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REVIEW

Cancer-targeted therapies and radiopharmaceuticals

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The treatment of bone metastases remains a clinical challenge. Although a number of well-established agents, namely bisphosphonates and denosumab, are available to reduce the occurrence of skeletal-related events, additional cancer-targeted therapies are required to improve patients' prognosis and quality of life. This review focuses on novel targets and agents that are under clinical evaluation for the treatment of malignant bone diseases such as activin A, src and endothelin-1 inhibition or agents that are clinically approved and may positively influence bone, such as the mTOR inhibitor everolimus. In addition, the potential of alpharadin, a novel radiopharmaceutical approved for the treatment of prostatic bone disease, is discussed.

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Introduction

Over the past years, there has been a growing awareness to consider the maintenance of bone health as an important issue in the management of patients with malignant diseases. As a number of cancer treatments, which effectively improve the prognosis of affected patients, have been shown to negatively affect bone metabolism, measures to counteract these effects are of special importance. In particular, hormone-ablative therapies, as commonly used for the treatment of breast and prostate cancer, result in a massive increase of bone turnover, which in turn leads to an increased risk of osteoporosis.[1]

On the other hand, there is an increasing interest in targets that may be suitable to act both as anticancer agents and positively affect bone health and bone metastases. A number of pharmacological compounds that are either already approved as cancer treatments or currently under clinical evaluation may fulfill these criteria (**Table 1**). This article aims to evaluate the effects of these substances on tumor growth and bone metabolism.

Activin A

Activin A is a member of the transforming growth factor-β ligand superfamily, which can bind to two activin receptors (activin receptors type I and II). Physiologically, activin A is an important regulator of reproduction, as it regulates the release of follicle-stimulating hormone in the pituitary gland. In addition, activins are also expressed in the bone microenvironment. *In vitro*, recombinant activin A strongly inhibits osteoblast mineralization, and osteoblast-derived activin A has been proposed to inhibit osteoblast formation in an autocrine manner.[2,3] This effect appears to be mediated, at least in part, by alteration of the extracellular matrix composition and by impairing matrix production.[4] Osteoclast differentiation, on the other hand, is promoted by the presence of activin A.[5] In addition, activin A has been shown to increase the expression of RANK on osteoclasts and thereby promote the formation and activity of osteoclasts in the presence of RANKL[6] (**Figure 1**).

There is additional data to indicate a role of activin A in malignancies. Prostate gland development is modulated by activin A, and while androgens regulate activin A they also stimulate androgen synthesis, which results in an enhanced growth of prostate cancer cells *in vitro*.[7] In a preclinical model of prostate cancer it was shown that, although prostate cancer cells, which overexpressed the activin type IB receptor, did not have increased levels of proliferation, they showed a higher level of migration and more commonly formed lymph node metastases than their parental cells. In addition, these cells showed an enhanced expression of genes associated with epithelial-to-mesenchymal transition.[8]

Activin A levels are increased in breast cancer tissue and serum from affected women.[9] In fact, levels of activin A were shown to be highest in patients with advanced breast and prostate cancer and bone metastases compared with those with non-metastatic disease.[10]

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Table 1 Novel targets for the treatment of bone metastases

Target	*Compound*	*Malignancy*	*Stage*	*Comment*	*Reference*
Activin A	Sotatercept	Myeloma	Phase 2	Positive effects on BMD, well tolerated	16
Endothelin-1	Atresantan	Prostate	Phase 3	Failed to show clinical benefit	23
	Zibotentan	Prostate	Phase 3	Failed to show clinical benefit	25
src	Dasatinib	Prostate	Phase 3	No improved survival	34
	Saracatinib	Breast/prostate	Phase 2	Results pending	NCT00558272
mTOR	Everolimus	Breast	Approved	*Post hoc* analyses revealed positive effects on markers of bone turnover	37
Bone	Xofigo	Prostate	Approved	Improved overall survival	46
		Breast	Phase 2	Decreased markers of bone turnover	50

In multiple myeloma, where osteolytic bone disease is a key finding, high circulating levels of activin A correlate with extensive bone involvement and poor survival.[11] In addition, it was recently shown that bone marrow macrophage-derived activin A mediates the osteoclastogenic effects of interleukin-3 in multiple myeloma and may thereby contribute to the extent of osteolytic disease.[12]

Consequently, the potential of an activin A blockade was evaluated in preclinical murine models of malignant bone disease. In a breast cancer model, inhibition of activin A by using a soluble activin receptor type IIA fusion protein (ActRIIA.muFc) resulted in a stimulation of bone formation and reduced the level of bone destruction resulting from myeloma cells, as well as the occurrence of bone metastases.[13] Therapeutic effects were confirmed in an *in vivo* humanized myeloma model, in which the use of an activin A-soluble decoy receptor reversed osteoblast inhibition, ameliorated myeloma bone disease and inhibited tumor growth.[14]

These findings led to the initiation of clinical trials involving the activin A inhibitor sotatercept (formerly called ACE-011). In a phase 1 trial, sotatercept increased bone-specific alkaline phosphatase by up to 36% in postmenopausal women.[15] A recent phase 2 trial investigated the effects of sotatercept in patients with myeloma bone disease. Compared with placebo, patients receiving sotatercept showed anabolic improvements in bone mineral density and bone formation, whereas it had little effect on bone resorption. In general, multiple doses of sotatercept were well tolerated by patients participating in this trial.[16] A number of other phase 2 trials assessing the effects of sotatercept on bone metabolism are currently underway.

Endothelin-1

Another protein that is better known for its nonosseous effect is endothelin-1 (ET-1), which is a potent vasoconstrictor that is highly expressed by endothelial cells. In bone, osteoblasts and osteoclasts also express endothelin type A receptors at high density. Although osteoclasts are inhibited upon ET-1 receptor binding, osteoblast differentiation and activity is promoted.[17] Serum levels of ET-1 are elevated in patients with prostate cancer bone metastases.[18] Overexpression of ET-1 induces new bone formation in osteosclerotic tumor models of prostate cancer.[19] Blocking of ET-1 signaling using an orally active endothelin A receptor antagonist on the other hand decreased bone metastases and tumor burden in mice. These findings led to the assumption that ET-1 may have a causal role in the pathogenesis of osteosclerotic bone metastases.[20] One proposed mechanism by which ET-1 may regulate osteoblast function is the inhibition of DKK-1 expression in bone marrow stromal cells.[21]

These promising preclinical findings were translated into a clinical trial program to assess the potential of ET-1 inhibition in prostate cancer. Atresantan, a highly selective and potent ET-A receptor antagonist, was tested in combination with zoledronic acid in a phase 2 trial for potential synergistic effects in men with metastatic prostate cancer. However, this trial failed to provide evidence for additive or synergistic effects of combination therapy with atrasentan and zoledronic acid on bone turnover markers.[22] Furthermore, a subsequent phase 3 trial, although showing evidence of biologic effects on PSA and bone markers, failed to delay disease progression in men with metastatic hormone refractory prostate cancer.[23] Another trial using the ET-1 receptor antagonist named zibotentan (ZD4054) failed to provide a significant clinical benefit in patients with prostate cancer when applied singularly and in combination with docetaxel.[24,25] In summary, although there is evidence of bone regulating potential by ET-1 modulation, up to date studies of ET-1 inhibition have failed to provide clinical benefit in patients with advanced prostate cancer.

Src

Another potential target for both bone and tumor biology is the nonreceptor tyrosine kinase Src. Src is a key regulator of normal osteoclast function, as seen in the osteoclast impairment in Src-deficient mice.[26] Furthermore, inhibition of Src enhances osteoblast differentiation and bone formation[27] (**Figure 1**).

In addition to its effects on bone, Src activity has been linked to the growth, migration and invasion of osteotropic malignancies such as prostate cancer.[28]

In breast cancer, Src kinases are higher expressed in malignant tissue compared with benign breast samples,[29] and in prostate samples increased Src expression correlates with a shorter time to castration response and poorer survival.[30] Dasatinib, an Src kinase inhibitor, prevented prostate cancer growth in a xenograft model in castrated mice.[31] Moreover, a number of clinical trials have assessed the potential of dasatinib in prostate cancer. In a phase 2 trial with 48 patients, dasatinib had modest effects on tumor progression (17% progression-free rate at 24 weeks) but did show a reduction in bone turnover markers.[32] In another phase 1/2 trial, when used in combination with docetaxel, dasatinib appeared to have more promising results, with PSA levels decreasing by $>50\%$ in 57% of patients.[33] However, in a larger phase 3 trial with 1522 patients, the combined treatment of dasatinib and docetaxel did not

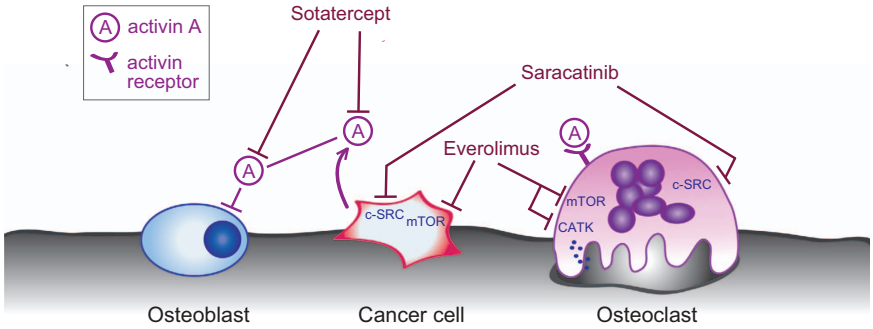

Figure 1 Cancer-targeted therapies in malignant bone disease. Sotatercept binds activin A and thereby prevents its inhibitory effects on osteoblasts and prosurvival effects on osteoclasts. Everolimus is an inhibitor of mTOR that exerts potent antitumor effects. Recent evidence indicates additional inhibitory actions on osteoclasts. Saracatinib inhibits c-src, which may positively influence the bone environment in addition to direct antitumor effects.

improve the overall survival (21.5 months vs 21.2 months) of the participants.[34]

Results from a phase 1 trial investigating the effects of Src inhibition on bone metabolism in 59 healthy young men by using an inhibitor named saracatinib (AZD0530) showed that saracatinib dose-dependently decreased serum concentrations of CTX by 88% and urinary excretion of NTX by 67% after 25 days. In addition, there was a difference in the concentrations of bone-formation markers between the use of saracatinib and placebo.[35] Another phase 1 trial investigated the effects of saracatinib on bone markers in patients with advanced malignancies. Again, the markers of bone resorption (sCTX and uNTX) were decreased at all times when measured, indicating an antiresorptive potential of Src inhibition.[36]

Furthermore, a phase 2 trial investigating the impact of saracatinib on metastatic breast and prostate cancer in conjunction with bisphosphonates has been completed, with results pending (NCT00558272).

mTOR Inhibition

Everolimus is an inhibitor of mTOR signaling, which is well known for its immunoregulatory abilities in patients requiring immunosuppression following transplantation.

Recent results from the BOLERO-2 trial have shown that inhibition of mTOR signaling using everolimus in combination with the aromatase inhibitor exemestane improved progression-free survival in patients with hormone receptor-positive advanced breast cancer by 4.1 months compared with the treatment of exemestane without everolimus (6.9 vs 2.8 months; 95% confidence interval 0.35–0.54, $P<0.01$).[37]

Strikingly, exploratory analyses of this trial evaluating changes in bone metabolism suggest beneficial effects of additional mTOR inhibition in a setting of hormone deprivation.[38] Although assessment of the bone markers BSAP, P1NP and CTX after 6 and 12 weeks of treatment revealed an expected increase in bone turnover markers in the group treated with exemestane and placebo, there was a significant decrease in those markers when exemestane was given in combination with everolimus. Furthermore, disease progression in bone was significantly lower in patients receiving the combination treatment compared with the application of only exemestane ($P=0.04$). Differences were evident by week 12 and remained nearly two-fold lower through week 30. Although the rates of on-study bone disease progression were higher for those with bone lesions at baseline, progression rates remained lower for those receiving mTOR inhibition ($P=0.02$).

Indeed, there is preclinical evidence to support these findings. The mTOR pathway has been linked to bone remodeling by promoting osteoclast survival and decreasing osteoclast apoptosis[39] (**Figure 1**). Inhibition of mTOR signaling modulates the RANKL/OPG pathway by increasing bone-protective osteoprotegerin. In addition, osteoclast-secreted cathepsin K, which mediates the collagen degradation during bone resorption, is decreased by everolimus. Of note, although mTOR inhibition exerts effects on osteoblasts *in vitro*, higher doses are required than for osteoclast inhibition. In an osteoporosis (OVX) rat model, everolimus prevented the induced loss of trabecular bone by 60%.[40]

Results from the BOLERO-2 trial are the first to provide sound clinical evidence that mTOR inhibition may have beneficial effects on bone metabolism in a setting in which hormone deprivation therapies normally place patients at a high risk of bone loss and consequently osteoporotic fractures.

The observed reduction in progression of bone disease is likely to derive from a combination of antitumor effects on one hand and antiresorptive effects on the other hand. These results warrant further research on the potential of mTOR inhibition in the setting of bone oncology.[41]

Alpharadin

Alpharadin, commercialized under the brand name of Xofigo, is a novel therapeutic approach for the treatment of bone metastases. Alpharadin is radium-233, which is an alpha particle-emitting radioisotope that acts as a calcimimetic. After uptake in the bone microenvironment, it forms complexes with hydroxyapatite. Uptake of radium-233 is especially high in areas with increased bone turnover as found in malignant bone disease.[42] As an alpha particle penetration into the surrounding tissue is limited, undesired off-target effects in the bone environment, especially on bone marrow cells, are minimized. This hypothesis was supported by findings from animal studies, in which radium-233 achieved therapeutically relevant effects on the bone surface, with only a small dosage of radiation absorbed by the bone marrow.[43]

A phase 1 trial in both breast and prostate cancer patients showed that radium-233 was well tolerated at therapeutically relevant dosages, which led to the initiation of phase 2 trials.[44] In these trials, four intravenous injections of radium-233,

given every 4 weeks in patients with castration-resistant prostate cancer and symptomatic bone metastases, resulted in a significant effect on bone-alkaline phosphatase concentrations.[45]

The therapeutic efficacy of radium-233 was assessed in a large phase 3 trial in patients with castration-resistant prostate cancer and symptomatic bone metastases, the ALSYMPCA study. The primary end point of this trial was overall survival, with secondary end points addressing skeletal events and bone markers. At the interim analyses, radium-233 significantly improved overall survival compared with placebo (14.0 months vs 11.2 months, $P = 0.002$). In addition, assessments of all main secondary efficacy end points also showed a benefit of radium-233 compared with placebo. Following the interim analyses, the study was terminated early because it reached the primary end point, and a cross-over to radium-223 was offered to placebo patients.[46] In terms of symptomatic skeletal events, radium-233 treatment significantly delayed the median time to first event compared with placebo (15.6 vs 9.8 months, $P < 0.001$).[47]

In general, radium-233 was well tolerated. The most relevant adverse event was myelosuppression, suggesting that clinically relevant off-target effects do occur. Although mild in most cases, 6% of the radium-233-treated group developed a grade 3 or 4 thrombocytopenia compared with 2% in the placebo group.[46] These results led to the approval of radium-233 in the United States and Europe for the treatment of men with castration-resistant prostate cancer with symptomatic bone metastases.

Radium-233 is currently not approved for the use in other malignancies, but there is emerging evidence to suggest efficacy in breast cancer. In a mouse model of established bone metastases, treatment with radium-233 significantly decreased osteolysis, tumor burden and tumor cachexia, as well as increasing the survival of treated mice.[48] Data from clinical trials in breast cancer patients are limited, but a case report described clinical benefit to young women suffering from hormone refractory and chemotherapy refractory metastatic breast cancer.[49] In a phase 2 trial in 23 advanced breast cancer patients with progressive bone-dominant disease, radium-223 led to a significant reduction in uNTX-1 and bone-alkaline phosphatase. Exploratory analyses of bone lesions by sequential FDG PET/CT scans revealed a 32.3% metabolic response rate after two radium-223 injections.[50]

Effects of New Hormonal Therapies on Bone Metastases

Recently, several new agents have been approved for the treatment of prostate cancer that act as cytochrome P (CYP17) inhibitors or direct androgen receptor inhibitors. Interestingly, phase 3 trials of these agents, namely abiterone and enzalutamide, not only showed an improved overall survival but also reduced the occurrence of symptomatic skeletal-related events. It remains to be elucidated whether these positive effects on SREs are mediated by direct antitumor effects or whether the vicious interaction between tumor and bone cells is interrupted by other mechanisms.[51]

Cabozantinib is a c-met inhibitor that resulted in a complete remission of detectable bone metastases in 12% of patients in a phase 2 trial.[52] These positive effects could not be confirmed in a recent phase 3 trial, in which cabozantinib failed to improve overall survival.[51]

Summary and Outlook

Bone provides an excellent microenvironment for tumor cell homing, expansion and survival in protected niches for tumor stem cells. Active malignant bone disease causes bone destruction, osteoporosis and pathological fractures owing to osteoclast-mediated osteolysis and simultaneous osteoblast overstimulation or inhibition. Bone-protecting treatment regimens using antiresorptive agents have been further developed during the past two decades but so far failed to confer significant antitumor activities in clinical studies. In particular, bisphosphonates require high local concentrations to translate the *in vitro* antitumor efficacy into clinical effects, although the characterization of sensitizers for such antitumor effects may stimulate further research and development.[53] The bone microenvironment and the vivid cross talk of tumor and bone cells provides a wealth of molecular mechanisms and signaling pathways that can be addressed as diagnostic and therapeutic targets to modulate this devastating disease. Activin antagonists and mTOR inhibitors, as well as the bone-seeking radioactive compound alpharadin, show promising data in advanced and ongoing clinical trials, whereas other substances that delivered preclinical and pathophysiological evidence, such as endothelin and Src signaling modulators, failed to ameliorate the clinical outcome.

Bone-targeted treatments so far aimed at preserving bone structure and fracture resistance and have been assigned "supportive" treatment strategies. Future bone-targeted treatments ideally would be part of an antitumor strategy designed to both protect and restore bone and at the same time provide antitumor efficacy or at least support the efficacy of classical chemotherapy. This could be achieved by intrinsic bone anabolic activity of antitumor agents or by bone anabolic principles with antitumor efficacy. The former has already been shown in multiple myeloma, where bortezomib shows both antitumor efficacy and stimulation of osteoblast activity.[1] The latter may be conveyed by antibodies against inhibitors of core osteogenic pathways such as sclerostin or DKK-1 that are abundant in metastatic bone disease. Research in tumor cell-homing mechanisms and their signaling pathways such as the SDF-1/CXCR4 axis and its cooperative activity with, for example, the mTOR pathway may also open up new avenues to influence tumor cell homing,[54] whereas combined osteoanabolic stimuli might reconquer hijacked regenerative niches and thus enable effective treatment of residual disease.

Conflict of Interest

The authors (or the respective institutions in their name) have received grants or honorariums by Amgen (LCH, TDR, FJ), Novartis (LCH, TDR, FJ), Lilly (FJ), Servier (FJ), Merck (LCH, TDR, FJ) and Nycomed (FJ) for participating in advisory boards or giving lectures.

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