Targeting Bone Metastatic Castration-Resistant Prostate Cancer

Leah M. Cook, PhD, and Conor C. Lynch, PhD

Abstract

Metastatic castration-resistant prostate cancer (mCRPC) is incurable and typically manifests in the skeleton. In the bone microenvironment, prostate cancer cells survive by promoting bone remodeling, resulting in the release of factors that drive CRPC growth. These lesions contain areas of extensive bone destruction and formation that can lead to pathological fracture, thereby greatly contributing to patient morbidity and mortality. Therapies that can treat the disease and extend overall survival are an urgent clinical need. To this end, recent advances in our understanding of how mCRPC survives and grows in bone have contributed to the development of promising therapeutics. Here, we briefly review current standard of care therapies, ongoing trials, and novel therapies for the treatment of mCRPC.

Introduction

Metastasis is responsible for more than 90% of cancer-related deaths. Prostate cancer is no exception, with approximately 26,120 men expected to succumb to the disease due to complications of metastasis in 2016.[1] Of these, it is expected that more than 90% will have evidence of skeletal lesions.[2] The median survival time for patients with active metastatic castration-resistant prostate cancer (mCRPC) is approximately 3 years. Understanding how metastatic prostate cancer cells grow and interact with the surrounding tumor microenvironment can identify key circuits driving the progression of the disease.[3] Research in this area has revealed targets for therapeutic intervention, the translation of which should enhance the overall survival (OS) of patients with mCRPC.

Androgen Deprivation Therapy (ADT) for Bone mCRPC

The National Comprehensive Cancer Network suggested guidelines[4] for the treatment of men given a diagnosis with bone mCRPC are immunotherapy (sipuleucel-T) followed by androgen deprivation therapy (ADT; abiraterone acetate and enzalutamide), chemotherapy (docetaxel with prednisone), radiopharmaceutical therapy (radium 223), suggestion of a clinical trial, or a potential secondary hormone therapy such as ketaconozole.

The upregulation of pathways involved in androgen synthesis or mutations/amplification in the androgen receptor (AR) itself allows cancer cells to continue feeding on androgens despite the systemic depletion of the ligand. Further, androgen interaction with AR-expressing bone-building osteoblasts promotes differentiation and bone formation.[5,6] Given the reliance of mCRPC cells on androgen for growth in bone, inhibitors that block androgen synthesis or the activity of mutant AR remain an intense area of investigation and clinical trial activity. For example, CYP17A1 is an important enzyme used by CRPC cells for the de novo synthesis of androgens, and this discovery led to the genesis of abiraterone, a small molecule inhibitor of CYP17A1 activity.[7] Abiraterone given in combination with prednisone, a corticosteroid, was first shown to increase the median OS by 4.6 months, compared with placebo plus prednisone, in mCRPC patients who had previously received docetaxel.[8] Median OS was increased to three years in chemotherapy-naïve patients, compared with placebo.[9] Additionally, abiraterone was shown to also significantly delay the time to first skeletal-related event (SRE).[10,11]

Enzalutamide, an AR antagonist, was first shown to increase median OS by 4.8 months in mCRPC patients who had previously received docetaxel, compared with the placebo group.[12] The time-to-disease progression, measured by prostate-specific antigen (PSA) levels, was increased by 5.3 months and radiographic progression-free survival (PFS) increased by 5.4 months, compared with placebo. In a phase III randomized trial, enzalutamide increased the time to the first occurrence of an SRE, suggesting an impact on disease progression in bone.[13] Given the success of these ADTs as single agents, they are now being investigated for their efficacy together or when combined with other therapies (Table).

Building on this approach, galeterone, a novel dual small molecule inhibitor of CYP17A1 and AR was compared with enzalutamide alone in clinical trials (ARMOR3-SV). The major endpoint for mCRPC patients was radiographic PFS but in July 2016, the trial was halted due to predicted failure to meet this goal.[14] The drug, however, remains in phase II clinical trials examining safety and response of the patients that have progressive CRPC but have failed oral therapy (ARMOR2; NCT01709734). Other AR and CYP17A1 targeted inhibitors, such as apalutamide and seviteronel, remain the

TABLE 1. An Overview of Ongoing Clinical Trials for Bone Metastatic CRPC (www.clinicaltrials.gov)

Drug	Class/Type	Target	Trial (Phase, Combination Drug)
Abiraterone acetate	Hormone therapy	CYP17A1	NCT02036060 (Phase 2, +/-Docetaxel); NCT01949337 (Phase 3, +/-Enzalutamide); NCT01972217 (Phase 2, +/- Olaparib); NCT01487863 (Phase 2, +/- Sipuleucel-T); NCT02415621 (Pilot-Adaptive Therapy)
Enzalutamide (MDV3100)	Hormone therapy	AR	NCT02116582 (Phase 4, post-abiraterone)
Galeterone	Hormone therapy	CYP17A1 and AR	NCT01709734 (Phase 2)
EPI-506	Hormone therapy	N-terminus of AR	NCT02606123 (Phase 1/2)
Seviteronel (VT-464)	Hormone therapy	CYP17A1 and AR	NCT02445976 (Phase 2); NCT02130700 (Phase 2); NCT02012920 (Phase 1/2)
Apalutamide (ARN-509)	Hormone therapy	AR	NCT02123758 (Phase 1, +Abiraterone or +Prednisone); NCT02106507 (Phase 1, +Everolimus); NCT01171898 (Phase 1/2); NCT01792687 (Phase 1)
Dovitinib	TKI	FGFR3	NCT01994590 (Phase 2, +Abiraterone/Prednisone)
Dasatinib	TKI	Bcr-Abl and SRC	NCT01685125 (Phase 2, +/- Abiraterone/Prednisone)
Cabozantinib	TKI	VEGFR2; cMET	NCT01630590 (Phase 2, +Androgen ablation); NCT01683994 (Phase 1/2, +/- Docetaxel/Prednisone)
Temsirolimus	RTK effectors	mTOR	NCT01174199 (Phase 1, +Vorinostat)
Cixutumumab	RTK effectors	IGF1R	NCT01026623 (Phase 1/2, +/-Temsirolimus)
Olaparib	PARP inhibitor	PARP	NCT01972217
Denosumab	Bone-targeted	OCL inhibitor	NCT02758132 (Correlative, +Enzalutamide, +/-Abiraterone/Prednisone)
Osteodex	Chemotherapy/Bisphosphonate	Tumor and OCL	NCT02825628 (Phase 2)
Radium-223 chloride	Radiopharmaceutical		NCT02043678 (Phase 3, +/- Abiraterone); NCT01106352 (Phase 1/2, +/-Docetaxel)
Sipuleucel-T	Immunotherapy	PAP	NCT01807065 (Phase 2, +/- external beam radiation therapy); NCT01981122 (Phase 2 +Enzalutamide)
PROSTVAC	Immunotherapy	PSA	NCT01322490 (Phase 3; +/-GM-CSF)
Nivolumab	Immunotherapy	PD-L1	NCT02601014 (Phase 2; +Ipilimumab in AR-V7 expressing patients)
Ipilimumab	Immunotherapy	CTLA-4	NCT02601014; NCT01498978 (Phase 2, +Androgen suppression therapy); NCT00064129 (Phase 1, +GM-CSF)

AR-V7, androgen-receptor splice variant 7; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; FGFR3, fibroblast growth factor receptor 3; GM-CSF, granulocyte-macrophage colony-stimulating factor; IGF1R, insulin-like growth factor 1 receptor; mTOR, mammalian target of rapamycin; OCL, osteoclast; PAP, prostatic acid phosphatase; PARP, Poly ADP ribose polymerase; PD-L1, programmed death-ligand 1; PSA, prostate-specific antigen; RTK, receptor tyrosine kinase; TKI, tyrosine-kinase inhibitor; and VEGFR2, vascular endothelial growth factor receptor 2.

focus of phase I and II clinical trials (**Table 1**).

While results for these inhibitors have been encouraging, a caveat has been the emergence of AR variants (AR-V) that, in some instances, lack ligand-binding domains but still drive the expression of AR-related genes. Recently, AR-V7 has been linked to acquired resistance to enzalutamide and abiraterone.[15] EPI-506 is a novel small molecule inhibitor that binds to the N-terminal domain of AR and therefore could be an effective treatment for mCRPC patients who have developed resistance to enzalutamide.[16] The long-term safety of EPI-506 is currently being studied (NCT02606123).

Targeting Bone mCRPC From the Outside In

Although the emphasis has remained on ADT, understanding ligands, receptors, and signaling pathways that control CRPC has revealed critical circuits controlling cancer well in survival and growth. The mutation/amplification/upregulation of several receptor tyrosine kinases (RTKs) have been implicated in the development, growth, and progression of prostate cancer and are the focus of clinical trials.[17] For example, dovitinib, a tyrosine kinase inhibitor (TKI) that binds fibroblast growth factor receptor 3 (FGFR3), is currently under investigation for efficacy in combination with abiraterone (NCT01994590), after being previously shown to improve bone scans and reduce SREs in 6 of 23 patients in a proof-of-principle study.[18] FGF signaling in bone stromal cells is an important regulator of bone formation, and it is possible dovitinib can impact prostate cancer cell growth and osteoblast behavior.[19]

Overall, TKI trial results for the treatment of mCRPC have been varied. Dasatinib, an inhibitor of multiple TKIs including SRC family kinases, reduced disease progression in 57% and bone lesions in 30% of mCRPC patients in a phase I trial.[20] However, in a recent phase 3 trial, the combination of dasatinib and docetaxel failed to provide a survival advantage compared with docetaxel and placebo.[21] Interestingly, dasatinib has been shown to induce differentiation of mesenchymal stromal cells in bone-forming osteoblasts, which may exacerbate prostate tumor-induced osteogenesis.[22] Combination therapy with an anti-androgen may circumvent this possibility. To that end, a combinational study of dasatinib and abiraterone/prednisone prior to chemotherapy is currently being investigated for impact on PFS as a primary outcome (NCT01685125).

Constitutive activation of multiple signaling pathways via different RTKs can provide a significant survival advantage for tumors; thus dual targeting of TKIs may be beneficial for impacting tumor growth. Cabozantinib, for example, is a dual TKI of VEGFR2, the receptor for angiogenic factor vascular endothelial growth factor (VEGF), and c-MET, a receptor for hepatocyte growth factor (HGF). In a phase II randomized trial, daily administration of cabozantinib improved bone scans in 68% of mCRPC patients (with complete resolution in 12%), reduced soft tissue lesions, and improved PFS.[23,24] However, cabozantinib failed to reach the primary endpoint of increasing OS, compared with prednisone alone, in a phase III randomized trial of mCRPC patients who had previously received docetaxel and abiraterone.[25] Trials examining cabozantinib in combination with androgen ablation (NCT01630590) or chemotherapies such as docetaxel (NCT01683994) are ongoing and recruiting. RTKs mediate their effects via cell signaling circuitry and inhibitors of RTK effectors — such as mTOR for example — are also being explored clinically (NCT01174199 and NCT01026623).

Under selective pressures induced by therapeutic regimens, prostate cancer cells often acquire resistance to programmed cell death. For example, upregulation of DNA repair mechanisms is a common way for prostate cancer cells to avoid apoptosis induced by environmental stress.[26,27] Currently, inhibitors of poly ADP ribose polymerases (PARPs) that repair DNA "nicks" are being investigated. PARP-1 is a nuclear enzyme that detects single- and double-strand DNA breaks and initiates repair mechanisms. Further, PARP-1 can bind and regulate AR transcriptional function. PARP-1 has also been shown to play a critical role in mesenchymal stem cell–driven osteogenesis, making it a promising target for treating bone mCRPC.[21,28] Olaparib (Lynparza), a PARP-1 inhibitor, was included in a phase II trial of 50 mCRPC patients, 16 of which had mutational defects in DNA-repair genes,[29] in bone and visceral organ metastasis biopsies measured before and after treatment. Olaparib produced a PSA response (decline of 50% or more) in 22% of the patients and reduced the numbers of circulating tumor cells in 29%. Eighty-eight percent of patients with defects in DNA-repair genes (including *BRCA1, ATM, CHEK2,* and *HDAC*) showed a positive response to olaparib, suggesting that mutations in DNA repair genes may serve as a biomarker for mCRPC response to PARP inhibition. A current trial is examining the efficacy, safety, and tolerance of olaparib given in combination with abiraterone and will be compared with placebo with abiraterone (NCT01972217).

Bone Microenvironment Targeted Therapies for mCRPC Treatment

The surrounding bone microenvironment is a key driver of CRPC growth, and as such, presents therapeutic opportunities.[3] Although a hallmark of mCRPC is bone formation, the lesions also contain areas of extensive osteolysis and osteoclast activity. The monoclonal antibody denosumab binds to the receptor-activator of nuclear factor kappa B-ligand (RANKL), a key regulator of osteoclast formation. By preventing interaction with its cognate receptor RANK, denosumab effectively inhibits osteoclast formation and activation. Denosumab has been proven to significantly increase the median time to 1 SRE by 18% (20.7 months vs 17.1 months) compared with bisphosphonates.[30] Despite these results, no impact on OS of the patients was noted compared with the control arm. Because denosumab is well tolerated, it is currently being explored in combination with other therapies such as abiraterone (NCT02758132).

Another class of bone-targeted inhibitors commonly used for the treatment of mCRPC is bisphosphonates. Bisphosphonates specifically target normal and pathological bone formation by binding to calcium in newly-formed bone, and upon resorption, they are taken up by osteoclasts, inducing their apoptosis.[31] Compared with placebo, bisphosphonates such as zoledronate significantly increased me-

dian time to a SRE (488 days vs 321 days for placebo treatment) and reduced the frequency of SREs (39% vs 49%). Similar to denosumab, zoledronate does not enhance OS of men with mCRPC.[32,33] Bisphosphonates are also well tolerated in patients and thus provide an advantageous strategy for delivering therapies to the bone tissue and, specifically, areas undergoing remodeling. Guanidine, for example, is a potent chemotherapy but noted side effects make applying the treatment to patients difficult.[34] Osteodex is a novel therapy that grafts guanidine onto a bisphosphonic foundation with the goal of specifically targeting bone metastases and avoiding dose-limiting toxicities via bone-specific delivery of guanidine. Osteodex is currently in phase II trials that are investigating time to SRE (NCT02825628).

A recent breakthrough has been the FDA approval of radium-223 dichloride. The radium isotope is similar in nature to calcium and is preferentially absorbed by bone tissue where it emits high-energy alpha particles, killing cancer cells within a short range (less than 100 mcm). Radium-223 was found to improve median OS by 3.6 months compared with placebo.[35] Because of its success, clinical trials are investigating the efficacy of radium-223 with other therapies such as abiraterone (NCT02043678) and docetaxel (NCT01106352).

Significant advances have been made in the past decade with the development of immune-targeted therapies aimed at activating anti-tumor immunity and inhibiting pro-tumoral immunity. The immunostimulant sipuleucel-T and immune vaccine, PROSTVAC, activate the immune system against 2 well-defined prostate antigens, prostate acid phosphatase (PAP) and PSA, respectively. Sipuleucel-T is a personalized treatment involving ex vivo culture of patient-derived antigen-presenting cells (APCs) with a fusion protein (PA2024) of recombinant PAP and granulocyte-macrophage colony-stimulating factor (GM-CSF), an immune stimulating factor. APC-expressing PA2024 cells are then transfused back into the patient where they induce immune activation against cancer-derived PAP. Compared with placebo, sipuleucel-T proved to be most beneficial for mCRPC patients with low disease burden, improving median OS by 4 months and 3-year survival, but has not been as successful for patients with more advanced disease (>20 detected bone lesions), demonstrating a need for greater understanding of mCRPC immunogenicity in bone.[36-38]

PROSTVAC utilizes 2 recombinant poxviruses: vaccinia (PROST-VAC-V), which primes the immune system; and fowlpox (PROST-VAC-F), an immune system booster. Each vector has been transduced to express 4 human genes: PSA and 3 costimulatory molecules that enhance T-cell activation (leukocyte function-associated antigen-3, [LFA3]; intercellular adhesion molecule-1, [ICAM1]; and B7-1).[39] In a phase II trial, PROSTVAC significantly improved median OS in mCRPC patients.[40] These findings contributed to the initiation of an ongoing phase III trial investigating the impact of PROSTVAC alone or in combination with GM-CSF on overall survival in asymptomatic mCRPC patients (NCT01322490).

A new wave of immunotherapies has arisen in recent years that specifically targets checkpoint inhibition, a mechanism of tumor immune evasion that prevents cytotoxic T-cell lympho-

cyte (CTL) activation. Although the percentage of T cells in the bone marrow is relatively low, CD4+ and CD8+ CTLs have been shown to exert anti-tumor effects in bone metastases of other cancers, such as breast and melanoma.[41-43] Ipilimumab, a monoclonal antibody against receptor cytotoxic T-lymphocyte antigen-4 (CTLA-4), a negative regulator of T-cell activation, inhibits regulatory T-cell function and activates cytotoxic T-cells. Although ipilimumab has been highly successful for the treatment of metastatic melanoma,[44,45] it has not proved efficacious for the treatment of mCRPC. In a recent phase III trial, ipilimumab failed to improve overall survival in comparison to placebo, yet reduced PSA, and improved 3-month progression-free survival.[42]

Several studies have demonstrated adverse effects that ended initial clinical trials but of note, a single patient had a dramatic response with a reduction in the number of bone lesions and disease free survival at 6 years.[46] Defining markers predictive of patient response to checkpoint inhibitors will be critical for their clinical application. Nevertheless, clinical trials are investigating the combination of ipilimumab with ADT on disease progression (NCT01498978) and the safety of using ipilimumab in combination with GM-CSF (NCT00064129). Another checkpoint inhibitor drug, nivolumab, a monoclonal antibody that targets PD-1/PD-L1, has shown little promise, as there has been no clear indication that CRPC tumors express PD-L1.[47] Targeting T-cell activation through 2 different mechanisms, APC-mediated activation and immune checkpoint inhibition, such as combinational PROSTVAC with ipilumimab treatment, may enhance drug efficacy over the individual compounds. Clinical trials are studying the efficacy of the checkpoint inhibitors combined (NCT02601014) or when added to ADT (NCT01498978).

Upcoming Opportunities and Threats for Bone mCRPC Treatment

Significant progress has been made in the development of therapies that target mCRPC growth in the bone microenvironment. Moving forward, the upfront application of therapies in combination — such as ADT with radium-223 — for example, may prove more effective than sequential treatments in extending OS. Molecular profiling of individual mCRPC patients and the identification of response predictors will clearly be beneficial for the smart application of targeted therapies, such as TKIs and immune check point inhibitors, in order to achieve maximal responses. A major challenge for the medical oncologists is mCRPC heterogeneity and the emergence of resistant disease.[48,49] Adaptive therapy aims to prevent the emergence of resistant subpopulations by maintaining therapy-sensitive populations.[50] The application of therapies, as needed, to stabilize disease progression, rather than continuously, is currently being explored in the clinic for abiraterone in mCRPC (NCT02415621). Further, novel computational modeling approaches to define optimal therapeutic strategies for heterogeneous bone metastatic prostate cancer are under investigation.[51-53]

In conclusion, a greater understanding of the molecular underpinnings of bone metastasis has contributed to an expansion of potential therapies for mCRPC. Defining the optimal sequence and combina-

tions needed for these therapies, identifying key characteristics of the tumor that could determine which patients would benefit most, and controlling tumor evolution in the bone microenvironment will no doubt improve the efficacy of current therapies and significantly extend the OS of men with mCRPC.

Affiliations: Leah M. Cook and Conor C. Lynch are with the Department of Tumor Biology, H. Lee Moffitt Cancer Research Center and Institute, 12902 Magnolia Dr, Tampa, FL 33612, USA.
Address correspondence to:
Conor C. Lynch, H. Lee Moffitt Cancer Research Center and Institute, 12902 Magnolia Dr, Tampa, FL 33612, USA; TEL: +813-745-8094; E-mail: conor.lynch@moffitt.org.

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