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Recent Advances in Bone-Targeted Therapies of Metastatic Prostate Cancer

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Abstract

Prostate cancer is one of the most common malignancies affecting men worldwide, with bone being the most common site of metastasis in patients that progress beyond organ confinement. Bone metastases are virtually incurable and result in significant disease morbidity and mortality. Bone provides a unique microenvironment whose local interactions with tumor cells offer novel targets for therapeutic interventions. Several attractive molecules or pathways have been identified as new potential therapeutic targets for bone metastases caused by metastatic castration-resistant prostate cancer. In this review, we present the recent advances in molecular targeted therapies for prostate cancer bone metastasis focusing on therapies that target the bone cells and the bone microenvironment. The therapies covered in this review include agents that inhibit bone resorption, agents that stimulate bone formation, and agents that target the bone matrix. Suggestions to devise more effective molecular targeted therapies are proposed. Hopefully, with better understanding of the biology of the disease and the development of more robust targeted therapies, the survival and quality of life of the affected individuals could be significantly improved.

Keywords

bone metastasis; molecular targeted therapy; osteoblasts; osteoclasts; prostate cancer; RANKL

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Conflict of Interest

None of the authors have conflicts of interest with the information presented in this manuscript.

Authors' contributions

All named authors conceived for the study, and were involved in collecting the data and drafting and revising the manuscript. All authors read and approved the final manuscript.

1 Introduction

Globally, 903,500 new cases of prostate cancer (PCa) and 258,400 deaths from PCa were estimated in 2011. In the United States, PCa is the most common malignancy affecting men, and is the second leading cause of cancer death among men, with an estimate of 240,890 new cases and more than 28,000 deaths in 2011 (1). In eastern countries such as China (2) and Japan (3), the incidence of PCa has dramatically increased over the past two decades, most likely due to economic development and lifestyle changes. In PCa, the development of metastasis essentially means the patient is incurable. The most common site of PCa metastasis is bone. Autopsy studies of men who died of PCa revealed radiological evidence of bone metastases in nearly 90% of the patients examined, a percentage much higher than that of other bone-metastasizing solid tumors (such as breast and lung cancer) (4). One of the hallmarks of prostate bone metastasis is the osteosclerotic (blastic) phenotype, which is the presenting manifestation for most PCa bone metastases. Even so, increased bone resorption is a prerequisite for the successful seeding of the PCa cells in blastic-predominant bone metastasis (5).

Bone metastasis significantly affects patients' quality of life through skeletal-related events (SRE) including bone pain, pathological fractures, nerve impingement and myelophthisis. More importantly, once tumors metastasize to bone, they are virtually incurable and result in significant disease morbidity prior to a patient's death (6). The main therapeutic option for bone metastasis in hormone-responsive PCa is androgen deprivation therapy (ADT). Despite initial response rates of 80–90%, virtually all treated patients progress to androgen-insensitive disease, a state referred to as castration-resistant PCa (CRPC) (7). Even with substantial progress in the understanding of the biology of PCa bone metastasis and the constant development of new therapeutic agents, treatments so far have had only modest effects on survival for patients with metastatic CRPC.

Several attractive molecules or pathways in the metastatic process of PCa have been identified as new potential therapeutic targets and significant progress has been made in the area of bone-targeted therapies of metastatic PCa especially in recent years. This review presents the recent advances in bone-targeted therapies for PCa bone metastasis, from preclinical *in vivo* investigations to clinical studies. Not covered in this review are advances in therapies that primarily target or modulate the tumor cells *per se*, such as androgen receptor inhibition, chemotherapy, and immunotherapy, and therapies that are currently being evaluated in other solid tumors that spread to bone but have not been tested in PCa. Interested readers may refer to other related reviews for these interesting topics (7–10).

2 Mechanisms Governing Bone Metastasis in Prostate Cancer

2.1 Why Bone?

Several theories have been proposed to explain the propensity of PCa to metastasize to bone. The most widely accepted theory is the “seed-and-soil” hypothesis, which was proposed more than a century ago by Stephen Paget and may be relevant in many cancer types including PCa (11). According to this theory, cancer cells (“seeds”) metastasize to locations (“soil”) that are biochemically and physiologically favorable for their implantation and

growth. Strong support for this theory was demonstrated by the seminal work of Fidler *et al.* some 80 years later who demonstrated that although tumor cells reached the vasculature of all organs, metastases selectively developed only in certain organs (12). “Osteomimicry” is another theory proposed to explain the preferential growth of PCa cells in bone. According to this hypothesis, metastatic PCa cells take on the properties and behaviors of osteoblasts or osteoclasts upon arrival in bone. These activities lead to enhanced turnover of the bone matrix and preferential growth of PCa cells in bone (11). The bone metastatic process may involve a combination of the above, and as yet undefined, mechanisms. Understanding these mechanisms may provide a basis for identifying therapeutic targets.

2.2 RANK/RANKL/OPG as the Essential Regulators of Prostate Cancer-Bone Interactions

The interaction between osteoclasts and osteoblasts during the processes of bone resorption and formation is key to normal bone turnover. The best understood molecular link between osteoblasts and osteoclasts is the RANK/RANKL/OPG triad (6). The receptor activator of NF- κ B (RANK) is a transmembrane receptor expressed on osteoclast precursor cells, while RANK ligand (RANKL) is expressed by osteoblasts and bone marrow stromal cells. Upon binding to RANK, RANKL leads to osteoclast maturation, activation, and survival. This process can be interrupted by osteoprotegerin (OPG), a soluble decoy receptor for RANKL, which is produced by mature osteoblasts and stromal cells to inhibit the maturation of osteoclasts. Upon binding RANKL, OPG inhibits RANKL’s ability to activate RANK. This, in turn, diminishes osteoclastogenesis and osteoclast activation. Increasing the ratio of OPG to RANKL has been shown to result in increased bone mass (13).

The dysregulation of the functional equilibrium in the RANK/RANKL/OPG triad is responsible for the pathological remodeling associated with malignant tumors and for the development of metastatic deposits in bone sites. Tumor cells release growth factors and/or cytokines into the bone microenvironment, which, in turn, stimulate the production of RANKL from osteoblasts. RANK stimulation by RANKL results in the differentiation of preosteoclasts into active osteoclasts, which resorb the mineralized bone matrix, thus releasing factors to promote further colonization and growth of tumor cells (14). Therefore, targeting the RANK/RANKL/OPG axis constitutes an important strategy for the management of PCa bone metastasis.

2.3 Other Molecules Important for Prostate Cancer Bone Metastasis

During the process of bone metastasis, tumor cells may acquire a specific phenotype favoring the secretion of specific cytokines and proteases that interact with the bone microenvironment (6). These factors include bone morphogenetic proteins (BMPs), transforming growth factor- β (TGF- β), protease-activated receptor (PAR), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), fibroblast growth factors (FGFs), Wnt1, parathyroid hormone-related protein (PTHrP) and prostate-specific antigen (PSA). In addition to tumor-produced factors, bone also provides a fertile “soil” for the “seeds”. Specifically, cytokines and non-collagen proteins released from the bone matrix or synthesized during bone turnover promote the colonization and growth of PCa cells in bone. This crosstalk between tumor cells and bone microenvironment creates a “vicious cycle” between the tumor cells and the bone microenvironment. For example, interactions

between stromal cell-derived factor 1 (SDF-1, also known as CXCL12), which is expressed by endothelial cells, osteoblasts, and stromal cells, and its receptor CXCR4, which is expressed by PCa cells, promote the directed migration (chemotaxis) and growth of metastatic deposits of PCa cells in bone (15). Proteases such as matrix metalloproteinases (MMPs) (16), cathepsins (17), and the urokinase-type plasminogen activator (uPA) (18) also significantly contribute to the growth and expansion of the metastatic deposit of PCa in bone. Src family kinases play an important role in PCa growth, invasion, and metastatic dissemination (19). Integrin $\alpha_v\beta_3$ expression in osteoclasts is also involved in the formation of PCa bone metastasis (20). In-depth review of the molecular mechanisms of PCa bone metastasis is not the focus of this review but can be found elsewhere (6, 21).

3 Agents Inhibiting Bone Resorption

3.1 Bisphosphonates as Inhibitors of Osteoclastic Activity

Bisphosphonates (BPs), synthetic non-hydrolyzable analogs of pyrophosphate with structural similarity to inorganic phosphate, are the most commonly used drugs in the management of bone complications in bone-metastasizing cancers. Nitrogen-containing BPs such as Zoledronic acid (ZA) inhibit farnesyl pyrophosphate synthase in osteoclasts, thereby preventing the formation of isoprenoid lipids required for the prenylation of small GTPases (22). In contrast, non-nitrogen-containing BPs, such as Clodronate, inhibit osteoclast activity through the intracellular formation of AppCCl₂p, an ATP analog.

The FDA approved ZA in 2002 to treat hypercalcemia and prevent SREs in patients suffering from bone-metastatic breast cancer, lung cancer, multiple myeloma, and PCa (23). Although ZA was associated with significant reductions in 6-month risks of SREs in bone-metastatic PCa patients, the observed improvement in overall survival (OS) was not statistically significant (24). Clodronate has initially been reported to have no effect on OS or disease-free survival. However, a prospective study of 140 high-risk PCa patients who had clinically organ-confined disease indicated that the addition of Clodronate delayed the appearance of the first bone metastasis by seven-fold (25). Data from two aligned Medical Research Council randomized trials revealed that although there was no evidence of a benefit of OS in the non-metastatic group, Clodronate conferred an overall survival benefit when given in addition to standard hormone therapy in men with metastatic CRPC (26).

3.2 Therapies Targeting the RANK/RANKL/OPG Triad

3.2.1 RANKL-neutralizing Antibody—Denosumab (Xgeva[®]) is a fully human monoclonal antibody that binds to and neutralizes RANKL, thereby inhibiting osteoclast function and preventing generalized bone resorption and local bone destruction. Denosumab represents a significant advance in the treatment of bone loss associated with hormone ablation therapy in women with breast cancer and men with PCa and the prevention of SREs in patients with bone metastases from solid tumors (27).

Three main Phase III clinical studies have been published for PCa. The first study was conducted to evaluate the effect of Denosumab on bone mineral density and fractures in men receiving ADT (28). The second study compared Denosumab to ZA for the prevention of SREs in men with bone metastases from CRPC (29). The third study assessed Denosumab

for the prevention of bone metastasis or death in non-metastatic CRPC patients (30). In this double-blind randomized study, Denosumab increased bone metastasis-free survival by a median of 4.2 months, compared with placebo and delayed time to first bone metastasis. However, OS was not significantly changed. The FDA voted against expanding the current indications of Denosumab since the benefits of this drug did not outweigh its risks, including osteonecrosis of jaw (ONJ) (<http://articles.latimes.com/2012/feb/08/business/la-fi-amgen-20120208>).

3.2.2 RANK-Fc—The efficacy of the extracellular domain of the recombinant soluble RANK fused with the immunoglobulin Fc domain (RANK-Fc) was tested as an adjuvant therapy for PCa bone metastasis in preclinical models. In a mouse intratibial injection model, the combination of RANK-Fc and Docetaxel reduced tumor burden in bone more efficiently than either treatment alone (31). In an osteolytic SCID mouse model of bone metastasis induced by intratibial injection of PC-3 cells, Virk *et al.* studied the effect of simultaneous blockade of the RANK/RANKL axis using subcutaneous administration of RANK-Fc and the BMP pathway using retrovirus-mediated overexpression of noggin, a cognate binding protein and an antagonist of BMP4. The combination therapy with RANK-Fc and noggin effectively delayed the development of osteolytic lesions and decreased the bone loss and tumor burden compared with noggin overexpression alone (32). Further, in a mixed lytic/blastic PCa lesion in bone induced by intratibial injection of C42b cells, the same group found that treatment with both RANK-Fc and noggin demonstrated delayed development of bone lesions, attenuation of osteolysis, inhibition of small soft tissue tumors, and preservation of bone architecture with less tumor-induced new bone formation (33). These studies suggest that combined inhibition of RANKL and the BMP pathway may be an effective biologic therapy to inhibit the progression of established osteolytic as well as mixed lytic/blastic PCa lesions in bone. The clinical significance of this combination therapy awaits further investigations.

3.2.3 OPG-Fc—In a murine model of PCa bone metastasis using luciferase expressing PC-3 cells, OPG fused to Fc (OPG-Fc) alone reduced bone resorption, inhibited progression of established osteolytic lesions, and reduced tumor area (34). OPG-Fc in combination with Docetaxel (Taxotere), the most commonly used chemotherapeutic drug for the treatment of metastatic hormone-refractory PCa, suppressed skeletal tumor burden and increased median survival time by 16.7% compared with Docetaxel alone. RANKL inhibition by OPG-Fc may enhance Docetaxel effects by increasing tumor cell apoptosis as evidenced by increased active caspase-3 (35). These studies show that inhibition of RANKL provides an additive benefit to Docetaxel treatment and support clinical evaluation of this treatment option in patients.

3.3 Agents Targeting the Src Family Kinases

The Src family kinases (SFKs) are a family of non-receptor tyrosine kinases involved in the regulation of a wide range of cellular activities including proliferation, adhesion, motility, and survival. High levels of Src are found in mature osteoclasts and Src activity is essential for bone remodeling through positively regulating osteoclasts and negatively regulating osteoblasts. Several lines of evidence indicate that the SFKs contribute significantly to the

increased osteoclastic activity associated with PCa bone metastases and play an important role in PCa growth, invasion, and metastatic dissemination (19). Therefore, Src/SFK inhibition represents a potentially useful therapeutic strategy for patients with advanced-stage PCa.

Of the Src inhibitors examined in clinical studies, the broadest range of data is available for Dasatinib (BMS-354825, SPRYCEL[®]), the dual Src and Bcr-Abl tyrosine kinase inhibitor. In Phase II monotherapy trials for CRPC patients, Dasatinib (100 mg once daily (36) or twice daily (37)) showed similar and encouraging results in a subset of patients. One Phase I-II trial assessed the combination of Dasatinib and Docetaxel, in which a 50% decline in serum PSA levels was observed in 57% of the patients and a partial response observed in 60% of the patients with measurable disease. Urinary N-telopeptide (NTx) levels decreased in 87% of the patients, and toxicity was considered manageable (38). An ongoing Phase III trial is assessing Dasatinib (100 mg once daily) in combination with Docetaxel, and the results are expected very soon (http://clinicaltrials.gov_NCT00744497).

Saracatinib (AZD-0530), another Src and Bcr-Abl tyrosine kinase inhibitor, has been tested in a Phase II trial for the treatment of patients with PCa that did not respond to hormone therapy (http://clinicaltrials.gov_NCT00513071). A further randomized Phase II trial evaluated the efficacy of Saracatinib in comparison with ZA on patients with metastatic breast cancer and PCa (http://clinicaltrials.gov_NCT00558272). Both of these studies have been completed, pending publication of the trial results.

KX2-391, the third Src inhibitor, is the only Src-specific inhibitor. Unlike other Src inhibitors, KX2-391 binds to the peptide substrate-binding side of Src rather than its ATP-binding site. A Phase II study is evaluating the safety and efficacy of KX2-391 (40 mg twice daily) in bone-metastatic CRPC patients who have not received chemotherapy (http://clinicaltrials.gov_NCT01074138). In a single-arm Phase II study in men with metastatic CRPC, KX2-391 used at the same dosage did not show antitumor activity, but had modest effects on bone turnover markers such as urinary NTx and osteocalcin. Higher once-daily dosing is planned for future trials using KX2-391 (39). Bosutinib (SKI-606), the fourth inhibitor of Src and Bcr-Abl, was shown to block PCa invasion, growth, and metastasis in preclinical studies (40). This leads to the proposal for its future evaluation as a potential therapeutic in PCa bone metastasis in clinic trials.

3.4 Agents Targeting Integrins

Integrins are heterodimeric adhesion receptors that mediate cell–matrix interaction. Integrin $\alpha_v\beta_3$ is known to be expressed on tumor cells as well as on osteoclasts and has been shown to induce RANKL expression in PCa cells (41). Integrin $\alpha_v\beta_3$ expression is involved in bone metastasis and subsequent bone resorption in PCa (20). Abegrin[™] (Etaracizumab, previously known as Vitaxin[®] or MEDI-522) is a novel humanized monoclonal antibody directed against the $\alpha_v\beta_3$ integrin. A Phase II, randomized, multicenter study has been launched to evaluate the effect of Abegrin in combination with Docetaxel, Prednisone, or ZA for the treatment of patients with metastatic CRPC. This clinical trial has been completed, pending publication of the trial result (http://clinicaltrials.gov_NCT00072930).

MK-0429 (Merck, NJ, US) is an $\alpha_v\beta_3$ integrin inhibitor under evaluation for its effect on bone turnover and disease activity in men with CRPC and bone metastases. There was some evidence of an early reduction of bone turnover, indicating a potential for clinical use in the treatment of bone-metastatic disease. MK-0429 was generally well tolerated, with the most common side effects being nausea and an unexpected increase in serum PSA level (20). EMD 525797 (DI17E6) is a novel humanized monoclonal antibody targeting the α_v integrin. A multicenter Phase I study in progressive CRPC with bone metastases revealed no dose-limiting toxicities (42). Further studies are warranted to evaluate the clinical activity of EMD 525797.

GLPG0187 is a non-peptide α_v -integrin antagonist. *In vitro* and *in vivo* data showed that GLPG0187 is a potent inhibitor of osteoclastic bone resorption and angiogenesis. In animal models of PCa bone metastasis, GLPG0187 markedly reduced the metastatic tumor growth of PC-3M-Pro4/luc cells in both preventive and curative protocols (43). These results suggested that GLPG0187 can inhibit PCa bone metastases by antitumor, antiresorptive, and antiangiogenic mechanisms.

4 Agents Stimulating Bone Formation

4.1 Endothelin A Receptor

Endothelins exert their activity through binding to two distinct G protein-coupled receptors (GPCRs): Endothelin A receptor (ETAR) and Endothelin B receptor (ETBR). Endothelin 1 (ET-1) is a proliferation signal for many cells including PCa cells and osteoblasts and is known to participate in the process by which PCa forms metastases in bone (44). It has been shown that higher tissue ETAR levels in PCa patients correlate with advanced tumor stage, grade, and metastases. Although preclinical data support the concept that inhibition of the endothelin signaling axis may be useful in controlling tumor growth in bone, clinical results to date have been mixed and are sometimes disappointing.

Atrasentan (ABT-627 or XinlayTM), which inhibits the binding of ET-1 to ETAR without affecting the receptor density, is the first oral selective ETAR antagonist in clinical use. A Phase I-II study of Atrasentan and Docetaxel in men with metastatic CRPC determined the maximum tolerated dose of every-3-week Docetaxel with 10 mg Atrasentan was 70–75 mg/m². OS and progression-free survival were not significantly improved compared to those seen with Docetaxel alone, whereas the rate of PSA decline was slightly lower than expected (45). In a Phase III study in CRPC, the only significant finding with Atrasentan administration (10 mg/day) was the suppression of BALP and PSA levels (46). Another phase III trial in non-metastatic CRPC that randomized 467 men to Atrasentan and 474 to placebo also failed to improve time-to-disease progression or OS (47). These unfavorable clinical outcomes and a correlation with increased adverse cardiovascular events such as vasodilation and fluid retention led the FDA to reject the approval of Atrasentan for the treatment of CRPC.

Zibotentan (ZD-4054) is a specific oral ETAR inhibitor that, unlike Atrasentan, demonstrates no detectable inhibition of the ETBR (48). In Phase II trials, in patients with asymptomatic or mildly symptomatic metastatic CRPC, Zibotentan (10–15 mg/day) did not

delay time to progression, but, conversely to Atrasentan, it was associated with a promising improvement in OS (49, 50). However, a recent Phase III study of 594 patients did not demonstrate any clinical benefit in patients with bone-metastatic CRPC (51). Another Phase III study has been performed to further evaluate the efficacy of Zibotentan in combination with Docetaxel in metastatic CRPC. Primary results indicated that Zibotentan plus Docetaxel did not result in a significant improvement in OS compared with Docetaxel plus placebo in patients with metastatic CRPC (52). These disappointing results have cast a shadow over the future of the endothelin receptor antagonists in PCa therapy.

5 Agents Targeting the Bone Matrix

5.1 Bone-homing Radionuclides

Bone-homing radionuclides are systemically administered radiopharmaceuticals currently under investigation for the palliative treatment of severe pain in osteosclerotic metastatic bone disease (53). Certain radionuclides such as the β -emitter Strontium-89 (^{89}Sr) and the α -emitter Radium-223 (Alpharadin or Xofigo[®]) integrate into the bone cavity, thus irradiating tumor cells that colonize in bone; others radionuclides, e.g., the β -emitter Samarium-153 (^{153}Sm), can be conjugated to ligands, such as ethylenediaminetetramethylene phosphonate (EDTMP), to direct selective delivery to bone lesions (54). ^{153}Sm -EDTMP, which was approved by FDA in 1997, is the preferred β -emitting radiopharmaceutical of many clinicians. The half-life is 1.9 days and pain relief is rapid, generally between 2 and 7 days. Although ^{153}Sm -EDTMP can control pain, it has failed to affect the survival of PCa patients when used alone (55). However, several Phase I–II trials have demonstrated that, when combined with other systemic therapies, such as Docetaxel, the efficiency of ^{153}Sm therapy can be increased, leading to a marked improvement in bone pain and a trend towards increased OS (56–58).

The α -emitter Alpharadin, which has a half-life of 11.43 days, naturally targets the hydroxyapatite in the bone matrix because it simulates the stoichiometry of calcium. Alpharadin is different from the β -particles in that it carries higher radioactive energy but travels shorter distances (<100 μm), allowing for greater killing to tumor cells but less toxicity to the surrounding healthy tissue (59). Another advantage of Alpharadin is that it does not require the cancer cell to cycle in order to achieve its antitumor effect. This distinct feature is of particular benefit in the treatment of PCa, which has a low overall proliferative rate. Alpharadin has been systematically evaluated for highly localized treatment of osteoblastic bone metastases and shown to be a promising candidate for managing bone metastases in patients with CRPC.

In a Phase II trial of 64 CRPC patients scheduled to receive local-field external-beam radiation therapy to relieve pain from bone metastasis, Alpharadin has been shown to prolong the time to SRE and increase OS compared with placebo. Importantly, very few patients had grade 3–4 hematological toxicities, with the most common adverse effects being nausea, bone pain, fatigue, diarrhea, vomiting, and constipation (60). Less hematological toxicities compared with the β -emitters ^{153}Sm and ^{89}Sr were confirmed in a more recent clinical study (61). The latest Phase III clinical trial results have been published, which indicated that, compared with placebo, Alpharadin significantly improved OS

(median 14.0 vs. 11.2 months) and was associated with low myelosuppression rates and fewer adverse effects in metastatic PCa (62). In May, 2013, the US FDA approved Alpharadin for the treatment of patients with CRPC, symptomatic bone metastases and no known visceral metastatic disease (<http://www.prnewswire.com/news-releases/bayer-receives-us-fda-approval-for-xofigo-radium-ra-223-dichloride-injection-as-a-new-treatment-for-castration-resistant-prostate-cancer-with-bone-metastases-207545191.html>).

5.2 Cathepsin K

Cathepsins have been shown to be important mediators of metastasis across a range of tumors, including PCa. The primary cathepsins found in the bone microenvironment are cathepsins D, K, and L. Cathepsin K is responsible for the production of NTx, a marker of bone turnover, which is generated from degradation of bone matrix collagen I. Increased type I collagen degradation of NTx associated with cathepsin K activity was demonstrated in the sera of patients with PCa bone metastases (17). The critical role of cathepsin K in degradation and/or mobilization of mineralized collagen I and other metastasis-related proteins, such as osteonectin, VEGF, osteopontin, SDF-1, during bone resorption makes the inhibition of its activity an attractive antiresorptive strategy in the treatment of metastatic bone disease.

Most of the investigations of cathepsin K inhibitors have been performed in breast cancer and osteoporosis patients, with novel inhibitors only recently being applied to PCa bone metastases. The only Phase III trial of testing the cathepsin K inhibitor, Odanacatib (MK-0822), which was initiated to assess its effect in prolonging time to first bone metastasis in men with CRPC, was closed before its completion and no further evaluation is ongoing in the oncology setting, likely owing to commercial considerations (http://clinicaltrials.gov_NCT00691899).

5.3 The MMP/TIMP System

The extracellular matrix (ECM) is a barrier for cancer cell spreading. Solubilization of the ECM is a prerequisite for cancer cell migration, invasion, and formation of metastatic deposits. MMPs are a family of zinc-dependent proteolytic enzymes that collectively degrade most of the components of the ECM (63). MMPs are expressed by cancer cells, stromal cells, infiltrating inflammatory cells, as well as osteoclasts. In general, the expression and activation of MMPs correlate with advanced tumor stage, increased tumor angiogenesis, invasion, metastasis, and poor prognosis. MMPs, especially MMP-1, MMP-2, MMP-7, MMP-9, and MT1-MMP, are expressed in human PCa tissues and are involved in the establishment and growth of metastatic PCa in bone (64). Therefore, MMPs constitute an attractive target for intervention in PCa bone metastasis.

A large number of MMP inhibitors (MMPIs) have been developed in recent two or three decades. Several generations of synthetic MMPIs were tested in clinical trials since the 1990's. These include the first-generation peptidomimetics (such as Batimastat and Marimastat), the second-generation nonpeptidomimetics (such as Tanomastat and Prinomastat), and the third-generation tetracycline derivatives (such as Minocycline and Metastat). These MMPIs have shown to be effective in blocking PCa bone metastasis in

preclinical studies. However, clinical trials were basically unsuccessful mainly because of the lack of overall response and the presence of dose-limiting toxicity. Consequently, all clinical trials on the use of synthetic MMPs have been terminated (65). Inhibition of MMPs using PCK3145 (66), a synthetic peptide, or Silibinin (67), a naturally occurring small molecule inhibitor, has been reported and the results showed that these agents inhibited PCa cell-induced osteolysis in preclinical studies.

In a chimeric SCID-human model of PCa bone metastasis, we investigated the effect of MMP inhibition by tissue inhibitors of metalloproteinases (TIMPs), the most important endogenous inhibitors of MMPs, on the interaction between PCa cells and the implanted bone. We found that recombinant adenoviruses expressing TIMP-1 or TIMP-2 (AdTIMP-1 or AdTIMP-2) in cultured bone fragments inhibited MMP-2 activity, bone turnover and degradation induced by PC-3 cancer cells. *In vivo*, AdTIMP-2 expression in bone significantly reduced PC-3-induced osteolysis, osteoclast recruitment, and bone turnover (68). This establishes that the TIMP pathway is an intriguing target for developing gene therapy in hopes of preventing PCa growth and spread in bone. However, strategies by which an adenoviral vector can survive the immunosurveillance of an affected individual and by which the body's entire bone marrow stroma can be transduced need to be carefully planned.

5.4 The uPA–uPAR System

uPA is a serine protease that binds to its cell surface receptor uPAR and promotes pericellular proteolysis and intracellular signaling, which both coordinate multiple processes including cell migration, proliferation, and survival during cancer progression (18). The clinical evaluation of uPA and uPAR in the serum of patients with PCa has revealed strong associations with multiple states of disease progression (69). The finding that the amino-terminal fragment (ATF) of uPA, which antagonizes the actions of uPA, drives osteoblastic responses in three dimensional co-cultures of PCa cells and osteoblasts led to its consideration as a target in osteoblastic metastatic bone disease.

In a mouse model of intratibial injection of PC-3 cells, Fritz *et al.* used murine mesenchymal stem cells (MSCs) engineered to express hATF to evaluate the effect of MSC-mediated delivery of uPA antagonist on PCa progression in bone (70). They demonstrated a significant decrease in tumor-associated angiogenesis and protection from tumor-induced osteolysis in MSC-hATF-treated mice. These studies suggested that the regulation of the uPA–uPAR system could provide potential treatments for bone-metastatic PCa.

5.5 TGF- β Signaling

TGF- β is a major bone-derived factor, which contributes to the “vicious cycle” of cancer bone metastasis. TGF- β released from the bone matrix stimulates tumor cells to produce osteolytic factors that further stimulate adjacent bone resorption. In addition, TGF- β also regulates key components of the metastatic cascade including epithelial-mesenchymal transition (EMT), tumor cell invasion, angiogenesis, and immunosuppression (71).

In an intracardiac injection model of PCa bone metastasis, the effect of TGF- β signaling inhibition on osteoblastic tumor growth was investigated. The results indicated that stable knockdown of TGF- β 1 with an shRNA resulted in decreased tumor incidence and bone formation. In addition, systemic administration of either a small inhibitor of TGF- β receptor type I kinase (T β RI-KI) or a pan TGF- β binding protein (BG_FR_{II}) decreased bone tumor growth and osteoblastic bone formation (72). More recently, Hu *et al.* examined whether Ad.sT β R-Fc, an oncolytic virus expressing soluble TGF- β receptor type II fused with human Fc, and TAd.sT β R-Fc, a variant of Ad.sT β R-Fc, can treat bone metastasis of PCa. Both Ad.sT β R-Fc and TAd.sT β R-Fc caused significant inhibition of tumor growth and inhibited tumor-induced hypercalcemia, with the former being more effective in inhibiting tumor growth (73).

LY2109761 is a selective TGF- β receptor type I kinase inhibitor. LY2109761 treatment significantly inhibited the growth of MDA PCa 2b (osteoblastic) and PC-3 (osteolytic) cells in the bone of SCID mice; moreover, it resulted in significantly less bone loss in the PC-3 tumor-bearing bones as well as an increase in normal bone mass. This increased mass in non-tumorous bone may be a desirable side effect of LY2109761 treatment for osteopenia or osteoporosis secondary to ADT (74). These results support the development of therapies targeting TGF- β for advanced PCa. It is anticipated that TGF- β signaling inhibitors will shortly enter clinical evaluation for metastatic PCa.

6 The Role of Androgens in Bone Metastasis

Androgens impact both PCa growth and bone remodeling. Thus, they have potential to modulate the progression of bone metastasis directly, through a direct proliferative effect on PCa cells and indirectly, through altering the bone metastatic microenvironment through bone remodeling. Androgen status can impact the signaling state and survival of PCa cells. For example, androgen deprivation therapy has been shown to alter the subcellular localization of ErbB3, a receptor tyrosine kinase, that has pro-tumorigenic effects and is upregulated in bone metastasis (75). It is not clear if androgen ablative therapies have any specific impact specifically on bone metastasis as opposed to metastasis in soft tissue sites. In one report, adding androgen ablation therapy to docetaxel treatment had no additive or synergistic effect on tumor response in a mouse model compared to docetaxel alone (76). In contrast, intratibial tumors in mice of the androgen-responsive PCa cell line, C42, responded to castration, providing evidence that PCa skeletal metastases can maintain an androgen sensitive state (77). There are no good clinical data to suggest that androgen deprivation therapy prevents bone metastasis; albeit, hormone naïve (i.e., no androgen deprivation therapy received by the patient) bone metastases can respond to androgen deprivation therapy.

7 Concluding Remarks and Perspectives

Novel therapies that target the bone cells and the bone matrix during PCa metastasis are summarized in Figure 1 and Table 1. Even with these developments, the management of PCa bone metastases still remains a dilemma for researchers as well as clinicians. In order to achieve the goal of curing bone metastasis in PCa patients, we propose that several factors

be taken into consideration. First, treatment of bone metastasis in CRPC is complicated by the fact that both osteoblastic and osteolytic responses are involved in different stages of bone metastasis. Therefore, overemphasizing on either arm will result in unsatisfactory outcome. Logically, one could expect that better disease control could be achieved by combination therapies that target both of the osteoblastic and osteolytic pathways during PCa progression. Second, PCa metastasis to bone is a complex process involving tumor-stromal cell, tumor-matrix, and tumor-vasculature interactions. Simultaneous targeting of the molecules that facilitate interactions between PCa cells and the bone microenvironment could significantly influence future therapeutic approaches. Third, current animal models of cancer metastasis (78) do not faithfully reflect what's really going on inside the patients' bone. Therefore, more robust models are needed that mimic the complex nature of PCa bone metastasis. These models should be more representative of patient disease progression. Hopefully, with better understanding of the biology of PCa bone metastasis and the development of more robust molecular therapies, the survival and quality of life of the affected individuals could be significantly improved.

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List of Abbreviations

ADT	Androgen deprivation therapy
BALP	Bone alkaline phosphatase
BMPs	Bone morphogenetic proteins
BPs	Bisphosphonates
CRPC	Castration-resistant prostate cancer
ECM	Extracellular matrix
ET-1	Endothelin-1
ETAR	Endothelin A receptor
ETBR	Endothelin B receptor
FDA	The US Food and Drug Administration
hATF	Human amino-terminal fragment
MMPs	Matrix metalloproteinases
MMPIs	Matrix metalloproteinase inhibitors
MSCs	Mesenchymal stem cells

NTx	N-telopeptide
OPG	Osteoprotegerin
OS	Overall survival
PCa	Prostate cancer
PSA	Prostate-specific antigen
RANK	Receptor activator of NF- κ B
RANKL	Receptor activator of NF- κ B ligand
SFKs	Src family kinases
SREs	Skeletal-related events
TGF-β	Transforming growth factor β
TIMP	Tissue inhibitor of metalloproteinases
TβRI	Transforming growth factor β receptor type I
uPA	Urokinase-type plasminogen activator
uPAR	Urokinase-type plasminogen activator receptor
ZA	Zoledronic acid

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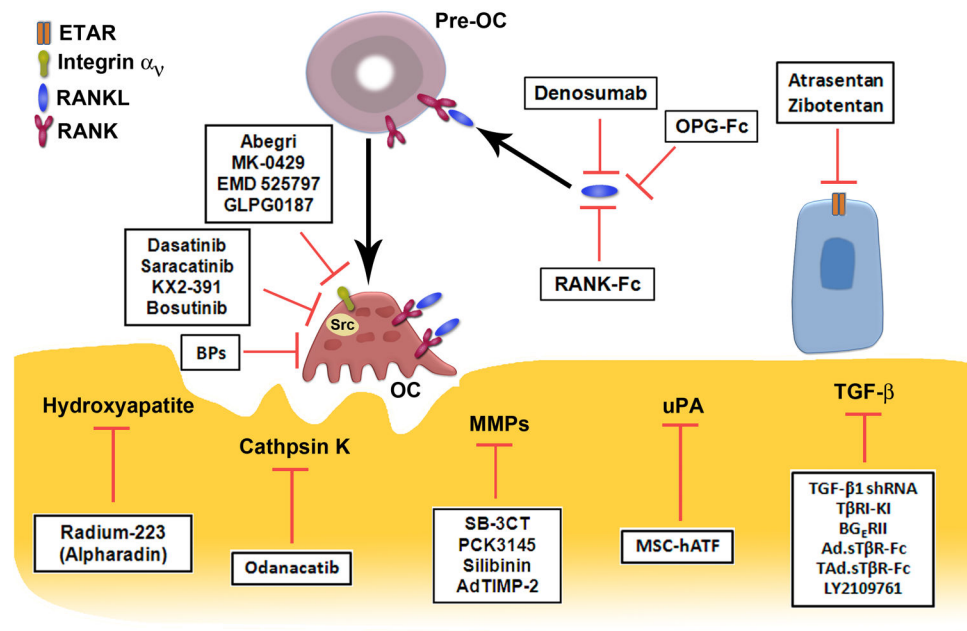


Figure 1. Summary of the agents used to target the bone for prostate cancer metastasis
 A schematic overview of the recent development of bone-targeted therapeutic strategies for prostate cancer metastasis that have been investigated in clinical or preclinical studies. A detailed description is provided in the text.

Table 1
Development of Agents for Bone-targeted Therapies of Metastatic Prostate Cancer

Agents	Mechanism of Action	Biochemical Property	Molecular Targets	Stage of Development	Refs.
Zoledronic acid	Inhibiting resorption	Synthetic analog of pyrophosphate (N-containing)	Farnesyl pyrophosphate synthase	Approved	23, 24
Clodronate	Inhibiting resorption	Synthetic analog of pyrophosphate (non-N-containing)	?	PR04/PR05	25, 26
Denosumab	Inhibiting resorption	Humanized monoclonal antibody	RANKL	Phase III	28–30
RANK-Fc	Inhibiting resorption	Recombinant protein	RANKL	Preclinical	31–33
OPG-Fc	Inhibiting resorption	Recombinant protein	RANKL	Preclinical	34, 35
Dasatinib (SPRYCEL®)	Inhibiting resorption	Tyrosine kinase inhibitor	Src, Abl	Phase III	36–38
Saracatinib (AZD-0530)	Inhibiting resorption	Tyrosine kinase inhibitor	Src, Abl	Phase II	*
KX2-391	Inhibiting resorption	Tyrosine kinase inhibitor	Src	Phase II	39, #
Bosutinib (SKI-606)	Inhibiting resorption	Tyrosine kinase inhibitor	Src, Abl	Preclinical	40
Abegri (Etaracizumab, Viaxin® or MEDI-522)	Inhibiting resorption	Humanized monoclonal antibody	Integrin alpha(nu)beta(3)	Phase II	&
MK-0429	Inhibiting resorption	Small molecule inhibitor	Integrin alpha(nu)beta(3)	Phase I	20
EMD 525797 (D117E6)	Inhibiting resorption	Humanized monoclonal antibody	Integrin alpha(nu)	Phase I	42
GLPG0187	Inhibiting resorption	Non-peptide integrin antagonist	Integrin alpha(nu)	Preclinical	43
Atrasentan (ABT-627 or Xinlay)	Stimulating bone formation	Small molecule inhibitor	ETAR>ETBR	Phase III, further approval rejected	46, 47
Zibotentan (ZD-4054)	Stimulating bone formation	Small molecule inhibitor	ETAR	Phase III	49–52
Samarium-153-EDTMP	Targeting bone matrix	Radiopharmaceutical (β-emitter)	?	Approved; Phase II for combination with Docetaxel	56–58
Radium-223 (Alpharadin, or Xofigo®)	Targeting bone matrix	Radiopharmaceutical (α-emitter)	Hydroxyapatite	Approved	§
Odanacatib (MK-0822)	Targeting bone matrix	Small molecule inhibitor	Cathepsin K	Phase III	▲
PCK3145	Targeting bone matrix	Synthetic peptide	MMPs	Preclinical	66
Silibinin	Targeting bone matrix	Small molecule inhibitor	MMPs	Preclinical	67
AdTIMP-2	Targeting bone matrix	Recombinant adenovirus expressing TIMP-2	MMPs	Preclinical	68
MSC-hATF	Targeting bone matrix	MSCs engineered to express uPA antagonist hATF	uPA-uPAR	Preclinical	70
TGF-β1 shRNA	Targeting bone matrix	shRNA	TGF-β	Preclinical	74

Agents	Mechanism of Action	Biochemical Property	Molecular Targets	Stage of Development	Refs.
TβRI-KI	Targeting bone matrix	Receptor kinase inhibitor	TGF-β receptor type I	Preclinical	74
BG ₁ RII	Targeting bone matrix	Pan TGF-β binding protein	TGF-β	Preclinical	74
Ad.sTβR-Fc	Targeting bone matrix	Oncolytic virus expressing soluble TGF-β receptor type II fused with Fc	TGF-β	Preclinical	75
TAd.sTβR-Fc	Targeting bone matrix	A variant of Ad.sTβR-Fc	TGF-β	Preclinical	75
LY2109761	Targeting bone matrix	Small molecule inhibitor	TGF-β receptor type I	Preclinical	76

Abbreviations

EDTMP, ethylenediaminetetramethylene phosphonate; ETAR, endothelin A receptor; ETBR, endothelin B receptor; hATF, human amino-terminal fragment; MMPs, matrix metalloproteinases; MSC, mesenchymal stem cell; OPG, osteoprotegerin; RANK, receptor activator of NF-κB; RANKL, receptor activator of NF-κB ligand; TGF-β, transforming growth factor β; TIMP-2, tissue inhibitor of metalloproteinase 2; TβRI, TGF-β receptor type I; uPA, urokinase-type plasminogen activator; uPAR, urokinase-type plasminogen activator receptor

* <http://www.clinicaltrials.gov/ct2/results?term=NCT00513071&Search=Search>; <http://www.clinicaltrials.gov/ct2/results?term=NCT00558272&Search=Search>

<http://clinicaltrials.gov/ct2/results?term=NCT01074138&Search=Search> & <http://www.clinicaltrials.gov/ct2/results?term=NCT00072930&Search=Search>

\$ <http://www.prnewswire.com/news-releases/bayer-receives-us-fda-approval-for-xofigo-radium-ra-223-dichloride-injection-as-a-new-treatment-for-castration-resistant-prostate-cancer-with-bone-metastases-207545191.html>

▲ <http://www.clinicaltrials.gov/ct2/results?term=NCT00691899&Search=Search>