radium-223. The bone-seeking properties of radium-223 (and other similar radiopharmaceuticals) make it useful for the treatment of bone metastases. Although most radiopharmaceuticals emit β particles, radium-223 emits α particles to deliver more localized radiation...
(< 100 µm distance) to induce cell death via DNA damage.\textsuperscript{37} In a study of men with mCRPC previously treated with radiotherapy, radium-223 showed improved rates of overall survival, time to PSA progression, and reduced alkaline phosphatase levels (a measure of bone remodeling).\textsuperscript{38} In addition, radium-223 delays the time to first SRE.\textsuperscript{35} Previous radiopharmaceuticals used to treat mCRPC were effective at reducing pain alone. Therefore, radium-223 represents an important step forward for the field.\textsuperscript{46}

Emerging Therapeutic Options

Despite the growing number of FDA-approved agents to treat mCRPC, room remains to improve upon the therapeutic options available to patients and clinicians. For example, although approximately 50% of patients with mCRPC will respond to docetaxel, most patients develop resistance and disease progression within 1 year of beginning treatment.\textsuperscript{36} However, some treatments that target cancer and support the microenvironment are currently in clinical trials that have the potential to provide health care professionals with new therapeutic options to treat men diagnosed with mCRPC (see Fig 1). A list of these experimental therapies appears in Table 2.\textsuperscript{32,48-57}

Orteronel

Similar to abiraterone acetate, orteronel inhibits CYP17A1 to reduce circulating levels of testosterone. However, orteronel possesses specificity toward lyase activity, leaving the synthesis of adrenal corticosteroids unaltered.\textsuperscript{17,20} Therefore, orteronel is less likely than abiraterone acetate to require the concomitant administration of corticosteroids.\textsuperscript{25,58} Phase 2 trials demonstrated a significant reduction in serum levels of PSA that led to 10 partial responses and 22 cases of stable disease in 51 patients.\textsuperscript{59} Decreases in circulating tumor cells were also observed, thus serving as a further indication of efficacy. Based on these positive data, phase 3 trials were initiated; however, the results of one of those phase 3 trials indicated that orteronel administered in combination with prednisone failed to significantly impact overall survival rates compared with placebo but did provide a benefit in radiographic progression free survival rates in both chemotherapy naive and postchemotherapy mCRPC.\textsuperscript{48,49}

<table>
<thead>
<tr>
<th>Drug</th>
<th>Target</th>
<th>Effect</th>
<th>Study Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cabozantinib</td>
<td>c-MET, VEGF-R2</td>
<td>Inhibits tyrosine kinase activity</td>
<td>Partial resolution of bone lesions, decreases number of CTCs, decreases pain\textsuperscript{51}</td>
</tr>
<tr>
<td>Custirsen</td>
<td>Clusterin</td>
<td>Improves response to docetaxel</td>
<td>Extended median survival, extends PFS, improves PSA declines\textsuperscript{10}</td>
</tr>
<tr>
<td>Ipilimumab</td>
<td>CTLA-4</td>
<td>T-cell activation</td>
<td>Ongoing\textsuperscript{44,55}</td>
</tr>
<tr>
<td>Nivolumab</td>
<td>PD-1</td>
<td>T-cell activation</td>
<td>Ongoing\textsuperscript{43}</td>
</tr>
<tr>
<td>Orteronel</td>
<td>CYP17A1 (17,20 lyase activity)</td>
<td>Reduces circulating testosterone levels</td>
<td>Decreases number of CTCs, improves radiographic PFS\textsuperscript{48,49}</td>
</tr>
<tr>
<td>Prostvac-VF</td>
<td>Delivery of PSA transgene</td>
<td>T-cell activation</td>
<td>Improves median survival\textsuperscript{12,58}</td>
</tr>
<tr>
<td>Tasquinimod</td>
<td>Thrombospondin S100A9</td>
<td>Antiangiogenic, reduces MDSC recruitment</td>
<td>Improves median PFS, stable bone alkaline phosphatase levels\textsuperscript{10,57}</td>
</tr>
</tbody>
</table>

CTC = circulating tumor cell, CTLA = cytotoxic T-lymphocyte antigen 4, MDSC = myeloid-derived suppressor cell, PD-1 = programmed cell death 1, PFS = progression-free survival, PSA = prostate-specific antigen, VEGF-R2 = vascular endothelial growth factor receptor 2.
to decrease or discontinue narcotics. An additional exploratory analysis updated the results of this phase 2 trial and indicated a reduction of more than 30% in the bone scan lesion area and also indicated a reduction in circulating tumor cells. Multiple phase 3 trials focused on the treatment of mCRPC with cabozantinib are either ongoing or in the recruiting stages (NCT01428219, NCT01703065, NCT01995058, NCT01605227, NCT01834651, NCT01599793, NCT01522443, NCT01683994). At the time of publication, NCT01605227 failed to reach efficacy in men with mCRPC.

**Custirsen:** Custirsen is an antisense oligonucleotide that targets clusterin, a chaperone induced by stress and detected at elevated levels in several tumor types, including prostate cancer. Studies of clusterin have demonstrated its antiapoptotic and prosurvival activities in prostate cancer that are believed to be associated with docetaxel resistance. As such, inhibiting clusterin concomitantly with docetaxel may increase the time until docetaxel resistance in mCRPC. Phase 2 trials of weekly intravenous custirsen plus docetaxel extended median survival rates from 16.9 months to 23.8 months compared with single-agent docetaxel. Subsequent to treatment, significant decreases in clusterin levels were noted in patients treated with custirsen. A second phase 2 trial evaluating custirsen plus prednisone compared with mitoxantrone plus prednisone in patients with mCRPC who previously failed first-line docetaxel showed an increase of 4.3 months in median overall survival and a 3.8-month increase in progression-free survival as well as improved declines in PSA. Phase 3 trials of custirsen are ongoing (NCT01578655), although its benefits may be limited to patients expressing high levels of clusterin.

**Prostvac-VF:** The use of cancer vaccines aims to generate an immune response to specific tumor antigens. The Prostvac vaccine uses a fowlpox and vaccinia platform to deliver the PSA transgene to antigen-presenting cells, which, in turn, express and present the antigen to T cells and T-cell activation. In addition to PSA, the vaccine has been engineered to include B7-1, ICAM-1, and LFA-3 antigen-presenting cell costimulatory molecules. Phase 2 trials in patients with mCRPC have shown improvements of 8 to 9 months in median survival rates. The results of these trials suggest that Prostvac offers an improvement compared with sipuleucel-T and have resulted in the initiation of a phase 3 trial (NCT01322490).

**Nivolumab:** Blocking the programmed cell death 1 (PD-1)/programmed death ligand 1 (PD-L1) immunosuppressive axis has received much attention in recent years. Nivolumab is a monoclonal antibody that inhibits the interaction between PD-L1 and T-cell expressed PD-1, preventing tumor-induced loss of T-cell effector function. In trials of melanoma, 80% of patients responded to nivolumab therapy. However, limited studies in CRPC have not been as promising; phase 1 studies have failed to reach objective responses and others have shown limited or lack of PD-L1 expression by CRPCs or the immune infiltrates. However, it is possible that prospective, individual patients with mCRPC with high levels of PD-L1 could benefit from nivolumab.

**Ipilimumab:** As cancer progresses, it can express inhibitory ligands such as B7-1, B7-2, and PD-L1 to suppress the immune system. Ipilimumab is a monoclonal antibody that inhibits T-cell–expressed cytotoxic T-lymphocyte antigen 4 from interacting with antigen-presenting cell B7-1 and B7-2 ligands but not those on tumor cells, allowing for the continued immune-mediated destruction of tumor cells. Ipilimumab has been studied in melanoma and is the only FDA-approved immune checkpoint inhibitor on the market. Despite encouraging results in early clinical trials, the results of a phase 3 trial of patients with mCRPC receiving bone-directed radiotherapy prior to 10 mg/kg ipilimumab or placebo revealed no significant improvement in overall survival rates. However, individual analysis of patient subsets indicated that ipilimumab may benefit men with low disease burden, thus emphasizing the importance of appropriate patient selection.

**Therapeutic Opportunities on the Horizon**

Treatment options to extend the overall survival of patients diagnosed with mCRPC remains a major clinical challenge. Therefore, understanding the factors that drive the process of metastasis, the homing of the metastasis to organs (eg, bone), and how prostate cancer cells form life-threatening active metastases once in the bone warrants extensive research to generate new therapies to cure the disease. Although metastasis is classically thought of as a linear sequence of events beginning with the dissemination and invasion of tumor cells from the primary site and ending with proliferation at the metastatic site, recent evidence suggests that the first steps of metastasis can occur before a patient’s tumor is diagnosed (Fig 2). This “step 0” of the metastatic cascade results in the nonrandom priming of future sites of metastasis, a concept known as the “premetastatic niche.”

**Premetastatic Niche**

Primary tumor-derived factors have been implicated in the development of premetastatic niches in distant organs. Through a series of in vivo experiments, it was illustrated that conditioned media derived from highly metastatic cancer cells lines, such as the B-16 melanoma cell line, could stimulate the mobilization of bone marrow–derived VEGF receptor 1+ VLA4+ Id3+...
hematopoietic precursor cells to develop premetastatic niche sites, including the lungs, liver, spleen, kidney, and testes. Cancer-derived exosomes have been implicated as the mechanism for facilitating long distance, tumor-stroma interactions and initiating the premetastatic niche. Exosomes are microvesicles measuring 30 nm to 100 nm that contain a variety of functional proteins and messenger/micro RNAs. In the context of premetastatic niche formation, B16-F10–derived exosomes have been labeled and shown to "home" to common sites of melanoma metastasis. Furthermore, in the premetastatic niche, exosomes can educate bone marrow–derived cells to support metastatic tumor growth via the horizontal transfer of the c-MET protein. c-MET inhibitors, such as cabozantinib, could be used to prevent the development of premetastatic niches and, thus, mitigate the ability of cancers to metastasize to new sites.

Exosome shedding has also been demonstrated in prostate cancer, and studies have shown the presence of microvesicles termed oncosomes (0.5–5 µm) in prostate cancer–conditioned media. Oncosomes contain a variety of signal transduction proteins, including Akt and Src, and can interact with tumor and stromal cells to elicit disease-promoting responses. In addition, a correlation exists between a Gleason score higher than 7 and the number of oncosomes present in patient plasma. Based on these findings, it is plausible that prostate cancer–derived exosomes can play a role in the formation of premetastatic niches in the bone microenvironment. Emerging evidence also suggests that prostate cancer cells homing to the bone microenvironment can occupy the endosteal niche, the vascular niche, or both.

Defining Factors Controlling the Homing of Bone Metastatic Castration-Resistant Prostate Cancer

An unsolved question regarding metastasis is why prostate cancer has such a predilection for the bone microenvironment. More than a century ago, Paget formulated the "seed and soil" hypothesis to address this question. His hypothesis suggested that metastasis is a challenging process that requires "fertile soil" for outgrowth but begins long before the "seed" meets the "soil." Ewing challenged Paget's hypothesis in the 1920s, proposing that metastasis was instead dependent on anatomy, vasculature, and lymphatics. Metastasis by anatomy would become the accepted model until the 1970s when modern experiments kindled interest in the "seed and soil" hypothesis, notably observing that circulating tumor cells reach the vasculature of all organs, but only certain organs are receptive for metastasis. In reality, prostate to bone metastasis occurs by a blend of both hypotheses: It metastasizes first to the pelvic lymph node and then to sites in the bone, including iliac crests, sacrum wings, L1 to L5 vertebrae, T8 to T12 vertebrae, ribs, manubrium, humeral heads, and femoral necks. Although 15% to 30% of prostate to bone metastases are due to cells traveling through the Batson plexus to the lumbar spine, it is clear that molecular factors, such as chemokines and integrins, underpin the propensity for prostate cancer cells to metastasize to the skeleton. Elucidating those factors could help identify new therapies to prevent bone metastatic CRPC.

Bone is the home of regulatory sites for hematopoietic stem cells (HSCs), which are cells localized to the vascular and endosteal niches where they either await hematopoietic demand or reside in a quiescent state. One well-defined signaling axis implicated in metastasis is that between stromal cell–derived factor 1/CXCL12 and its receptor CXCR4, a system normally utilized by HSCs homing to the niche. CXCL12 expression is increased in the premetastatic niche, and studies in prostate cancer have demonstrated that tumor cells with high bone-homing capacity express CXCR4 and CXCR7 to parasitize the HSC niche. Furthermore, CXCR4 expression correlates with poor prognosis. Additional axes, including MCP-1/CCR2 and CXCL16/CXCR6, have also been found to contribute to the progression of prostate cancer through increases in proliferation, migration, and invasion.

Disseminated Tumor Cells and Dormancy

Evidence suggests that tumor cells disseminated from the prostate localize to the bone marrow niche, displace HSCs, and either proliferate to form a metastatic mass or enter a state of dormancy. Dissemination from the primary site to reside in distant environments is an early event seen in prostate cancer, as patients who undergo prostatectomy may present with metastases many years later. Disseminated tumor cells (DTCs) reside in the bone marrow niche where they can remain dormant and resistant to chemotherapy for long periods of time (> 10 years) before emerging to form metastatic outgrowths. Although most patients with prostate cancer harbor DTCs, not all will develop metastases, suggesting that mechanisms exist to maintain DTC dormancy as well as to promote awakening.

Several bone marrow–dependent mechanisms have been identified as modulators of prostate cancer DTC dormancy. In the endosteal niche, the osteoblast expression of Anxa2 combined with the expression of the Anxa2 receptor (Anxa2R) by HSCs is important in regulating HSC homing to the niche. Anxa2R expression is elevated in metastatic prostate tumor cells and, as such, the Anxa2/Anxa2R axis can be hijacked to promote the homing of prostate tumor cells to the niche. Interrupting the interaction between Anxa2 and Anxa2R is sufficient to reduce tumor burden in the niche. Evidence has revealed that the ligation of Anxa2 with Anxa2R stimulates the expression of...
the Axl receptor tyrosine kinase.\textsuperscript{97} Axl, along with Tyro3 and Mer, are receptors for osteoblast-expressed growth arrest-specific 6 (GAS6).\textsuperscript{98} As was the case with Anxa2/Anxa2R, the GAS6/Axl interaction typically occurs between HSCs and osteoblasts and is one mechanism of controlling HSC dormancy.\textsuperscript{99} Engaging osteoblast-expressed GAS6 and tumor cell–expressed Axl yields a similar result that includes growth arrest and enhanced drug resistance in prostate cancer cells.\textsuperscript{97} Following-up on these observations, data show that these activities may be specific to the Axl receptor compared with other GAS6 receptors.\textsuperscript{98} A high ratio of Axl to Tyro3 expression encourages maintenance of a dormant state, whereas reducing the expression of Axl and increasing the expression of Tyro3 has been shown to promote outgrowth.\textsuperscript{98}

Interactions between osteoblasts and tumor cells may be important to DTC dormancy. Prostate cancer cells that bind with osteoblasts also upregulate the expression of TANK-binding kinase 1 (TBK1). In vitro and in vivo knockdown of TBK1 resulted in decreased drug resistance, suggesting that TBK1 may also play a role in dormancy and drug resistance.\textsuperscript{100} A high p38:ERK ratio has been shown to maintain dormancy of squamous carcinoma cells, whereas interactions with the microenvironment can stimulate a switch to high ERK:p38 and reverse dormancy.\textsuperscript{101} Bone marrow–derived transforming growth factor (TGF) \(\beta\)2 has been implicated in maintaining the dormancy of DTCs by p38 activation, and inhibiting either the TGF-\(\beta\) receptor 1 or p38 leads to the proliferation and metastasis of DTCs.\textsuperscript{102} Similarly, bone morphogenetic protein 7 triggers prostate cancer DTC dormancy in part by activating p38.\textsuperscript{103}

Although much focus has been on the endosteal niche, the vascular niche also has implications for DTC dormancy. Through the use of advanced imaging techniques, dormant DTCs have been shown to home to perivascular niches in the bone marrow and the lungs.\textsuperscript{104} These niches promote dormancy through the expression of TSP-1; however, dormancy is lost in regions of sprouting vasculature due to a loss of TSP-1 and the activation of TGF-\(\beta\) and periostin.\textsuperscript{104}

In vivo experiments in mice receiving bone marrow transplantation revealed that fewer HSCs successfully engraft in tumor-bearing mice, suggesting that the tumor cells occupying the niche outcompete HSCs for residence.\textsuperscript{105} In addition, expanding the endosteal osteoblast niche with parathyroid hormone (PTH) promoted metastasis, whereas decreasing the size of the niche using conditional osteoblast knockout models reduced dissemination.\textsuperscript{105} Tumor cells can also be forced out of the niche using methods to mobilize HSCs, perhaps offering an opportunity for therapeutic intervention.\textsuperscript{105} Filgrastim is an agent that mobilizes HSCs out of the niche, and plerixafor blocks the interaction with stromal cell–derived factor 1 by acting as a CXCR4 antagonist to mobilize HSCs.\textsuperscript{106} Both agents have been approved by the FDA and may serve as a method of awakening and forcing the DTCs into circulation where they would become vulnerable to chemotherapy. A small molecule inhibitor specific to CXCR6 but not other chemokine receptors was developed for investigating the CXCL16/CXCR6 axis.\textsuperscript{107} Although the clinical utility of such an inhibitor must be investigated, the selectivity of small molecule antagonists could aid in the targeting of dormant tumor cells.

**Therapeutic Opportunities for “Active” mCRPC**

Although therapies to prevent the homing and establishment of mCRPC in the bone microenvironment are important clinical tactics, many patients in the clinical setting present with “active” bone metastases that cause extensive bone remodeling. Defining the mechanisms that control cell–cell communication between the metastases and the microenvironment are also likely to reveal important therapeutic targets.

**Osteomimicry:** A recurring theme in bone metastasis is the hijacking of normal bone mechanisms by tumor cells. The concept of osteomimicry is that bone metastatic prostate cells acquire the ability to produce proteins typically restricted to bone cells, such as osteoblasts, to survive and proliferate in the otherwise restrictive bone microenvironment.\textsuperscript{108} Select genes normally expressed in bone have been detected in prostate cells, including osteocalcin, osteopontin, bone sialoprotein, osteonectin, RANK, RANKL, and PTH-related protein.\textsuperscript{108,111} The expression of these genes appears to be associated with the metastatic capacity of the cells. Studies in both the PC3 and LNCaP cell lines have shown that the expression of osteonectin is highest in the more invasive and metastatic sublines, including the LNCaP metastatic variant C4-2B.\textsuperscript{109} Analysis of patient samples support these findings, showing that osteonectin staining in prostate to bone metastases was more intense than from soft-tissue metastases.\textsuperscript{109} In addition to changes in gene expression, prostate tumor cells may adopt biological activities usually specific to bone cells. In vitro studies indicate that human C4-2B prostate tumor cells are capable of depositing hydroxyapatite and contributing to mineralization, a common feature of the sclerotic lesions observed in vivo.\textsuperscript{110}

Due to the shared expression of specific bone genes between tumor and stroma cells, these common proteins could be used to simultaneously target both compartments. Understanding that soluble factors like bone morphogenetic protein 2, RANKL, TGF-\(\beta\), granulocyte colony-stimulating factor, and granulocyte-macrophage colony-stimulating factor are partially responsible for inducing osteomimetic genes may also provide options to specifically target osteomimicry and establish bone outgrowths.\textsuperscript{111} It has
been suggested that promoters for the common genes between the tumor and stroma cells could be utilized to drive the expression of therapeutic genes; thus targeting both the stroma and tumor cells.108

Halting the Vicious Cycle of Bone Metastases:

Once the DTCs awaken and establish micrometastases, continued outgrowth arises through the interaction with multiple stromal cell types, growth factors, and enzymes in a process known as the vicious cycle model.112 Prostate to bone metastases are characterized by areas of mixed osteogenesis and osteolysis that give rise to painful lesions.113 A number of tumor-derived factors, including PTH-related protein, interleukin (IL) 1, IL-6, and IL-11, have been shown to interact with osteoblasts and stimulate the production of RANKL.114 RANKL is a crucial molecule for osteoclast differentiation; therefore, it contributes to the extensive bone remodeling seen in bone metastasis. In addition to bone destruction, osteoclast-mediated bone resorption also releases a multitude of bone-derived factors such as TGF-β, insulin growth factor, platelet-derived growth factor, and fibroblast growth factor. These factors provide positive feedback via interaction with their respective receptors on the surface of tumor cells, thus promoting the proliferation and continued production of tumor-derived factors.111 The vicious cycle is continually evolving to include other cell types, cytokines, proteases, and therapeutics.115-118 Several studies have shown contributory roles for highly expressed host matrix metalloproteinases (MMPs) in the vicious cycle, including the regulation of latent TGF-β and VEGF-A bioavailability by MMP-2 and MMP-9, and the generation of a soluble form of RANKL by MMP-7, which promotes osteoclastogenesis and mammary tumor-induced osteolysis in vivo.119-121 In recent years, the interactions with immune cells have become an integral part of the vicious cycle. For example, T cells stimulate and inhibit the formation of osteoclasts, and the recruitment of regulatory T cells to bone marrow may inhibit osteoclastogenesis. Myeloid-derived suppressor cells suppress T cells and release angiogenic, tumor-promoting factors. Recruited myeloid-derived suppressor cells have also been shown to differentiate into osteoclasts.118

Although the need for therapies aimed at the early stages of metastasis has been emphasized, patients will still present in the later stages of the disease; therefore, improving therapies for these patients must still remain a priority. The interactions between tumor and stromal cells in the vicious cycle model offer many opportunities to intervene. Therapies such as zoledronic acid and denosumab interfere with the osteolytic component of the vicious cycle; however, therapies to inhibit the unique osteosclerotic component of prostate to bone metastases are lacking. Many roles for specific MMPs have been elucidated in the vicious cycle,115,120,121 and the development of MMP inhibitors with improved specificity is perhaps a promising method to modulate the vicious cycle.122

From these discoveries, it is becoming evident that the metastasis of prostate cancer is not a linear, stepwise procedure. Defining the mechanisms that control CRPC metastasis may help elucidate new therapeutic targets that directly impact the cancer cells and the processes that facilitate the formation of a premetastatic niche, niche seeding, dormancy, and the vicious cycle.123 Such new discoveries are highly likely to impact the clinical treatment of patients with mCRPC.

Upcoming Challenges

Our knowledge of the mechanisms driving the progression of prostate cancer is growing. Although several new therapies that target both the cancer cells and the supporting microenvironment and are likely to increase overall survival rates for men with mCRPC, new challenges are also emerging, particularly within the context of tumor heterogeneity. Heterogeneity is a key aspect of cancer evolution and is a clinical reality in many cancers, including prostate cancer.124-126 Greater heterogeneity facilitates the evolution of the treatment resistance of cancer but also gives the cancer a number of phenotypic strategies that allow for growth in select microenvironments (eg, bone).

Emerging studies suggest that most patients would be best served by therapies tailored toward cancer cells harboring common aberrations as well as by therapies geared toward smaller subpopulations who could potentially become the dominant-resistant population.127 The therapies described herein constitute new ways in which to expand the number of potential options for the treatment of heterogeneous bone metastatic CRPCs. However, a challenge emerging with the advent of these therapies is how to rationally design a treatment strategy for individual patients. Current guidelines from the National Comprehensive Cancer Network provide recommendations for applying the sequence of existing therapies to patients with mCRPC based on individual patient parameters. However, some studies suggest that altering the sequence or the combination of existing therapies can have a profound impact on overall survival rates.128 To circumvent costly and time-consuming clinical trials assessing the combination and sequence alterations of a new line of targeted therapies currently in clinical trials, alternative approaches are required. In this regard, integrating computational models and genetic algorithms with individual patient-derived biological data might lead to the rapid optimization of therapy choice and sequence. In the preclinical setting, the power of this integrated approach has been demonstrated. Recent studies have discovered how appropriate drug combinations guided by com-
Conclusions

Metastatic castration-resistant prostate cancer is an incurable disease, but the advent of new therapies, combined with an enhanced understanding of the underlying biology, suggests that significant improvement in overall survival is within reach. An increase in the number of available treatment options will be challenging from a clinical perspective with regard to patient stratification and in selecting the optimal therapy sequence, combination, or both. However, integrating computational models and genetic algorithms based on individual patient data may help overcome this challenge and allow for the delivery of individualized treatment for patients with this disease.

References


