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REVIEW

Bone-Targeted Therapies in Cancer-Induced Bone Disease

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Abstract Cancer-induced bone disease is a major source of morbidity and mortality in cancer patients. Thus, effective bone-targeted therapies are essential to improve disease-free, overall survival and quality of life of cancer patients with bone metastases. Depending of the cancer-type, bone metastases mainly involve the modulation of osteoclast and/or osteoblast activity by tumour cells. To inhibit metastatic bone disease effectively, it is imperative to understand its underlying mechanisms and identify the target cells for therapy. If the aim is to prevent bone metastasis, it is essential to target not only bone metastatic features in the tumour cells, but also tumour-nurturing bone microenvironment properties. The currently available bone-targeted agents mainly affect osteoclasts, inhibiting bone resorption (e.g. bisphosphonates, denosumab). Some agents targeting osteoblasts begin to emerge which target osteoblasts (e.g. romosozumab), activating bone formation. Moreover, certain drugs initially thought to target only osteoclasts are now known to have a dual action (activating osteoblasts and inhibiting osteoclasts, e.g. proteasome inhibitors). This review will focus on the evolution of bone-targeted therapies for the treatment of cancer-induced bone disease, summarizing preclinical and clinical findings obtained with anti-resorptive and bone anabolic therapies.

Keywords Bone metastasis · RANKL · Sclerostin · DKK1 · Bisphosphonates · mTOR inhibitors · Denosumab · Romosozumab · Radium 223 · Cathepsin k inhibitors · c-Src inhibitors

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Introduction

Bone metastases are frequent complications of solid tumours [1, 2]. For example, the incidence of bone metastases is 65–75% in breast cancer [1], 65–90% in prostate cancer [2], 20–25% in renal cell carcinoma [3], 14–45% in melanoma [4], 65% in thyroid cancer [4], 17–64% in lung cancer [4], 40% in bladder cancer [4], 10% in colorectal cancer and the incidence of cancer-induced bone disease in cases of multiple myeloma is 70–95% [4]. Moreover, osteosarcoma, a primary bone cancer accounts for 2% of childhood cancers, and the average 5-year survival rate for patients with localized disease is 60–80%, and for metastatic patients 15–30% [5]. Therefore, providing appropriate relief and/or preventing the appearance of bone metastases or cancer-induced bone disease in high-risk cancer patients is a major challenge in the field. Adding to the deleterious effects of bone metastases (e.g. bone pain, fractures, spinal cord compression, hypercalcemia), certain anti-cancer therapeutic regimens also worsen bone health (e.g. treatment induced bone loss [6]). Thus, bone-targeted agents are also needed in such cases to preserve bone quality.

The multifactorial, multi-step nature of bone metastasis has unveiled different cellular and molecular targets in the

metastatic cascade such as osteoclasts, osteoblasts, osteocytes, endothelial cells and other bone microenvironment elements (e.g. immune cells, extracellular matrix). This bone tropism of solid tumours, such as breast and prostate cancers, has been explained by several concepts: 1- the "seed and soil" theory; 2- the osteomimicry of tumour cells; and 3- the establishment of a vicious cycle between tumour and bone cells. Bone marrow acts as a fertile soil for the seeding of circulating tumour cells (CTCs), expressing anchoring receptors and providing growth factors for the establishment and subsequent growth of metastatic foci. Recent developments in the cancer research field highlighted the concept of pre-metastatic niches in which primary tumour cells secrete factors or activate immune cells, that prime distant sites, rendering them more nurturing to CTCs [7–9]. Several lines of evidence have shown that breast and prostate cancer cells, under the selective pressure of the bone microenvironment, acquire an osteoblast-like phenotype by overexpressing bone-related genes, which ultimately facilitate tumour cells to thrive and subsequently cause overt bone metastases [10, 11]. In bone, tumour cells activate osteoclast differentiation and bone-resorbing activity leading to the release of growth factors previously embedded in the bone matrix, stimulating tumour growth, inhibiting osteoblast activity and thereby perpetuating a cycle of osteoclast activation and tumour growth, ultimately leading to bone destruction (Fig. 1) [12].

Because bone destruction is the main skeletal-related event associated with bone metastasis bone-targeted therapies have been essentially aimed at inhibiting the bone resorptive activity of osteoclasts. Anti-resorptive drugs used in the treatment of bone metastasis in preclinical and clinical settings include bisphosphonates, the anti-RANKL antibody denosumab, cathepsin K inhibitors, mTOR inhibitors and Src inhibitors (Fig. 1). However, with the growing evidence of the involvement of other cell types, particularly in the early steps of the bone metastatic cascade, new targets arose in osteoblasts, osteocytes, endothelial cells, immune cells, etc. Moreover, some osteoclast-targeted agents were also found to affect osteoblasts, macrophages and other cells directly or indirectly [13, 14].

This review will cover currently approved bone-targeted therapies in cancer-induced bone disease, bone-targeted therapies currently in the clinical trial pipeline, as well as emerging therapies in the discovery/preclinical phases.

Approved Bone-Targeting Therapies

Molecular-Targeted Therapies

Bisphosphonates

Bisphosphonates (BPs) have a high affinity for hydroxyapatite crystals and mainly target bone-resorbing

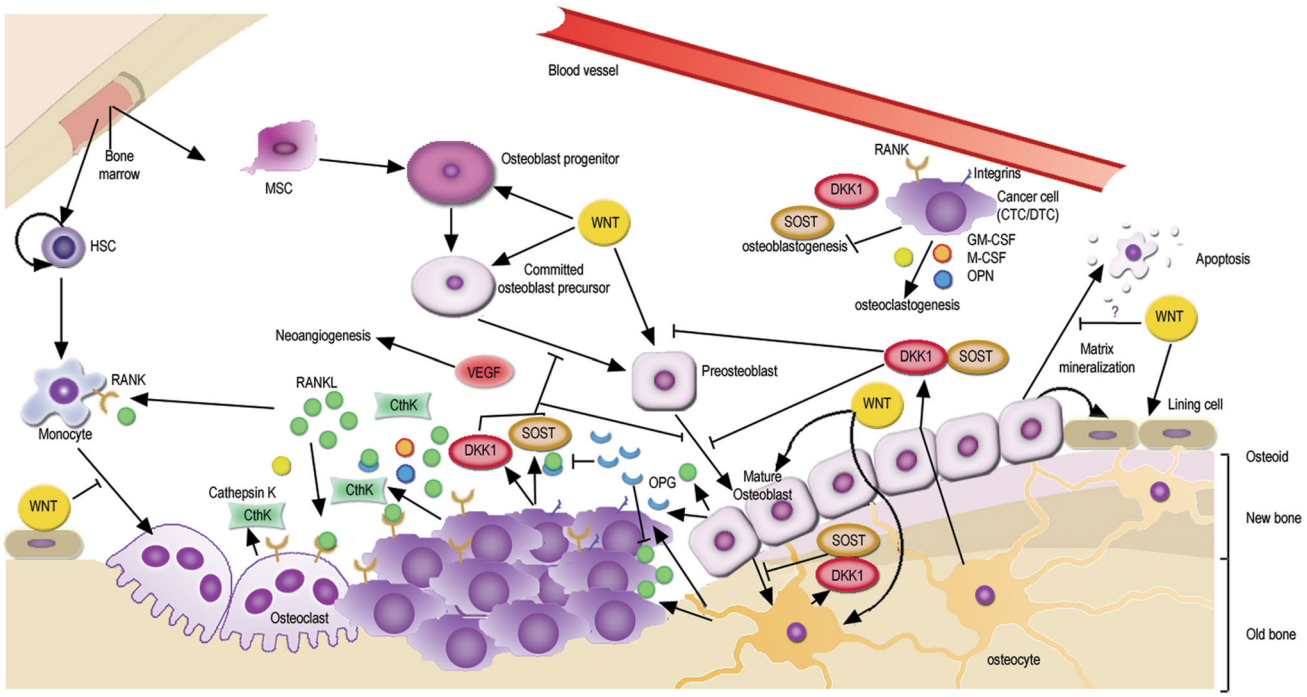

Fig. 1 Cancer-induced disease bone marrow microenvironmental targets

osteoclasts. Depending on their structure the bone mineral affinity, potency and mechanisms of action of BPs are different [15]. Non-nitrogen containing BPs (non-N-BPs) are metabolized to AppCp-type ATP analogues, which are cytotoxic via inhibition of the mitochondrial adenine nucleotide translocase (ANT), thereby inducing apoptosis. Nitrogen containing BPs (N-BPs) inhibit the mevalonate pathway enzyme farnesyl pyrophosphate synthase (FPPS) leading to inhibition of protein prenylation and accumulation of isopentenyl pyrophosphate (IPP) and triphosphoric acid 1-adenosin-5′-yl ester 3-(3-methylbut-3-enyl) ester (ApppI) [16]. ApppI evokes apoptosis similarly to the ATP analogues formed from non-nitrogen containing BPs. Table 1 provides a list of the different bisphosphonates and their anti-tumour and/or bone-preserving activity.

In the past 2–3 decades, novel cellular targets for BPs emerged both in vitro and in vivo. Among such targets are tumour-associated macrophages [26], neutrophils [27], γδ-T cells [28–31], endothelial cells [24] and osteoblasts [32]. The direct anti-tumour effects of N-BPs relied on frequent low doses of the drugs, which lead to high concentrations within tumours [33].

Preclinical evidence demonstrated the benefit of bisphosphonates in the management of established bone metastases of solid tumour and multiple myeloma. This has prompted several clinical trials in the late 90s early 2000s, leading to the EMEA (2001) and FDA (2002) approval of bisphosphonates, particularly zoledronic acid (ZOL) for the prevention of skeletal-related events (SRE) in patients with advanced malignancies involving bone and treatment of tumour-induced hypercalcaemia.

Further preclinical [34] and clinical studies (Table 2) showed that bisphosphonates prevent bone metastasis as well as have extra-skeletal benefits in defined microenvironmental contexts, such as oestrogen deprivation.

A recent meta-analysis of individual patient data from randomized trials of adjuvant BP use in early breast cancer has further proved a reduction in the risk of bone and other metastases and breast cancer mortality only in older or oestrogen-deprived patients [35].

Two prospective clinical studies [36, 37] explored the anti-tumour potential of ZOL in the neoadjuvant setting. The first study by Winter and colleagues [36] showed a synergistic effect of ZOL treatment followed by chemotherapy possibly due to an increased apoptosis and reduced proliferation and a reduction of the VEGF levels. The second study [37], enrolling fifty-three breast cancer patients (thirty-three with locally advanced and twenty with a first bone-only relapse) demonstrated that a single 4 mg dose of ZOL 14 days prior to any further treatment increased the number of apoptotic CTCs and primary tumour cells, reduced tumour and endothelial cell proliferation. ZOL antiangiogenic effects in a neoadjuvant setting were also suggested in prostate cancer patients, where ZOL treatment decreased the number of circulating endothelial and endothelial precursor cells, both markers of ongoing pathological neoangiogenesis [38].

Additionally, a meta-analysis of randomized trials of ZOL plus neoadjuvant chemotherapy in breast cancer patients revealed benefits in terms of pathological complete response in the breast only for postmenopausal women [39].

Overall, the use of BPs (mainly oral CLO and intravenous ZOL) in the adjuvant and neoadjuvant settings of large phase-III, prospective clinical trials in early breast cancer shows that these agents exhibit anti-cancer activity in patients with hormone-responsive breast cancer who had low levels of reproductive hormones at study entry, achieved either through natural menopause or ovarian suppression therapy (Table 1). The mechanisms behind the improved overall survival of these patients in a low oestrogen environment who received a BP are unknown. In stark contrast, the use of ZOL in the adjuvant treatment of patients with high-risk, castration-sensitive prostate cancer,

Table 1 Bisphosphonate classes and their anti-tumour and/or bone-preserving activity

BP class	BP	Anti-tumour or bone-preserving activity
Non-N-BPs	Clodronate	In experimental breast cancer bone metastasis, daily regimens decrease skeletal tumour growth [17]
N-BPs	Alendronate	Decrease breast cancer cell adhesion to bone matrices [18]
	Pamidronate	
	Olpadronate	
	Ibandronate	Decreases breast cancer cell adhesion to bone matrices and bone metastasis via induction of osteoclast and breast cancer cell apoptosis [18, 19]
	Zoledronate	Induces breast, prostate, lung, colon, osteosarcoma and myeloma cancer cell apoptosis [20–22] and decreases osteolytic lesions and bone tumour burden, preserving bone structure in breast cancer bone metastasis models [23–25]

BP bisphosphonates, *Non-N-BPs* non-nitrogen containing BPs, *N-BPs* nitrogen containing BPs

Table 2 Effects of bisphosphonates in adjuvant and neoadjuvant settings of breast and prostate cancer clinical trials

Clinical trial	Patient population	BP (dose, treatment duration)	Results
Breast cancer			
Oral CLO in adjuvant treatment [40]	Operable breast cancer patients	CLO (1600 mg/day *po*, 3 years)	↑Recurrence-free, bone metastasis-free and non-bone metastasis-free intervals in women > 50 years
Z/ZO-FAST [41, 42]	Postmenopausal early breast cancer patients	Immediate or delayed ZOL (4 mg *iv q* 6 months, 5 years)	Preserved BMD and ↑DFS
AZURE [43]	Early breast cancer patients	ZOL (4 mg *iv* q 3-4 weeks × 6, 4 mg *iv q* 3 months × 8, 4 mg *iv q* 6 months, 5 years)	↑IDFS and ↓extra-skeletal metastases in postmenopausal women ↓Bone metastases in the whole population
ABCSG-12 [44]	Premenopausal early breast cancer patients	ZOL (4 mg *iv q* 6 months, 3 years)	↑DFS in oestrogen-deprived women ZOL ↑efficacy of tamoxifen
Prostate cancer			
Adjuvant Effect of IV CLO [45]	Castration-sensitive prostate cancer patients	CLO (1500 mg *iv q* 3 months for 50–124 months)	Delayed time to first bone metastasis
MRC PR04, PR05 studies [46]	Castration-sensitive prostate cancer patients	CLO (2080 mg/day *po*, 5 years)	No prevention of bone metastasis
RADAR [47]	Castration-sensitive prostate cancer patients	ZOL (4 mg *iv q* 3 months, 18 months)	ZOL treatment prevented the sustained BMD loss caused by 18 months of ADT
ZEUS study [48]	High-risk, castration-sensitive prostate cancer patients	ZOL (4 mg *iv q* 3 months, 4 years)	Ineffective in the prevention bone metastasis
STAMPEDE study [49]	High-risk, castration-sensitive prostate cancer patients	ZOL (4 mg *iv q* 3 weeks × 6, 4 mg *iv q* 4 weeks, 2 years)	ZOL shows no evidence of survival improvement

BP bisphosphonate, *CLO* clodronate, *q* every, *po* per os, *iv* intravenously, *ZOL* zoledronic acid, *BMD* bone mineral density, *DFS* disease-free survival, *IDFS* invasive disease-free survival, *OS* overall survival, *SRE* skeletal-related events, *ADT* androgen-deprivation treatment

regardless of whether these men received or not androgen-deprivation therapy, does not provide a benefit on disease-free survival (Table 2). The reasons for these marked differences in the clinical outcome of breast and prostate cancer patients receiving an adjuvant BP treatment are unclear.

Despite being the gold standard in bone-targeted therapy, bisphosphonates have side-effects, which may limit their use in certain patient groups. The most common adverse effects are fatigue, fever, nausea/vomiting, anaemia, bone/joint pain, osteonecrosis of the jaw and atypical femur fractures. Osteonecrosis of the jaw and atypical femur are rare but preoccupying side-effects that are associated with bisphosphonate long-term use [50].

Due to their high affinity to hydroxyapatite, bisphosphonates are also exploited as bone-targeting moieties, for decades as radiotracers, and more recently to deliver chemotherapy and other toxic cargo to bone resident tumour cells. So far, the latter approach remains in the preclinical realm [51]. However, future studies may change this scenario.

Anti-RANKL Antibody (Denosumab)

The triad receptor activator of nuclear factor-Kβ (RANK), RANK ligand (RANKL) and osteoprotegerin (OPG, RANKL decoy receptor) has been shown to regulate osteoclast maturation, differentiation and survival. RANK is expressed by osteoclasts and osteoclast precursors, whereas RANKL is produced by osteoblasts and osteocytes [52]. The generation of the RANK- and RANKL-knockout mice revealed that, other than osteopetrosis, the other tissue compartments that exhibited functional defects were the peripheral lymphatic tissue and the development of the mammary gland during pregnancy [53]. These findings are in line with the observation that activated T cells and mammary epithelial cells express RANK/RANKL [54]. Additionally, RANK and RANKL mRNAs are expressed in other tissues, including skeletal muscle, thymus, liver, heart, brain and adrenal glands [54].

RANKL binding to RANK activates osteoclastogenesis and promotes osteoclast survival and activity, leading to increased bone resorption, a feature of osteoporosis and breast cancer-induced bone disease. 1,25-dihydroxyvitamin D3, parathyroid hormone-related protein (PTHrP), interleukins 1 and 6 (IL-1, IL-6), tumour necrosis factor (TNF), prolactin, corticosteroids and prostaglandin E2 increase RANKL expression. Oestrogens, calcitonin, transforming growth factor β (TGFβ), platelet-derived growth factor (PDGF) and calcium-induced OPG decrease RANKL/RANK binding thus preventing excessive bone resorption [54]. Upon RANK/RANKL interaction osteoblasts secrete further RANKL, which initiates a cancer cell osteoblast osteoclast vicious cycle and promotes osteoclastogenesis and osteoclast activity. Increased osteoclast-mediated bone resorption further fuels the vicious cycle by the release of growth factors from the resorbed bone, which stimulate tumour growth (Fig. 2).

Originally, due to the osteoclast-activating role of RANK/RANKL, and the known part played by osteoclasts in mediating osteolytic bone metastasis, OPG a decoy receptor for RANKL was used in preclinical breast cancer bone metastatic models [55]. Due to the ubiquitous expression of RANK in a variety of tissues, targeting the circulating and local levels of RANKL seems a safer therapeutic approach than directly inhibiting the receptor. OPG-Fc was the first of such attempts, but the induction of an immune response against OPG was a potential safety risk. Its development was therefore discontinued in favour of anti-RANKL human monoclonal antibodies with better pharmacokinetic profiles and higher anti-RANKL affinity/specificity e.g. denosumab. The fully human anti-RANKL IgG2 antibody, denosumab, binds RANKL as the endogenous decoy receptor OPG, blocking RANKL-RANK interaction and thus inhibiting osteoclastogenesis and osteoclast activity, which in turn reduces bone resorption.

As the murine RANKL is not recognized by human monoclonal antibodies against RANKL, the preclinical development of such therapeutic agents relied on the use of primates (cynomolgus monkey) [56, 57] or humanized mice [58, 59]. Most of the preclinical evidence of blocking RANKL in oncology derived from OPG-Fc and RANK-Fc treatments [60, 61] and only recently made use of the humanized mouse model suitable for denosumab treatments [62]. A strong inhibition of osteoclast differentiation and activity with these experimental treatments has prompted the use of denosumab in several oncology phase-III clinical trials. These trials focused on the prevention of SRE by denosumab in comparison with ZOL in patients with advanced cancer and bone metastasis, including multiple myeloma [63–66], breast [63, 66–69], prostate [70–73] and other metastatic cancers [64, 74]. Denosumab was found to be superior to ZOL in delaying the onset of the first SRE in breast and prostate cancer. In other metastatic solid tumours (excluding breast and prostate cancer) and multiple myeloma, denosumab was non-inferior to ZOL [69]. Denosumab has also shown positive results in the treatment of patients in which bone loss is prevalent, including women with breast cancer receiving aromatase inhibitor therapy and men with prostate cancer undergoing androgen-deprivation therapy (Table 3). In these placebo-controlled randomized phase-III trials denosumab increases bone mineral density (BMD) and decreases bone resorption (Table 3). Denosumab also increases bone metastasis-free survival and delays time to first bone metastasis compared with placebo in men with non-metastatic castration-resistant prostate cancer at high risk of bone metastasis (prostate-specific antigen [PSA] $\geq$ 8.0 μg/L or PSA doubling time $\leq$ 10.0 months, or both) (Table 3).

An ongoing clinical trial (D-CARE, NCT01077154) is testing denosumab in high-risk early breast cancer patients receiving neoadjuvant or adjuvant therapy (Table 3). Its

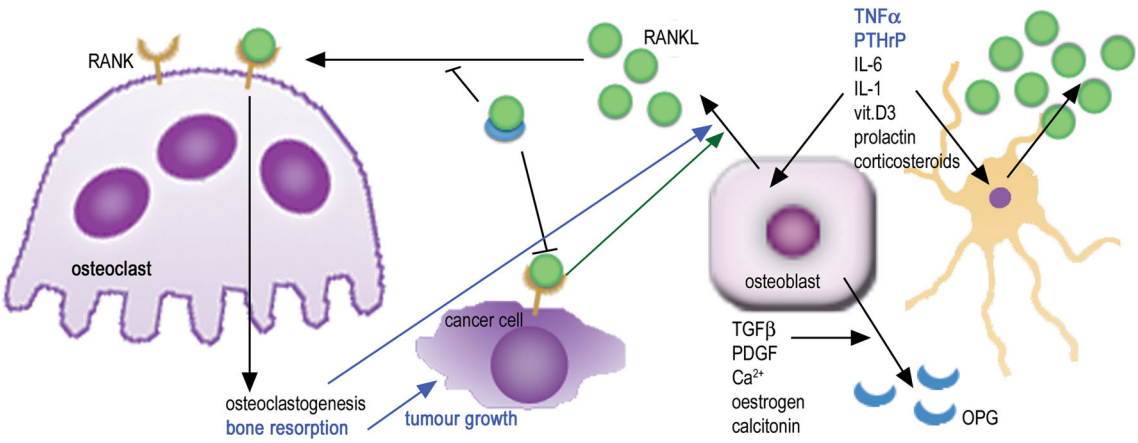

Fig. 2 RANK-RANKL signalling in cancer-induced bone disease

Table 3 Effects of denosumab in adjuvant settings in breast and prostate cancer clinical trials

Clinical trial	Patient population	Dose	Results
Breast cancer			
HALT, NCT00089661 [75, 76]	Hormone receptor-positive non-metastatic breast cancer patients	Denosumab (60 mg *sc q* 6 months) versus placebo plus AI	↑BMD and ↓bone remodelling markers
ABCSG-18 [77]	Postmenopausal breast cancer patients	Denosumab (60 mg *sc q* 6 months) versus placebo plus AI	↓AI-induced fractures
Prostate cancer			
NCT00089674 [78]	Non-metastatic CRPC patients	Denosumab (60 mg *sc q* 6 months) plus androgen-deprivation therapy	Rapid and sustained decrease of bone turnover markers by denosumab
Smith et al. [72]	Non-metastatic CRPC patients at high risk of bone metastasis	Denosumab (120 mg *sc q* 4 weeks) versus placebo, plus ADT	Increase bone metastasis-free survival
D-CARE NCT01077154	High-risk early breast cancer patients	Denosumab (120 mg *sc q* 4 weeks, 6 months, then 120 mg *sc q* 3 months, 18 months) versus placebo, plus neoadjuvant or adjuvant therapy	August 2017

sc subcutaneously, *q* every, *AI* aromatase inhibitors, *ADT* androgen-deprivation therapy, *BMD* bone mineral density, *CRPC* castration-resistant prostate cancer

estimated primary completion date is August 2017 and its estimated study completion date August 2022.

Cumulative in vitro, in vivo and retrospective clinical data link RANK overexpression by breast cells with stem cell properties, epithelial to mesenchymal transition [79], malignant transformation and ultimately with disease progression, particularly in progesterone driven breast cancers [53] and BRCA mutants [80]. Therefore, certain experts suggest the use of denosumab for breast cancer chemoprevention in high-risk women (BRCA1, BRCA2 mutation carriers) as an alternative to radical prophylactic mastectomy. An ongoing prospective clinical trial has started to address the efficacy of such a strategy (BRCA-D trial, ACTRN12614000694617).

In oncology denosumab is currently FDA approved for (1) treatment of adults and skeletally mature adolescents with giant cell tumour of bone that is unresectable or where surgical resection is likely to result in severe morbidity,(2) prevention of SREs (pathological fracture, radiation to bone, spinal cord compression or surgery to bone) in adults with bone metastases from solid tumours and (3) increasing bone mass in patients at high risk for fracture due to ADT for non-metastatic prostate cancer or AI therapy for breast cancer. Similar indications have been approved by EMEA, except for its use in unresectable giant cell tumour of bone and breast cancer patients receiving AI.

The most common adverse effects of denosumab are similar to those of BPs: nausea, diarrhoea, fatigue and osteonecrosis of the jaw, the latter being equally rare [81].

Mammalian Target of Rapamycin (mTOR) Inhibitors

The dysregulation of the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mTOR pathway is a common feature of cancer [82]. Additionally, it has been implicated in normal and pathological osteoclastogenesis [83]. Indeed, mTOR is an antiapoptotic downstream target of M-CSF, RANKL and TNF-α, which is essential for osteoclast differentiation, survival and activity [84, 85]. mTOR inhibition leads to increased OPG expression, osteoclast apoptosis and might also promote osteoblastogenesis [86, 87].

Rapamycin and its analogues (e.g. sirolimus, temsirolimus, everolimus, deforolimus) are mTOR inhibitors, which block the translation of survival factors and apoptosis inhibitors [84].

Preclinical evidence demonstrated an effect of mTOR inhibition in cancer-induced bone diseases. For instance, in the 4T1 orthotopic breast cancer model, rapamycin treatment of tumour-bearing animals reduces the number of osteolytic lesions and increased survival [88]. In osteoblastic and osteolytic osteosarcoma models, everolimus plus ZOL combination treatment slowed tumour progression and increased bone mass [89]. In prostate cancer, everolimus alone or in combination with docetaxel and/or ZOL decreased tumour burden and cachexia [90]. In oral squamous cell carcinoma, temsirolimus slowed tumour growth and inhibited osteolysis [91]. In neuroblastoma, despite the known direct effects of mTOR inhibition on osteoclasts and osteoblasts and their respective precursors the overall effects on cancer-induced bone disease may well be a combination of direct anti-tumour effects and

microenvironmental effects as the PI3K/AKT/mTOR pathway also regulates cell growth and apoptosis.

Due to its in vivo synergistic effect with other bone resorption inhibitors, chemotherapy and hormonal therapy, several clinical trials evaluated the effect of mTOR inhibitors in advanced cancers. In breast cancer, the efficacy of everolimus vs placebo in combination with trastuzumab plus paclitaxel as first-line treatment for patients with HER2-positive advanced breast cancer (BOLERO-1 trial) was investigated [92]. Although everolimus did not improve progression-free survival (PFS) of the whole population, compared to placebo there was a 7-month PFS prolongation in hormone receptor negative HER-2 positive breast cancer patients [92]. The BOLERO-2 trial led to the approval of everolimus in combination with exemestane, for patients with advanced hormone receptor-positive/HER2-negative (HER2−) breast cancer who progressed on prior endocrine therapy with either letrozole or anastrozole [93–95]. Additionally, exploratory analyses in BOLERO-2 evaluated the effect of everolimus on bone marker levels and progressive disease in bone [6]. Data obtained show that, compared to exemestane alone, everolimus plus exemestane has beneficial effects on bone turnover and progressive disease in bone in patients, irrespective of the use of bisphosphonates [6]. The effect of everolimus, as a single agent, was also studied in a double-blind, placebo-controlled, phase-II trial in HER2-negative breast cancer patients with bone metastases only (RADAR study) [96]. The results suggest that patients with bone metastases only may retrieve long-term benefit from everolimus.

The efficacy and safety of everolimus has also been examined in other advanced cancers with bone metastasis, including renal cell carcinoma, non-small cell lung carcinoma and prostate carcinoma [97–99]. Compared to everolimus alone, the combination of everolimus and ZOL significantly reduces bone resorption markers and prolongs tumour control in renal cell carcinoma patients with bone metastases [97].

Similar synergistic effects of everolimus and ZOL in delaying bone metastasis and prolonging OS were seen in patients with advanced non-small cell lung carcinoma and bone metastases [99]. In contrast, the addition of everolimus to carboplatin demonstrated minimal clinical efficacy in metastatic castrate-resistant prostate cancer patients. However, in this trial, patients were not pre-selected for: PTEN deletion, TSC1 mutations, and lack of pAKT staining, all potential biomarkers for mTOR inhibition response. Thus, the studied population could be biased to everolimus non-responders [100].

Phase-I clinical trials also explored the use of everolimus in relapsed and/or refractory multiple myeloma patients [101, 102]. Although these trials were designed to evaluate feasibility, anti-myeloma activity, defined as clinical benefit, was documented when everolimus was used as a single agent or in combination with lenalidomide [102].

The adverse events profile of mTOR inhibitors includes stomatitis, infection, rash, non-infectious pneumonitis, hyperglycaemia and hyperlipidaemia, most reversible by dose adjustment and supportive care measures. The metabolic abnormalities are of utmost concern in post-menopausal women, who have an already increased risk for such conditions. Thus, special attention must be paid in the management of this patient population [103].

Radium-223 and Other Radiopharmaceuticals

External beam radiation therapy has been in clinical practice to treat bone pain in metastatic patients. However, its use is limited to localized disease due to the toxicity to healthy surrounding tissues. Low linear energy transfer β-emitter radionucleotides like strontium-89 (calcium mimetic that binds to bone mineral) and bisphosphonate-conjugated samarium-153 enabled targeted delivery of radiotherapy to bone and showed benefits in the palliative treatment of bone metastatic patients. The wide radiation range of the latter two agents causes dose-limiting toxicities, thus their use is restricted. On the contrary, high linear energy transfer radionucleotides (particularly α-emitters) such as radium-223 have advantages in terms of relative biological effectiveness, enhanced bystander effect, reduced oxygen enhancement ratio and shorter range of radiation emission. This decreases toxicity and delivers higher energy to target tumour cells even in hypoxic sites, with lower probability to target healthy bone marrow cells [104, 105].

Radionucleotides act by inducing DNA double strand breaks which when accumulated in target cells lead to cell death. Due to unknown mechanisms, bystander cells not directly irradiated may also undergo apoptosis, via the formation of foci of γ-phosphorylated histone protein (γH2A) [104].

Preclinical studies demonstrated radium-223 distribution to osseous sites [106, 107] and its effectiveness in decreasing osteolytic lesions in experimental models of bone metastasis [108]. In the clinic, a phase-II trial in castration-resistant prostate cancer patients with symptomatic bone metastases (ALSYMPCA) showed that radium-223 improves OS, compared to the placebo group receiving the best standard of care including bisphosphonates or denosumab [109, 110]. Additionally, radium-223 improves quality of life, provides pain relief and is well tolerated in this patient population. Results from the ALSYMPCA study led to the FDA approval of radium-223 as a single agent in patients with hormone refractory prostate cancer and bone metastases. Similar encouraging

results were observed in metastatic breast cancer [111–113]. Experimentally, radium-223 alone or in combination with ZOL or doxorubicin, increases OS, decreases osteolysis and skeletal tumour burden and prevents tumour-induced cachexia in a breast cancer model of bone metastasis [111]. In an open-label phase-IIa study on breast cancer patients with bone-dominant disease, who have progressed on endocrine therapy, radium-223 treatment consistently reduced bone resorption and bone formation markers as well as metabolic changes associated with osteoblastic bone metastases [113]. Large randomized clinical trials in bone metastatic breast cancer patients are ongoing in order to examine the effect of radium-223 in combination with endocrine therapy and exemestane plus everolimus (NCT02258464 and NCT02258451 trials, respectively) [105]. Little is known on the effect of radium-223 in other cancer types. However, trials are ongoing in osteosarcoma (NCT01833520) and thyroid cancer with refractory bone metastases (NCT02390934) [105].

At the recommended doses (six injections of radium-223 at 50 kBq/kg every 4 weeks) data from the ALSYMPCA trial indicate low-grade diarrhoea and low-grade myelosuppression as the most common adverse effects of radium-223 [110].

Proteasome Inhibitors (Bortezomib)

The proteasome is an ATP-dependent enzymatic complex responsible for the degradation of ubiquitinated proteins. Malignant cells are generally more sensitive to proteasome inhibition due to their higher proliferation and protein synthesis rates. This is particularly true in multiple myeloma [114].

Bortezomib was the first proteasome inhibitor approved in the treatment of multiple myeloma. Bortezomib reversibly binds to the chymotrypsin-like subunit of the proteasome inhibiting its catalytic activity. Bortezomib dose-dependently inhibits RANKL-induced osteoclastogenesis as it prevents the degradation of the NF-κB inhibitor I-κB, which blocks the binding of NF-κB to the promoters of target genes. Additionally, bortezomib promotes bone formation via an increase in both Runx2 activity and expression of osteoblast markers such as type I collagen, as well as by increasing BMP-2 secretion by osteoblasts and inhibiting multiple myeloma-induced osteocyte death [114]. Due to its direct anti-tumour and anti-osteoclastic effects and its stimulatory effect on osteoblasts, bortezomib (as a proteasome inhibitor) seems ideal in the management of multiple myeloma. However, cancer patients have intrinsic or acquired resistance to bortezomib therapy. The mechanisms for this resistance are not fully understood, but include: upregulation of constitutive proteasome; point mutations in the bortezomib binding pocket; downregulation of the immunoproteasome; cellular extrusion of bortezomib by the drug efflux transporter P-glycoprotein; activation of pro-survival pathways (particularly in the bone marrow microenvironment: MAPK, insulin like growth factor and Akt/PI3 K/signalling upregulation, increased IL-6 secretion, and expression of miRNA 15a); loss of Xbp1; increased expression of (phosphorylated) MARCKS; and autophagy [115].

Hundreds of clinical trials with bortezomib in combination therapy regimens are ongoing in multiple myeloma. Addressing them all is out of the scope of this review. A recent meta-analysis demonstrated that bortezomib improves OS, PFS and response rate in multiple myeloma patients, compared to those who did not receive bortezomib. The most common side-effects of bortezomib are increased risk of thrombocytopenia, neutropenia, gastrointestinal toxicities, peripheral neuropathy, infection and fatigue [116].

Hormone-Related Therapies

Anti-androgens

Sex steroids are essential for bone homeostasis. In males, testosterone is the most abundant sex steroid and it confers protection against osteoporotic fractures mainly via aromatization to oestradiol, which will act on oestrogen receptors. Testosterone also acts on the androgen receptor present in osteoblasts and osteocytes as well as in other cell types [117]. Abiraterone acetate is an irreversible inhibitor of cytochrome P17, which blocks androgen biosynthesis (Fig. 3) leading to undetectable levels of androgens [118].

Enzalutamide is an inhibitor of the androgen receptor (Fig. 3), which has shown to have bone specific effects, blocking the aromatization independent effects of testosterone in bone [119].

Prostate cancer is an androgen-dependent pathology, which initially responds to surgical or chemical castration. Androgen altered signalling is a validated therapeutic target in metastatic castration-resistant prostate cancer.

Initially thought to target only prostate cancer cells, abiraterone acetate directly inhibits osteoclastogenesis and promotes osteoblastogenesis and bone matrix deposition [120]. The use of anti-androgens like abiraterone acetate increases OS and delays time to development of SRE in metastatic castration-resistant prostate cancer patients [121]. However, despite their approved use in metastatic castration-resistant prostate cancer, the existence of androgen receptor splice variants (e.g. AR.V7) and mutants leads to the quick development of resistance to anti-androgens [122].

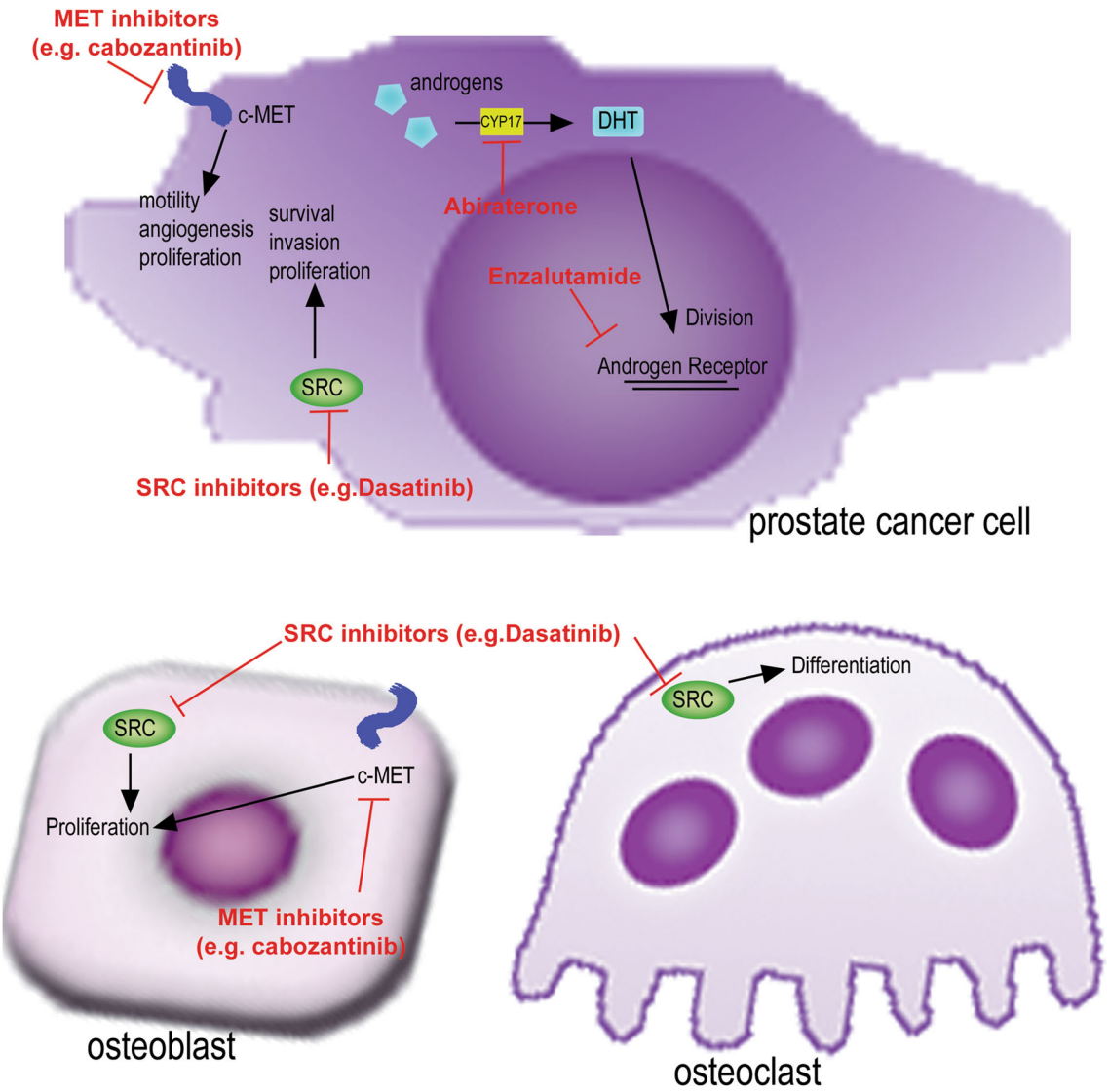

Fig. 3 Simplified mechanisms of action of c-SRC inhibitors, MET inhibitors and anti-androgens in prostate cancer-induced bone disease

Bone-Targeting Therapies in Clinical Trials

Molecular-Targeted Therapies

Cathepsin K Inhibitors

Cathepsin K is a lysosomal cysteine proteinase essential for osteoclast activity. Osteoclast secrete protons providing the optimal acidic microenvironment for cathepsin K degradation of the demineralized collagenous matrix by cleavage at multiple sites and release of N-telopeptide collagen fragments. Moreover, cathepsin K is also expressed by bone metastatic breast and prostate cancer cells, thus cathepsin K activity is elevated in osteolytic bone metastases and its targeting may provide dual cell targeting (osteoclasts and cancer cells) [123–125]. Additionally, in osteosarcoma patients, cathepsin K seems to be predictive of poor prognosis [126].

The design of cathepsin K inhibitors was initially challenging due to high similarity of substrate and mechanisms between members of the cysteine cathepsin family. Additionally, species differences (87–88% human/rodent homology) make human cathepsin K inhibitors less potent than rodent cathepsin K inhibitors. The initial cathepsin K inhibitors bound to the catalytic site irreversibly, which made them non-attractive to chronic use. Later generations of inhibitors (e.g. dutacatib, odanacatib, balicatib) reversibly bind to the catalytic site of cathepsin K, blocking substrate binding and subsequent cleavage. Another issue with these drugs is their high lysosomotropism. Prolonged lysosomal trapping of cathepsin K inhibitors leads to off-target effects and to skin adverse effects, common to

lysosomal targeting drugs [127]. For instance, balicatib a basic lysosomotropic cathepsin K inhibitor successfully completed a phase-II clinical trial for osteoarthritis (NCT00371670), increasing BMD and decreasing bone resorption, but was discontinued due to rash and morphea-like skin changes [127].

In preclinical models of breast cancer bone metastasis, treatment and preventive protocols with the cathepsin K inhibitor dutacatib (AFG495) 50 mg/kg, twice daily intraperitoneally alone or in combination with ZOL (single dose of 100 μg/kg) decreased tumour-induced osteolysis and skeletal tumour burden and did not affect primary breast tumour growth [124]. Cathepsin K inhibition did not affect breast cancer cell proliferation, thus pointing to a microenvironmental effect of decreased bone resorption leading to a less nurturing soil for tumour cell implantation [124]. Odanacatib is another cathepsin K inhibitor that is as effective as ZOL to reduce bone resorption in breast cancer patients with bone metastases, as assessed by measuring urinary N-telopeptide of type I collagen [128]. Interestingly, cathepsin K inhibitor L-235, which is structurally related to odanacatib but with higher potency against the rodent enzyme, was tested in a breast cancer model of bone metastasis and results were very similar to those previously reported for AFG495 [124, 129]. Additionally, L-235 inhibited breast cancer cell invasion in vitro, thus pointing to a dual targeting of breast cancer cells and osteoclasts [129].

The promising preclinical results obtained with non-basic/non-lysosomotropic cathepsin K inhibitors such as odanacatib led to several clinical trials in osteoporosis and cancer-associated bone metastases. As aforementioned, the safety and efficacy of odanacatib on inhibition of biochemical markers of bone turnover in patients with breast cancer and established bone metastases has been reported [128]. Similarly, a phase-II study in postmenopausal osteoporosis reported that a 5-year continuous therapy with odanacatib inhibited bone resorption and increased BMD [130]. A phase-III Long-Term Odanacatib Fracture Trial (LOFT) enrolling 16,713 participants from 387 centres was therefore conducted [131]. Unfortunately, phase-III results showed that while the drug could reduce osteoporotic fractures, it also increased the risk of atrial fibrillation and stroke. The big pharma Merck, the odanacatib manufacturer, therefore decided to discontinue the development of this drug. For undisclosed reasons the metastatic bone disease trials (NCT01552122, NCT00691899 and NCT00692458) were also withdrawn.

c-Src Inhibitors

c-Src was the first proto-oncogene to be identified and it is a non-receptor protein tyrosine kinase crucial in several cellular processes such as proliferation, migration, invasion and survival. c-Src knockout mice have defective osteoclasts, impaired bone remodelling, are osteopetrotic and toothless [132]. Osteoclastic c-Src activation is important for osteoclast differentiation and activity; it recruits several signalling proteins for motility and cytoskeletal rearrangement (e.g. podosome formation). RANK/RANKL signalling leads to c-Src activation, which in turn triggers PI3 K/Akt/mTOR signalling, promoting osteoclast survival [133]. Therefore, c-Src inhibition has the potential to impair osteoclast-mediated bone resorption. Additionally, c-Src inhibition has been shown to affect osteoblasts, suppressing proliferation and enhancing differentiation (Fig. 3) [134].

Several orally active c-Src/multikinase inhibitors are FDA-approved (bosutinib, dasatinib, ponatinib and vandetanib) or are in clinical trials for various malignancies. Due to the high similarity of Src family tyrosine kinases, none of the current inhibitors is uniquely selective for Src, Table 4 shows their known targets and potential therapeutic indications [135].

Pharmacological inhibition of c-Src or intra-cardiac injection of dominant-negative kinase dead c-Src breast cancer cells, reduces bone and visceral metastatic incidence as well as morbidity and lethality. Additionally, subcutaneous injection of c-Src overexpressing breast cancer cells originates larger and more proliferating breast tumours than wild type cells. Breast cancer cell c-Src overexpression affects osteoclastogenesis (via osteoblast inhibition) and endothelial proliferation. Thus, c-Src pharmacological inhibition may decrease bone metastasis by acting directly in tumour cells, osteoclasts, osteoblasts and endothelial cells [136].

In addition to osteoclasts, platelets and tumour cells, c-Src is also expressed by neurons. Therefore, a recent preclinical study evaluated the use of saracatinib in cancer-induced bone pain. Intra-tibial injection of rat mammary cancer cells induced thermal hyperalgesia and mechanical allodynia via phosphorylation of the GluN1 subunit of the *N*-methyl-D-aspartate receptor. Saracatinib treatment reduced this phosphorylation levels and reversed the thermal hyperalgesia while having no anti-tumour or bone preservation effect at any of the doses used [137]. This prompted the initiation of a clinical trial for the use of saracatinib in cancer-induced bone pain NCT02085603.

Preclinical studies with the PC-3 human bone metastatic prostate cancer model and two different c-Src inhibitors bosutinib [142] and saracatinib [143] demonstrated that c-Src inhibition decreases prostate cancer cell proliferation, migration and invasion in vitro and reduces tumour burden (orthotopically and in bone) and tumour-induced osteolysis in bone metastatic animals. Moreover, c-Src inhibition decreased the phosphorylation levels of several signalling

Table 4 c-Src/multikinase inhibitors (adapted from [135])

Inhibitor	Known targets	Potential therapeutic indications	Clinical trials in bone disease
Bosutinib	BCR-Abl, Src, Lyn, Hck, Kit, PDGFR	Ph^+, CML, ALL, breast cancer, glioblastoma	Yes [138]
Dasatinib	BCR-Abl, Src, Fyn, Yes, Lck, Arg, Kit, EphA2, EGFR, PDGFRβ	Ph^+, CML, ALL, breast, colorectal, endometrial, head and neck, ovarian, and small cell lung cancers, glioblastoma, melanoma and NSCLC	Yes [139–141]
Ponatinib	BCR-Abl, Src family kinases, VEGFR, PDGFR, FGFR, Eph, Kit, RET, Tie2, Flt3	Ph^+, CML, ALL, endometrial, GIST, hepatic biliary, small cell lung and thyroid cancers	No
Vandetanib	RET, Src family kinases, EGFR, VEGFRs, Brk, Tie2, EphRs	Medullary thyroid cancer, breast, head and neck, kidney cancers, NSCLC and several other solid tumours	Yes (NCT00659438)
Saracatinib (AZD0530)	Src, BCR-Abl	Colorectal, gastric, ovarian, small cell lung cancers, NSCLC, and metastatic osteosarcoma in lung	Yes (NCT00559507, NCT00397878, NCT02085603, NCT00558272)

Ph^+ Philadelphia chromosome positive leukaemia, *CML* chronic myelogenous leukaemia, *ALL* acute lymphoblastic leukaemia, *NSCLC* non-small cell lung cancers, *GIST* gastrointestinal stromal tumour

molecules (AKT, mitogen-activated protein kinase MAPK, focal adhesion kinase FAK) as well as the transcription of genes essential for tumour progression (urokinase receptor uPAR, matrix metalloproteinase 2 MMP-2, MMP-6, bone morphogenetic protein 2 BMP-2, BMP-6, interleukin 8 IL-8 and TGF-β) in prostate cancer cells. An orally bioavailable c-Src inhibitor (KX2-391), which targets the substrate binding site instead of the ATP-binding site, as all the other c-Src inhibitors, has been also investigated in castration-resistant prostate cancer patients with bone metastases. KX2-391 40 mg po twice daily did not however have an anti-tumour effect and had only modest effects on inhibition of bone turnover markers [144]. The study of its pharmacokinetic demonstrated that the median maximum concentration (C_{max}) achieved was inferior to the C_{max} necessary for inhibition of tubulin polymerization, which may explain these modest inhibitory effects [144].

Overall, phase-I/II trials in bone metastatic patients, using dasatinib and saracatinib showed these drugs are safe, well tolerated and have encouraging results in terms of delaying disease progression. The use of these drugs in large phase-III trials is awaited.

Anti-sclerostin Antibodies

Sclerostin (SOST) is an extracellular wingless pathway (Wnt) inhibitor, which acts by binding to low-density lipoprotein receptor-related proteins 5 and 6 (LRP5 and LRP6), preventing Wnt ligands binding and activation of canonical Wnt signalling (Fig. 4) in bone thus decreasing bone formation through osteoblast inhibition. SOST is a protein mainly secreted by osteocytes [145]. Nevertheless, multiple myeloma cells [146] and breast cancer cells also secrete SOST [147], SOST becomes therefore an interesting target in cancer-induced bone disease, as SOST inhibition could potentially lead to increased bone. For example, anastrozole treatment of postmenopausal women with hormone receptor-positive non-metastatic early breast cancer leads to increased SOST serum levels [148]. This suggests a role for osteocytes in bone turnover of breast cancer patients, which could be therefore modulated by anti-SOST therapy. Similarly, in symptomatic multiple myeloma patients, high SOST circulating levels positively correlated with altered bone remodelling and advanced disease [149, 150].

The role of SOST in prostate cancer is more controversial. Studies show an increase of serum SOST levels in prostate cancer patients, particularly those under androgen-deprivation therapy [151] or with high bone turnover [152]. However, a transcriptional and proteomic study shows no significant difference in the local levels of SOST in prostate cancer bone osteolytic and osteoblastic metastases [153]. A confounding factor could be the use of androgen depriving therapy, which could mask differences between baseline circulating and local SOST levels. Additionally, according to Hudson and colleagues SOST and Dickkopf 1(DKK1, another Wnt inhibitor) have opposing effects in prostate cancer bone metastasis. DKK1 promotes prostate cancer invasion and metastasis and SOST evokes the opposite effect [154]. This surprising result warrants further investigations into the role of SOST in prostate cancer, especially as studies demonstrate that Wnt activation evokes prostate cancer soft tissue and bone metastasis [155].

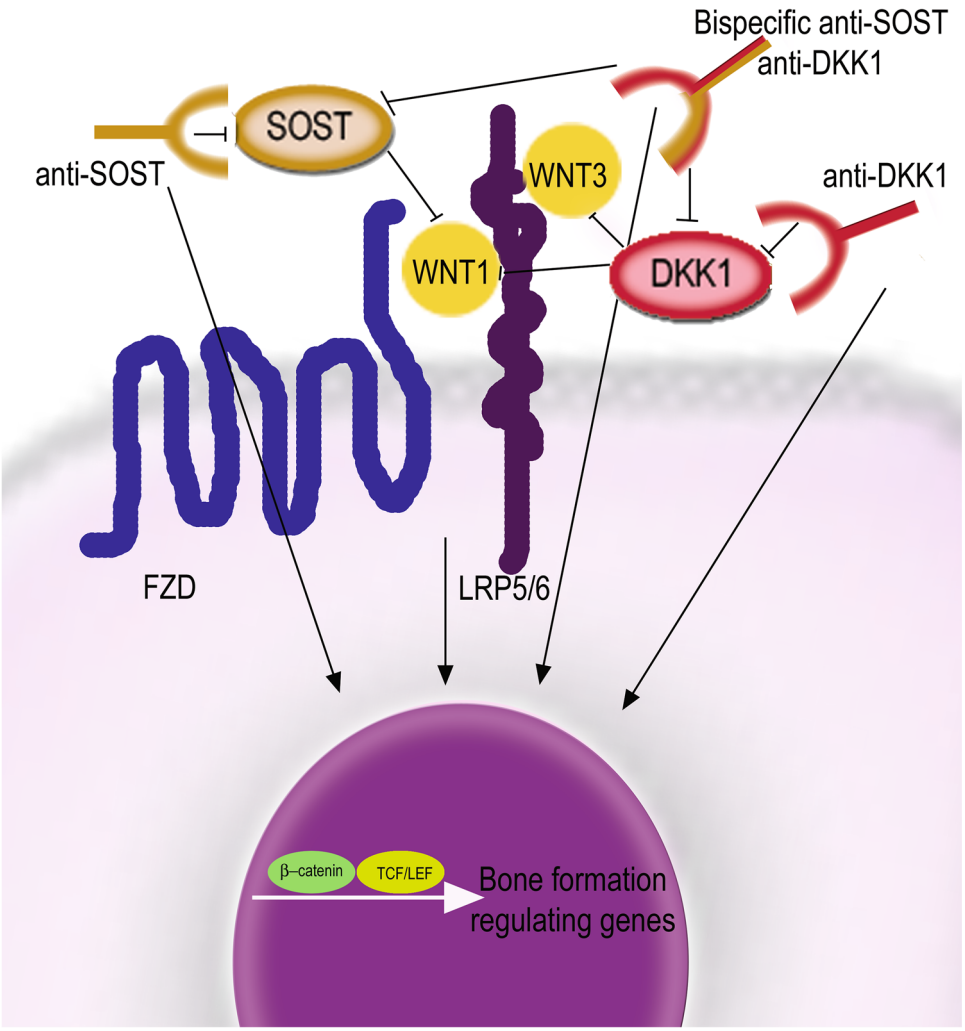

Fig. 4 Wnt signalling in bone, mechanisms of action of anti-SOST, anti-DKK1 and bispecific antibodies

There is some preclinical evidence that anti-SOST antibodies exhibit bone anabolic effects in animal models of bone loss [156–158]. A study in a multiple myeloma model demonstrated that an anti-SOST antibody can increase bone volume in tumour-bearing mice to levels similar to naïve mice and it decreased tumour burden, thus slowing disease progression [159]. Human SOST neutralizing monoclonal antibodies, such as romosozumab, blosozumab and BPS804 are in clinical development for osteoporosis and osteogenesis imperfecta [160–162].

Pending of the results of these ongoing clinical trials, anti-SOST therapy in cancer patients with bone metastasis could be then considered.

Emerging Bone-Targeting Therapies

Molecular-Targeted Therapies

Anti-Dikkopf-1 Antibody

Dikkopf-1 (DKK1), another extracellular Wnt inhibitor, produced by breast [163], prostate and multiple myeloma cancer cells leads to pronounced osteoblast inhibition, which is essential for the establishment of osteolytic bone disease. Wnt inhibition evokes OPG downregulation and RANKL upregulation, thus indirectly controls osteoclastogenesis [145].

Preclinical evidence in multiple myeloma shows that DKK1 inhibition reduces cancer-induced bone destruction and promotes bone formation [164]. In breast cancer bone metastasis DKK1 inhibition is controversial, as in vitro studies show that DKK1 inhibits tumour growth [165], which poses issues to the clinical utility of an anti-DKK1 treatment. In a PDX model of human osteosarcoma, the use

of a monoclonal antibody against human DKK1 (BHQ880) slowed the orthotopical tumour growth and inhibited metastasis, while increasing bone differentiation markers [166].

Phase-I (NCT00741377) and -II (NCT01302886, NCT01337752) clinical trials were conducted in multiple myeloma with the BHQ880, alone or in combination with ZOL and anti-myeloma therapy. The phase-I trial showed that BHQ880 is well tolerated and has potential clinical activity in multiple myeloma [167]. Phase-III clinical trials are missing to further ascertain the clinical utility of BHQ880.

Due to compensatory mechanisms in the expression of Wnt inhibitors upon their inhibition in monotherapy, a bispecific monoclonal antibody targeting SOST and DKK1 is in preclinical development. So far the dual inhibition of the Wnt inhibitors by bispecific antibodies has shown a further increase in bone mass (Fig. 4) [168].

MET/VEGFR Inhibitors

MET also known as hepatocyte growth factor receptor (HGFR) and vascular endothelial growth factor receptors (VEGFRs) are tyrosine protein kinase receptors. Expression of VEGFRs especially by bone marrow-derived endothelial progenitor cells and hematopoietic progenitor cells has been linked with the establishment of pre-metastatic niches. Particularly, $VEGFR1^+$ cells are required for extravasation, and $VEGFR2^+$ cells for neovascularization [169]. Moreover, VEGF/VEGFR signalling contributes to prostate cancer-induced osteoblast differentiation and activity [170]. MET is overexpressed in prostate cancer primary tumours and bone metastases and correlates with higher tumour grade [171]. MET/VEGFR targeting has thus the potential to impair tumour-induced neoangiogenesis, pre-metastatic niche formation, and osteoblast differentiation and activity (Fig. 3).

Initial studies with VEGFR inhibitors (e.g. sunitinib, sorafenib) and anti-VEGF therapy (e.g. bevacizumab) evoked an increased invasion and metastasis probably due to a rebound vascularization via activation of the MET pathway.

Cabozantinib is a tyrosine protein kinase receptor inhibitor, which preferentially targets VEGFR2 and MET. AXL, FLT-3, KIT and RET tyrosine kinase receptors are also inhibited but with less potency [172]. Cabozantinib treatment suppresses tumour growth and angiogenesis in multiple tumour types. Contrary to VEGFR/VEGF inhibition, cabozantinib also decreases metastasis [172]. In bone metastatic prostate cancer models, cabozantinib inhibited bone- and subcutaneous tumour growth. Additionally, cabozantinib altered bone remodelling in tumour free and tumour-bearing bones. In tumour free bones, cabozantinib increased bone volume/tissue volume ratio (BV/TV). In tumour-bearing bones, cabozantinib effects on bone remodelling were dependent of the tumour type: in LuCaP 23.1 tumours (osteoblastic, androgen sensitive) cabozantinib decreased BV/TV; in C4-2B tumours (mixed osteoblastic/osteolytic, castration resistant) it tended to increase BV/TV. The authors speculated that the different effects seen in the LuCap 23.1 and C4-2B models were due to the higher bone remodelling induced by the former, thus an effect on the tumour growth (smaller bone tumours, lower osteoblastic reaction, lower bone formation) led to a decrease on BV/TV. However, since the treatment also affected normal bone remodelling, one should consider that, the overall effect is a combination of cabozantinib on tumour and bone cells [173]. Dai and colleagues have obtained similar results with the additional demonstration of an effect of cabozantinib in osteoblasts [174]. Further studies in normal bones have shown that this agent reversibly: increases bone volume; increases osteoblast numbers (in male mice); decreases the number of osteoclasts (in female mice); and alters the bone marrow composition of treated animals, originating vascular ectasia, spillage of mature red blood cells in the extra vascular bone marrow and increasing the number of megakaryocytes [32]. Additional proof for the direct effect of cabozantinib in prostate cancer tumour cells, endothelial cells and osteoblasts was provided by patient derived xenografts and clinical trials [175].

A study with TAS-115, another MET/VEGFR inhibitor that also inhibits FMS, demonstrated an osteolysis prevention effect (PC-3 model) due to prostate cancer cell and osteoclast targeting (the latter via inhibition of FMS-dependent RANKL-induced pre-osteoclast to osteoclast differentiation) [176]. Similar results emerged from a model of bone metastatic lung carcinoma [177].

At least eight clinical trials of cabozantinib in bone metastatic cancers (prostate, breast, lung, multiple myeloma) are currently active. In the phase-III clinical trial in heavily treated (with docetaxel and Abiraterone acetate and/or enzalutamide) bone metastatic castration-resistant prostate cancer patients the results of cabozantinib versus prednisone were somehow disappointing, as the primary outcome of improved OS was not met. Cabozantinib improved bone scan response, radiographic PFS, symptomatic skeletal events, CTCs and bone biomarkers. The investigators argue that patient selection may be an issue and that treatment discontinuation in the cabozantinib group may be a confounding factor [178]. Nevertheless, the results of this trial led to the termination of several other trials in bone metastatic castration-resistant prostate cancer. Further clinical development of cabozantinib is pending on the use of a specific biomarker to select patients, on the clarification of the best diagnostic tool to determine its

effectiveness in bone metastatic castration-resistant prostate cancer (as bone scans seem inappropriate), and of its use in combination therapy [179, 180].

The most common adverse events of cabozantinib reported in the phase-III clinical trial were decreased appetite, nausea, diarrhoea, fatigue, vomiting, asthenia, decreased weight, constipation and anaemia [178].

Anti-integrins

Integrins are heterodimeric cell-surface receptors that mediate adhesion to the extracellular matrix and immunoglobulin superfamily molecules. Structurally, integrins are composed of non-covalently bound α and β subunits forming 24 different heterodimers. Each has an extracellular domain, a single trans-membrane region and a short cytoplasmic tail. The extracellular domain shows high affinity for a defined RGD-motif expressed by vitronectin, fibronectin, osteopontin and other extracellular matrix components. Ligand binding propagates outside-in and inside-out intracellular signalling. The unique integrin repertoire of a given cell determines the extent of adhesion and migration of that cell in different matrices. Abnormal integrin overexpression by tumour and/or host cells has been associated with tumour proliferation, survival, angiogenesis, migration and metastasis [181]. Particularly, in osteotropic tumours, overexpression of αvβ3 integrin has been linked with increased bone colonization by breast, prostate and lung cancer cells and osteomimetism by multiple myeloma cells. The interaction of cancer cells with stromal cells and immune cells via αvβ3 integrin induces the production of cytokines and growth factors that recruit and differentiate osteoclast precursors, thus inducing lytic lesions [182]. Other integrins, such as αvβ5, αvβ6, α5β1, α6β4, α4β1, have also been implicated in the tumour progression of different tumour types [181]. Therefore, integrin-targeting strategies either by the use of RGD-motif peptidomimetics or of monoclonal antibodies has reached preclinical and clinical development. αvβ3 integrin is the predominant integrin in osteoclasts and it is involved in osteoclast attachment to the bone matrix [183].

Preclinical evidence has established αvβ3 as a target in breast cancer bone metastasis. Tumour αvβ3 [184, 185] expression was essential for bone homing but not for bone colonization of breast and ovarian cancer cells. Short-term preventive treatment with a peptidomimetic antagonist of αvβ3 (PSK1404) inhibited tumour cell invasion in vivo while it did not inhibited bone resorption in ovariectomized mice. However, tumour-induced bone resorption was inhibited via the decrease in the secretion of osteoclast-activating factors by tumour cells. Continuous treatment, with a dose that inhibits bone resorption in ovariectomized mice, led to an even more effective reduction in skeletal tumour burden probably due to a dual inhibitory effect in tumour cells and osteoclasts [184].

Treatment with the selective peptidomimetic inhibitor for αv integrins, cilengitide, leads to decreased lung metastasis in an osteosarcoma model. In osteosarcoma patient specimens, αvβ5 and αvβ3 integrins are expressed by tumour cells and stromal cells, respectively. Dual inhibition of these integrins did not however affect primary tumour growth. In contrast, it decreased lung metastasis, thus advocating for a role of cilengitide in the treatment metastatic osteosarcoma patients [186].

Inhibition of α5β1 integrin with a novel peptidomimetic antagonist also showed preclinical benefits in breast cancer cell invasion and angiogenesis, decreasing lung colonization and bone metastasis progression [187].

A peptidomimetic of the α2β1 integrin-binding domain is efficacious at inhibiting ovariectomy induced bone loss, and impairs breast primary tumour growth and bone metastasis in mice. Combination with a suboptimal dose of doxorubicin increased OS to levels similar to the optimal doxorubicin dose [188].

A Phase-II clinical trial of MEDI-522 (also known as etaracizumab), a human monoclonal antibody directed against the human αvβ3 integrin, in combination with docetaxel, prednisone, and ZOL in the treatment of patients with metastatic castration-resistant prostate cancer has been completed (NCT00072930) although no results are publicly available. Phase-I/II Clinical studies in colorectal cancer and melanoma have also been completed. The outcome of phase-II clinical trials showed increased median survival of metastatic melanoma patients with minimal side-effects. An in vitro study demonstrated that anti- αvβ3 integrin antibody treatment reduced bone resorption without affecting osteoclastogenesis, simply by the inhibition of osteoclast attachment to bone surfaces [189].

Cilengitide in asymptomatic bone metastatic castration-resistant prostate cancer patients was well tolerated in a phase-II clinical trial, and the higher dose had a modest clinical effect in stabilizing disease, with no apparent effects on bone markers [190].

Overall, no clinical trials point to an efficacy of an integrin-targeting strategy being efficient in the management of cancer-induced bone disease, at least not in monotherapy. However, large phase-III clinical trials are lacking. Additionally, integrin targeting could be of use in theranostics. Integrin peptidomimetic inhibitors conjugated with radioligands have been used to target tumour and tumour endothelial cells aiding imaging and targeted radiotherapy in breast [191] and prostate cancer [192].

Table 5 Currently ongoing clinical trials of bone-targeted agents for cancer-induced bone disease

Bone-targeted therapy	Patient population	Clinical trial acronym/ number	Phase	Clinical trials.gov link
Denosumab	High-risk early breast cancer	D-CARE NCT01077154	3	https://clinicaltrials.gov/ct2/show/NCT01077154?term=NCT01077154&rank=1
Radium-223	Bone metastatic breast cancer with endocrine therapy	NCT02258464	2	https://clinicaltrials.gov/ct2/show/NCT02258464?term=NCT02258464&rank=1
Radium-223	Bone metastatic breast cancer treated with exemestane	NCT02258451	2	https://clinicaltrials.gov/ct2/show/NCT02258451?term=NCT02258451&rank=1
Radium-223	Osteosarcoma	NCT01833520	1–2	https://clinicaltrials.gov/ct2/show/NCT01833520?term=radium+223&cond=osteosarcoma&rank=1
Radium-223	Thyroid cancer refractory bone metastases	RAD-THYR NCT02390934	2	https://clinicaltrials.gov/ct2/show/NCT02390934?term=NCT02390934&rank=1
Bortezomib	Relapsed multiple myeloma (comparison carfilzomib and dexamethasone versus bortezomib)	NCT01568866	3	https://clinicaltrials.gov/ct2/show/NCT01568866?term=Bortezomib&recrs=d&cond=Multiple+Myeloma+in+Relapse&phase=2&rank=1
Bortezomib	Relapsed multiple myeloma (addition of daratumumab to bortezomib and dexamethasone)	NCT02136134	3	https://clinicaltrials.gov/ct2/show/NCT02136134?term=Bortezomib&recrs=d&cond=Multiple+Myeloma+in+Relapse&phase=2&rank=2
Bortezomib	Relapsed multiple myeloma (pomalidomide, bortezomib and low-dose dexamethasone)	OPTIMISMM NCT01734928	3	https://clinicaltrials.gov/ct2/show/NCT01734928?term=Bortezomib&recrs=d&cond=Multiple+Myeloma+in+Relapse&phase=2&rank=3
Bortezomib	Relapsed multiple myeloma (comparison carfilzomib, dexamethasose and once weekly bortezomib versus twice weekly bortezomib)	ARROW NCT02412878	3	https://clinicaltrials.gov/ct2/show/NCT02412878?term=Bortezomib&recrs=d&cond=Multiple+Myeloma+in+Relapse&phase=2&rank=4
Bortezomib	Relapsed multiple myeloma patients(pomalidomide, bortezomib and low-dose dexamethasone versus high-dose dexamethasone)	NIMBUS NCT01311687	3	https://clinicaltrials.gov/ct2/show/NCT01311687?term=Bortezomib&recrs=d&cond=Multiple+Myeloma+in+Relapse&phase=2&rank=5
Saracatinib	Cancer-induced bone pain	SarCaBon NCT02085603	2	https://clinicaltrials.gov/ct2/show/NCT02085603?term=NCT02085603&rank=1
Cabozantinib	Bone metastatic castration-resistant prostate cancer	NCT01599793	2	https://clinicaltrials.gov/ct2/show/NCT01599793?term=cabozantinib&cond=Bone+Metastases%2C+cancer&rank=1
Cabozantinib	Advanced solid (non-breast, non-prostate) malignancies and bony metastases	NCT01588821	2	https://clinicaltrials.gov/ct2/show/NCT01588821?term=cabozantinib&cond=Bone+Metastases%2C+cancer&rank=4
Cabozantinib	Non-metastatic and metastatic castration-resistant prostate cancer	NCT01703065	Pilot	https://clinicaltrials.gov/ct2/show/NCT01703065?term=cabozantinib&cond=Bone+Metastases%2C+cancer&rank=5
Cabozantinib	Metastatic colorectal cancer	CaboMAb NCT02008383	1	https://clinicaltrials.gov/ct2/show/NCT02008383?term=cabozantinib&recrs=abd&draw=1&rank=2
Cabozantinib	Multiple myeloma	NCT03201250	1-2	https://clinicaltrials.gov/ct2/show/NCT03201250?term=cabozantinib&recrs=abd&draw=3&rank=11
Cabozantinib	Androgen-dependent metastatic prostate cancer	NCT01630590	2	https://clinicaltrials.gov/ct2/show/NCT01630590?term=cabozantinib&recrs=abd&draw=3&rank=12
Cabozantinib	Metastatic hormone receptor-positive breast cancer	NCT01441947	2	https://clinicaltrials.gov/ct2/show/NCT01441947?term=cabozantinib&recrs=abd&draw=3&rank=15
Cabozantinib	Relapsed osteosarcoma or Ewing sarcoma	NCT02243605	2	https://clinicaltrials.gov/ct2/show/NCT02243605?term=cabozantinib&recrs=abd&draw=4&rank=22
Sotatercept	Refractory multiple myeloma treated with lenalidomide or pomalidomide and dexamethasone	NCT02406521	1	https://clinicaltrials.gov/ct2/show/NCT01562405?term=NCT01562405&recrs=abd&rank=1

Hormone-Related Therapies

Activin A Inhibitors

Activin A is a member of the TGFβ superfamily. In vitro, activin A promotes bone remodelling, increasing both osteoclastogenesis and osteoblastogenesis [193]. In vivo, blocking activin A increases bone formation [194]. Activin A binds to activin receptor type IIA, recruits activin-like kinase 4 which phosphorylates Smad2/3 leading to the nuclear translocation of Smad 4. In cancer, activin A has paradoxical effects. Despite decreasing cell proliferation, activin A promotes migration and invasion of cancer cells in bone matrices. Increased levels of circulating and intratumour activin A are associated with prostate [195] and breast cancer bone metastasis, as well with multiple myeloma-induced bone disease [196, 197]. Moreover, the decreased ratio of inhibins/activins is linked with the increased bone resorption, observed around perimenopause. Activin A is generally secreted by bone marrow stromal cells, inhibiting osteoblastogenesis and increasing osteoclastogenesis. Multiple myeloma cells stimulate this production [194, 198]. Inhibition of activin A signalling rescues multiple myeloma- and breast cancer-induced bone lytic disease [194, 196].

Cumulative preclinical evidence steered clinical trials in multiple myeloma and metastatic breast cancer (terminated due to slow recruitment) of sotatercept, a recombinant activin receptor type IIA (ActRIIA) ligand trap comprising the extracellular domain of the high-affinity human ActRIIA and the human immunoglobulin G Fc domain which binds activin A/B and other TGFβ superfamily members with high affinity. In the phase-II clinical trial sotatercept showed anabolic improvements in bone mineral density, while barely affecting bone resorption. The most serious adverse events seen in sotatercept-treated patients (grade 4) were neutropenia, granulocytopenia, and atrial fibrillation. Excessive increases in haemoglobin levels led to dose interruption in certain patients and suggested the use of sotatercept as an erythropoietic agent [199].

Lenalidomide, an approved multiple myeloma therapy, was shown to increase activin A production by bone marrow cells and therefore decrease osteoblastogenesis. Preclinical evidence suggested a combination of lenalidomide with sotatercept to promote bone formation. This led to the ongoing clinical trial of the combination treatment of lenalidomide or pomalidomide with dexamethasone plus sotatercept in refractory multiple myeloma (NCT01562405) [200].

Conclusion

Thanks to the existing clinically approved bone-targeted therapies, bone metastatic patients currently have fewer skeletal-related events and prolonged disease-free survival than in the era where such agents were unavailable. Table 5 provides a selection of ongoing clinical trials of bone-targeted therapies in cancer-induced bone disease, whose results may contribute to the approval of new drugs or new indications for currently used agents. With the growing improvement of bone anabolic agents and constant discovery of novel cellular and molecular targets in the bone metastatic cascade, one may expect that in the forthcoming years we will be able to further improve patient overall survival, quality of life and ideally prevent cancer-induced bone disease.

Conflict of interest The authors declare that they have no conflict of interest.

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