Bone Metastasis: mechanisms, therapies and biomarkers.

Philippe Clézardin,[1,2] Rob Coleman,[2] Margherita Puppo,[2] Penelope Ottewell,[2] Edith Bonnelye,[1] Frédéric Paycha,[3] Cyrille B. Confavreux,[1,4] Ingunn Holen.[2]

[1] INSERM, Research Unit UMR_S1033, LyOS, Faculty of Medicine Lyon-Est, University of Lyon 1, Lyon, France.

[2] Department of Oncology and Metabolism, University of Sheffield, Sheffield, UK.

[3] Service de Médecine Nucléaire, Hôpital Lariboisière, Paris, France.

[4] Service de Rhumatologie Sud, CEMOS - Centre Expert des Métastases Osseuses, Centre Hospitalier Lyon Sud, Hospices Civils de Lyon, Lyon, France.

Abbreviated title: bone metastasis

Key words: metastatic niche, dormancy, invasion, aerobic glycolysis, immunosurveillance, osteomimicry, osteoclast, osteoblast, osteocyte, bisphosphonate, denosumab, non-coding RNA

Corresponding author:

Prof Philippe Clézardin

INSERM, UMR_S1033, UFR de Médecine Lyon-Est, 7 Rue Guillaume Paradin,

69372 Lyon cedex 08, France

(Tel.: +33 478785737; e-mail: philippe.clezardin@inserm.fr)

Department of Oncology and Metabolism, The Medical School, Beech Hill Road, Sheffield S10 2RX, UK

(Tel.: +44 1142229242; e-mail: p.clezardin@sheffield.ac.uk)

Abstract:

Skeletal metastases are frequent complications of many cancers, causing bone complications (fractures, bone pain, disability), which negatively affect the patient's quality of life. Here, we first discuss the burden of skeletal complications in cancer bone metastasis. We then describe the pathophysiology of bone metastasis. Bone metastasis is a multistage process; long before the development of clinically detectable metastases, circulating tumor cells settle and enter a dormant state in normal vascular and endosteal niches present in the bone marrow, which provide immediate attachment and shelter, and only become active years later as they proliferate and alter the functions of bone-resorbing (osteoclasts) and bone-forming (osteoblasts) cells, promoting skeletal destruction. The molecular mechanisms involved in mediating each of these steps are described and we also explain how tumor cells interact with a myriad of interconnected cell populations in the bone marrow, including a rich vascular network, immune cells, adipocytes and nerves. We discuss metabolic programs that tumor cells could engage with to specifically grow in bone. We also describe the progress and future directions of existing bone-targeted agents and report emerging therapies that have arisen from recent advances in our understanding of the pathophysiology of bone metastases. Finally, we discuss the value of bone turnover biomarkers in detection and monitoring of progression and therapeutic effects in patients with bone metastasis.

I. INTRODUCTION

During metastatic dissemination, cancer cells from the primary tumor must first undergo epithelial-to-mesenchymal transition (EMT) to invade the surrounding tissue and enter the microvasculature (intravasation) of the blood and/or lymphatic systems (49, 268). Once in the bloodstream, cancer cells may disseminate to distant organs, exit from blood vessels (extravasation) and settle in the foreign microenvironment where they enter a dormant state or proliferate to subsequently form macroscopic secondary tumors (metastases) (49). It has been estimated that only 0.02% of cancer cells entering the circulation produce clinically detectable metastases (217). Metastasis formation is therefore a highly inefficient process. However, when metastases do occur, they are responsible for 90% of cancer-associated mortality (49). There is therefore an urgent need to increase our understanding of the cellular and molecular mechanisms associated with metastasis formation, in order to develop therapies that will improve patient outcome.

Bone metastases occur in more than 1.5 million patients with cancer worldwide (361). They are frequent complications of many cancers but are especially common from tumors arising in the breast and prostate. Weakened bones due to skeletal metastases can lead to occurrence of skeletal-related events, such as fractures, spinal cord compression, bone pain and disability, contributing substantially to both morbidity and mortality in patients with advanced cancer (150, 361). In adults, the bone mass is maintained by continuously shaping and reshaping the overall bone structure through a process called bone remodeling, which is a balance between the resorption of mineralized bone by bone-resorbing cells (osteoclasts) and formation of new bone by bone-forming cells (osteoblasts) (76). Bone remodeling is tightly regulated by systemic and local factors in order to maintain this balance at its physiological steady state (76, 150). The late Greg Mundy pioneered the field of cancer and bone, demonstrating that skeletal-related complications associated with bone metastasis were a consequence of a distortion in bone remodeling caused by interactions between cancer cells and cells within the bone microenvironment (236).

In this review, we provide a broad overview of the current understanding of cancer-associated bone metastasis. We first review the incidence of bone metastasis in different cancer types and discuss the burden of skeletal complications in cancer bone metastasis. Current knowledge of the pathophysiology of bone metastasis is then described in detail. Bone metastasis is a stepwise sequence of events that include tumor cell colonization of the bone marrow, adaptation to the microenvironment, construction of a cancer niche, disruption of normal bone homeostasis through tumor cell interactions with bone cells (osteoclasts, osteoblasts and osteocytes, the latter being osteoblasts that have undergone a dramatic morphological transformation into stellate cells) and the release of signals from the resorbed bone matrix that promote skeletal tumor growth. We describe molecular mechanisms that are involved in mediating each of these steps and explain how bone marrow cells (*e.g*. immune cells, endothelial cells, adipocytes, and nerve cells) contribute to tumor development through multiple interactions. We also highlight metabolic adaptations of cancer cells that facilitate tumor progression in bone. Finally, we review current and future therapies for the treatment and prevention of bone metastasis and discuss the clinical utility of bone turnover biomarkers to predict the risk of disease relapse in patients with cancer. Given the vast collection of literature existing on the pathophysiology of bone metastasis we focus here on cellular and molecular mechanisms that are the most relevant to human cancer. However, it is important to note that we also cover emerging research areas where many mechanisms are derived from model systems, which still remain to be validated in human systems but could ultimately yield clues for better understanding and prevention of bone metastases.

II. BONE METASTASIS INCIDENCE AND CONSEQUENCES FOR BONE HEALTH

A. Common Ground – Bone Metastasis in Different Cancer Types

Bone is one of the most common sites for metastasis in cancer. Much of the work performed to describe the natural history of bone metastases is based on autopsy studies and large case series

from single institutions conducted several decades ago. Although bone is a frequent location for metastases from many malignancies, there are specific types of cancers that have a predilection for metastasis to the skeleton (150, 361). In particular, bone metastases are frequent complications of breast (especially estrogen receptor positive) and prostate cancer. In their retrospective study, Coleman and Rubens found in breast cancer a bone metastasis incidence of around 70% (68). These findings were consistent with the post-mortem examination from Galasko (115), who reported bone metastasis incidences of 73% and 68% of bone metastasis in breast and prostate cancer, respectively. Autopsies allowed the identification of a second group of osteophilic tumors with a postmortem prevalence of bone metastases of 60% in thyroid cancer, 30-40% in lung cancer, 40% in bladder cancer, 20-25% in renal cancer and 14-45% in melanoma (65). Apart from osteoblastic bone metastases in prostate cancer, bone metastases from other cancers are mainly osteolytic or a mix of lytic and blastic changes to the bone structure (Figure 1).

With the exception of a few relatively rare malignancies such as high-grade lymphoma or germ cell tumors affecting bone, metastatic bone disease is currently incurable. However, for many patients the median prognosis after development of bone metastasis is measurable in years, especially in those patients with metastatic breast or prostate cancers or multiple myeloma who, with modern treatment approaches, can often be expected to survive more than 5 years after bone involvement is diagnosed (65). Furthermore, new drugs, such as tyrosine kinase inhibitors and immune checkpoint inhibitors, have prolonged primary disease control in patients considerably, resulting in longer survival and consequently living long enough for bone metastasis to become clinically relevant (338). Thus, the epidemiology of bone metastases is evolving. In the coming years we may therefore expect an onset of bone metastases in patients who would have never developed clinically detectable bone metastases some years ago because they would have died from their cancer at a time when they only had (sub-clinical) bone marrow micrometastases. As a result, the prevalence of bone metastasis is increasing

and, in many cancers, the dominant site of disease requires specialist expertise and multidisciplinary management (72).

B. Cancer-Related Skeletal Complications

Bone metastases may be identified when asymptomatic through imaging tests such are computerized tomography (CT), ^{18}F-fluorodeoxyglucose (FDG)-positron emission tomography (PET) scanning, or radionuclide bone scanning. However, most patients with bone metastasis present with bone pain (150, 236). Usually the onset of pain is insidious, may be localized or multifocal, and is often confused with benign causes such as osteoarthritis. With time the pain typically worsens and becomes persistent, frequently reaching a severe level that may not be relieved by opioids (150, 236).

Bone metastasis is associated with impaired quality of life, reduced physical function and loss of autonomy (68, 150). Because of the proximity between bone and neurological structures (spinal cord and nerve roots), bone metastasis often causes neurological pain, such as paresthesia and tingling or burning sensations induced by epiduritis (68). Fractures are major complications of bone metastases and are commonly a result of osteolytic lesions in the vertebrae and weight-bearing bones, such as the proximal femur (68). The humerus is also at risk because of the forces applied through the arm use in daily life. Once pathological fractures have occurred, bone healing is compromised, and surgical intervention often required. Pathological fractures can be devastating complication for cancer patients, typically worsening their quality of life and increasing mortality (68, 150).

Hypercalcemia is an important metabolic complication of bone metastases (337). Symptoms include a wide spectrum of presentations from subtle changes in mood and gastrointestinal symptoms of nausea and constipation to a life-threatening state with vomiting and dehydration, acute renal insufficiency, disordered consciousness and ultimately coma (337). In bone metastases, hypercalcemia usually results from increased osteoclastic bone resorption but may be exacerbated by the

paraneoplastic secretion of parathyroid hormone-related peptide (PTHrP) or an abnormal activation of 25-OH vitamin D (345). The use of systemic anti-resorptive drugs has considerably reduced the number of patients with hypercalcemia (61, 345).

Most clinical studies use the composite endpoint Skeletal-Related Events (SREs) to establish the efficacy of systemic anti-resorptive drugs (61, 345). SREs are defined as pathologic fractures, spinal cord compression and the requirement for radiation therapy and/or surgery to bone; episodes of hypercalcemia may also be considered within the definition (220). Early placebo-controlled bisphosphonate clinical trials estimated that 50 to 56% of patients with bone metastases from solid tumors suffer from at least one SRE during follow-up on standard anti-cancer treatments without the addition of a bone targeted treatment (157, 279). SREs can occur quite early and indeed can be the presenting event in a patient with bone metastasis. In these trials, the median time to occurrence of the first SRE ranged from 5 to 7 months (157, 279). Moreover, patients with a first SRE are at increased risk for subsequent events, strengthening the importance of primary and secondary SRE prevention in cancer patients with bone metastases (68, 72). In addition to reducing a patient's quality of life and social and functional independence, the management of SREs consumes considerable health care resources (68, 72).

Besides analgesics and anticancer treatments, bone metastases benefit from systemic anti-resorptive treatments (bisphosphonate or denosumab) and local treatments such as radiotherapy, surgery or interventional radiology (cementoplasty, radiofrequency, ablation, cryotherapy). Optimal care should be discussed in a Bone Metastasis Multidisciplinary Board in order to reach a personalized strategy for every patient (72). Bone-targeted agents such as bisphosphonates and denosumab have been shown to be very effective in preventing and reducing SREs and are now the standard of care for the treatment of patients with malignant bone disease (see sections IX-A.1 and A.2 for further discussion).

III. TAKING OVER THE NEIGHBORHOOD - BONE COLONIZATION BY TUMOR CELLS

Bone colonization by tumor cells is a stepwise sequence of events that include *(i)* the formation of a pre-metastatic niche in the bone marrow to attract circulating tumor cells, *(ii)* the extravasation of these tumor cells from the circulation and homing to the pre-metastatic niche, and *(iii),* following tumor cell engraftment, the evolution of this pre-metastatic niche into a metastatic niche, the latter being conducive to the survival of these tumor cells. Each of these events is discussed below (Figure 2).

A. Preparing the Soil – the Concept of the Premetastatic Niche

The concept of premetastatic niche was first described by Dr Lyden and colleagues showing that vascular endothelial growth factor (VEGF)-A and placental growth factor (PlGF) secreted from primary tumors mobilize bone marrow-derived VEGF receptor 1 (VEGFR-1)-positive hematopoietic cells to the lungs before the arrival of tumor cells (169). Furthermore, an upregulation of fibronectin in resident fibroblasts at these premetastatic sites subsequently supports adhesion of VEGFR-1-positive cells (169). This localized accumulation of bone marrow-derived hematopoietic cells and stromal fibronectin creates docking sites for the future engraftment of tumor cells in lungs (169). Since then, many other tumor-derived factors, including cytokines, chemokines, extracellular matrix components, small noncoding RNAs and tumor-shed extracellular vesicles have been shown to act as systemic signals that trigger the formation of premetastastic niches in lung, liver or lymph nodes in different preclinical models (149, 261). Clinical evidence for the existence of premetastatic tissues comes from patients with meningioma (a benign brain tumor) who later progress with tumor-to-meningioma metastasis of breast, lung or renal cancer (88, 264, 270). It is suggested that the presence of pro-inflammatory macrophages and the high microvascular density in meningioma contribute to metastasis formation (88). Similarly, the existence of a premetastatic tissue in sentinel lymph nodes resected from patients with solid tumors has been reported (302). Thus, there is preclinical and clinical evidence that primary tumors may remotely induce the formation of a permissive environment within distant organs for future metastasis.

Multiple molecular mechanisms involved in the formation of a premetastatic niche in bone have been described (98, 137, 151, 216, 247, 248, 250, 251, 261, 271, 327, 336, 341, 371). Some of them already exist in the normal bone marrow (216, 248, 250, 251), whereas others are initiated by systemic signals coming from primary tumors (98, 137, 151, 327, 336, 341, 371). For example, interleukin (IL)-6 secreted from senescent osteoblasts promotes osteoclast-mediated bone resorption that, in turn, increases tumor cell seeding and subsequent breast cancer bone metastasis formation in animals (216). Similarly, in the absence of estrogen or androgen, osteoclast activity and bone resorption are increased, which leads to the release of bone-derived factors from resorbed bone that shape a favorable environment for tumor cells to survive and grow (248-250). Additionally, soluble factors secreted from primary tumors can target stromal and/or bone cells to support future metastatic colonization in the bone marrow. For example, in breast cancer models, tumor-derived IL-1β drives bone metastasis formation *in vivo* (151, 336). Blocking IL-1β activity with the anti-IL-1 receptor antagonist Anakinra or the IL-1β specific antibody Canakinumab inhibits tumor cell dissemination from the primary site into the circulation and blocks spontaneous formation of metastases to human bone implants in treated mice, compared to the placebo-treated group (336). Hypoxia-induced lysyl oxidase (LOX) can be secreted from primary tumors into the circulation from which LOX primes distant organs for metastatic colonization, including bone (73, 98, 263, 274). The primary function of LOX is to drive collagen crosslinking and extracellular matrix stiffness (7). In bone, tumor-derived LOX cooperates with receptor activator of nuclear factor kappa-B (RANK) ligand (RANKL) to accelerate osteoclastic bone resorption, and the formation of premetastatic osteolytic lesions in animal models of breast or colon cancer (73, 274, 335). A function-blocking antibody (AB0023) directed against LOXL2 (another member of the LOX family) inhibits breast cancer bone metastasis formation in animals (10), suggesting that LOXL2 could also contribute to the formation of a pre-metastatic niche in the bone marrow. Phase II clinical trials with simtuzumab, a humanized anti-LOXL2 monoclonal antibody, showed however that the addition of this antibody to current treatments of patients with metastatic pancreatic or colorectal cancer

does not improve clinical outcomes (ClinicalTrials.gov identifiers NCT01472198 and NCT01479465, respectively). Conversely, the anti-IL-1β antibody canakinumab has been shown to significantly reduce the incidence of lung cancer and lung cancer mortality in patients with atherosclerosis (ClinicalTrials.gov identifier NCT01327846). It remains to be established whether blocking IL-1β, LOX or LOXL2 impedes progression of bone metastasis in breast cancer patients.

It also appears that factors contained within cancer cell-derived exosomes can influence bone cell activity before tumor cells arrive at this site. Exosomes are small extracellular vesicles (30-120 nm) containing DNA, RNA [mRNA, miRNA and other noncoding RNAs], lipids and proteins that are released by all types of cells and taken up by recipient cells (368). For example, exosomal amphiregulin secreted by non-small cell lung carcinoma (NSCLC) cells or amphiregulin-containing exosomes released in plasma of NSCLC patients promote the differentiation of human peripheral blood monocytes into osteoclasts (327). In melanoma, the transfer of the MET oncoprotein from tumor-shed exosomes to bone marrow progenitor cells can reprogram these cells towards a prometastatic phenotype in lungs and bone *in vivo* (261). Similar findings were reported with tumor-derived exosomal miRNAs (miR-21, miR-141, miR-192, and miR-940) (22, 137, 341, 371, 376). In particular, exosomal miR-141 and miR-940 produced by prostate cancer cells promote osteoblast differentiation and proliferation, facilitating the formation of bone metastases with an osteoblastic phenotype in mouse models (137, 376). MiRNAs mainly act as negative regulators of gene expression (14). In this respect, tumor-derived exosomal miR-141 promotes osteoblast differentiation by inhibiting DLC1 mRNA expression that, in turn, leads to p38MAPKinase activation and increased osteoprotegerin (OPG) expression in osteoblasts (376). Tumor-derived exosomal miR-940 promotes osteogenic differentiation of mesenchymal stem cells by directly inhibiting ARHGAP1 (Rho GTPase Activating Protein 1) and FAM134A (Family with Sequence Similarity 134 Member A) mRNA expression (137).

Overall, these experimental findings strongly suggest that, in addition to molecular mechanisms already existing in the normal bone marrow, primary tumors can also remotely control the formation of a

premetastatic niche through the release of systemic factors that induce a distortion in bone remodeling. Research designed to determine the mechanisms by which primary tumors promote the formation of pre-metastatic niches in bone is still in its infancy and further investigations using *in vivo* model systems are required to gain a more comprehensive understanding of this process. As tumor cell dissemination into bone is believed to be an early process, likely to occur before the clinical detection of primary tumors, the detection of these molecules in the primary tumor and/or blood may provide useful biomarkers to predict future relapse in bone. Further clinical trials are needed to test this hypothesis.

The premetastatic niche: current understandings & open questions

- Preclinical and clinical studies support the existence of premetastatic tissues for future metastasis.
- The general applicability of these mechanisms associated with the formation of a premetastatic niche remains to be validated *in vivo* for other model systems and for other cancer types.
- Beside the observation that primary tumors can generate systemic changes that modify the bone microenvironment, there is also some preclinical evidence suggesting that bone may remotely control growth of primary tumors at distant sites (85, 97, 166, 257). These latter observations are intriguing and clearly deserve further study.

B. Mechanisms of Tumor Cell Extravasation and Homing to the Bone Marrow

In response to pro-migratory and pro-inflammatory molecules produced by the pre-metastatic niche, circulating tumor cells (CTCs) cross the endothelial cell barrier and basement membrane of blood vessels (a process called extravasation) in order to home in the newly invaded parenchyma where they interact with specific extracellular matrix components that facilitate their survival.

1. Tumor cell extravasation

In the bone marrow, the vascular endothelium that constitutes blood vessels (called sinusoids) is predominantly discontinuous and fenestrated, which facilitates the traffic of hematopoietic stem cells (HSCs) (241). Therefore, the sinusoids are likely to be more permissive to CTCs, suggesting there is a limited requirement of extravasation mechanisms for tumor cells to invade the bone marrow (241, 273). Indeed, tumor cells hijack molecular mechanisms that are used by HSCs. In particular, E-selectin (Endothelial selectin) and CXCL-12 are constitutively expressed on sinusoidal endothelial cells, aiding the homing of HSCs in the bone marrow (301, 316). Similarly, E-selectin- and CXCL-12-expressing bone marrow endothelial cells mediate attachment of breast and prostate cancer cells through interaction with E-selectin ligands and CXCR-4, respectively (267, 273). Using high-resolution real-time fluorescence microscopy to track breast cancer cell migration in the calvarial bone marrow *in vivo*, Price *et al*. (267) showed that 2 hours after intracardiac injection, tumor cells interacted with endothelial cells in sinusoidal vascular and perisinusoidal vascular regions where expression of E-selectin and CXCL-12 is high. Of special interest, the preventive treatment of mice with a selective inhibitor of E-selectin, before tumor cell injection, substantially blocked tumor cell interaction with E-selectin-expressing endothelial cells, whereas pretreatment of animals with a small molecule inhibitor of CXCR-4 (AMD3100) did not inhibit breast cancer cell homing to the bone marrow *in vivo* (267). By contrast, AMD3100 treatment of mice after tumor cell engraftment forced breast cancer cells residing in perivascular niches to migrate from the bone marrow into the peripheral circulation. Overall, these findings demonstrate that E-selectin is critical for allowing breast cancer cells to extravasate in the bone marrow, whereas CXCR-4/CXCL-12 maintains tumor cells in the perivascular environment and controls their exit from the bone marrow (267). The CXCR-4/CXCL-12 axis is the most well-described and prominent mechanism involved in regulating tumor cell entry in the bone marrow environment (235). However, it should be noted that, not all breast cancers that metastasize to bone express CXCR4 (243), and other chemokines produced by the bone microenvironment (CXCL-5, CXCL-10, CXCL-13, CX3CL-1, CCL-2) have also been implicated in mediating tumor cell colonization in the bone marrow (158, 159,

193, 213, 232, 275, 300) (Table 1). However, correlations between expression of these chemokines and relapse in bone, in clinical samples, remains to be established.

Another factor that has been implicated in tumor cell extravasation is the cytokine IL-1β whose expression in breast cancer cell lines and primary breast carcinomas is strongly associated with bone metastasis (151, 336). IL-1β drives metastasis by inducing epithelial-to-mesenchymal transition and increasing dissemination of breast cancer cells into the circulation (151, 336). Once in bone, IL-1β facilitates adhesion of CTCs to sinusoidal endothelial cells by inducing the expression of vascular cell adhesion molecules [intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin] (273). Then, IL-1β stimulates expansion of the metastatic niche, increasing proliferation of blood vessels and osteoblasts, thereby promoting tumor cell extravasation and metastatic outgrowth of tumor cells that disseminated in this site (273, 336). Interestingly, IL-1β-expressing E0771 primary breast tumors spontaneously metastasize to bones in IL-1β-knockout animals and to an extent similar to that observed in normal mice, indicating that tumor-derived IL-1β rather than IL-1β from the bone marrow microenvironment promotes bone metastasis formation (336). Although these mechanisms have all been identified in mouse models it is likely that these, at least in part, explain why increased IL-1β in primary breast tumors associates with recurrence in bone in cancer patients (151, 336).

2. Tumor cell homing

Bone extracellular matrix proteins (*e.g.*, type I collagen, tenascin C, periostin, fibronectin, SIBLINGs) by binding to tumor cell surface integrins play an important role in mediating tumor cell attachment to the bone matrix (17, 57, 221, 255, 260, 285, 311, 317, 333). In particular, type I collagen, which is the most abundant protein among bone extracellular matrix components, mediates attachment of human prostate cancer cells through binding tumor cell α2β1 integrin (311). Fibronectin in the bone marrow mediates survival of human triple negative breast cancer cells by binding to tumor cell surface

α5β1 integrin (255). Tenascin C is a hexameric protein that fosters the early colonization of prostate cancer cells in the bone marrow through binding tumor cell surface α9β1 integrin (285). Like tenascin C, periostin is produced by stromal cells and mediates tumor cell adhesion by binding tumor αvβ3 integrin (221). As such, integrins have been therefore considered as attractive drug targets. For example, αvβ3 integrin recognizes an Arg-Gly-Asp (RGD) peptide motif expressed by extracellular matrix proteins and the treatment of animals with RGD-based peptide antagonists (PSK104, S247, GLPG0187) of αvβ3 integrin suppresses breast and prostate cancer bone metastasis formation, supporting the crucial role played by this integrin in bone metastasis formation (136, 303, 344, 388). However, despite these encouraging experimental studies, ensuing clinical trials that used integrin antagonists have been mainly unsuccessful (132). There may be a need to develop alternative strategies that target specific integrin signaling pathways promoting tumor cell survival and drug resistance (132). Integrins are also emerging as valuable cancer imaging probes probes to identify bone metastases in clinical studies. For example, integrin αvβ3 is highly expressed in osteotropic tumor cells and osteoclasts and, using PET/CT imaging, this property has been used to show that an RGD peptide-containing αvβ3 integrin tracer (^{99m}Tc-3P-RGD$_2$) is superior to ^{99m}Tc-bisphosphonates to detect osteolytic bone metastases in patients with advanced lung cancer (297). Similarly, PET/CT imaging with a tracer targeting gastrin-releasing peptide receptor and integrin αvβ3 (^{68}Ga-BBN-RGD) shows a significant uptake in bone metastases from patients with advanced breast cancer (384).

RANK and RANKL have an established role in regulating bone remodeling (183). RANKL secreted by osteoblasts and osteocytes binds to its receptor RANK on osteoclast precursors, leading to the formation of mature osteoclasts and osteoclast-mediated bone resorption (183). Interestingly, tumor cells can also express RANK in breast, prostate, and lung carcinomas and RANKL triggers *in vitro* migration of RANK-expressing breast and prostate cancer and melanoma cells (21, 164, 325, 346). In the case of breast cancer, a high RANK expression in hormone receptor-negative primary tumors is associated with poor relapse-free survival and high risk of bone metastasis in patients (272, 286, 347). It

has been therefore suggested that RANK-expressing cancer cells may be specifically attracted to bone where high local concentrations of RANKL exist (346). This contention was supported by the observation that inhibition of RANK/RANKL signaling by soluble decoy receptor OPG, which binds to RANKL, reduces skeletal tumor burden and bone metastasis in a melanoma model that does not activate osteoclasts, whereas metastasis in other organs (ovaries, brain, adrenal glands) remain unchanged (164). However, administration of OPG to a mouse model of breast cancer did not reduce the number of tumor cells that disseminated in bone (249). In clinical studies, the RANKL inhibitor denosumab, when given in patients with early-stage breast cancer or non-metastatic castration-resistant prostate cancer (CRPC), had no effect on disease recurrence in either pre- or postmenopausal women with breast cancer (63) and only modestly increased bone metastasis-free survival in CRPC patients (306), thereby suggesting that RANK/RANKL does not play a major role in tumor cell colonization in bone.

Tumor cell extravasation and homing: current understandings & open questions

- Recent studies on the bone microvasculature in mouse models have shown that there are particular vessel subtypes within the same vascular bed, termed H ($CD31^{hi}Endomucin^{hi}$) and L ($CD31^{lo}Endomucin^{lo}$) vessels, which have different characteristics (182). It will be of particular interest to determine whether disseminated tumor cells preferentially associate with a particular vessel subtype.
- Results obtained with PET/CT imaging using integrin-binding tracers to detect skeletal lesions and evaluate treatment response in patients with advanced cancer and bone metastases are very encouraging and deserve further investigations.

C. Hiding in Plain Sight –Tumor Cell Dormancy and Dormant Cell Reactivation Mechanisms in Bone Marrow Niches

Entering a foreign environment, such as the bone marrow, poses tumor cells with numerous challenges regarding survival and proliferation. It is hypothesized that tumor cells in the bone marrow compete with HSCs for occupancy in the vascular and endosteal niches. Once in the niche, tumor cells can enter a dormant state as a mechanism to help them survive until environmental conditions are sufficiently permissive for proliferation and tumor outgrowth (298, 309). This hypothesis of tumor dormancy is supported by an extensive body of clinical research. First, long before the development of clinically detectable metastases, tumor cells disseminate to the bone marrow, only becoming active following years or decades after primary tumor diagnosis (247). Second, disseminated tumor cells (DTCs) are present in the bone marrow of patients with various types of cancer and are predictive of future relapse, yet some of these cancer types will never develop clinically detectable bone metastases (205). Lastly, for those cancer types that have a high propensity to develop overt bone metastases, such as breast and prostate cancers, the rate of detection of DTCs in the bone marrow is higher than the proportion of patients who subsequently develop skeletal lesions, suggesting the bone marrow microenvironment influences DTC fate (32, 228). Thus, these clinical observations provide strong evidence of tumor dormancy in the bone marrow. However, DTCs isolated from the bone marrow of non-metastatic patients with cancer (breast, prostate, melanoma) fail to generate tumor xenografts in immunodeficient mice (364). Consequently, only tumor cell lines derived from overt metastases from patients with advanced cancer (breast, prostate) have been used in animal models to study tumor dormancy in bone (31, 46, 117, 119, 163, 177, 267, 320, 381, 382). The use of these metastatic cell lines, therefore, poses a major limitation as metastatic cancer cells and DTCs have different genotypic and phenotypic traits (309). Determining the different mechanisms controlling the ability of tumor cells to seed in the bone marrow and those responsible for metastatic outgrowth may require the development of more clinically relevant models. Yet, tumor dormancy is an emerging research area and we believe that unravelling molecular mechanisms associated with tumor dormancy using these existing preclinical models may still help us better understand the earliest stages that precede the clinical development of

446 bone metastases. Below we describe our current understanding of the mechanisms involved in
447 mediating tumor cell interactions with cells from bone marrow niches (Figure 2) and then detail
448 molecular signaling mechanisms proposed to keep these tumor cells in a dormant state (Figure 3).
449 Finally, we describe how osteoclast-mediated bone resorption creates an environment that promotes
450 dormant cell reactivation.

451 ## *1. Tumor cells interactions with cells from bone marrow niches*

452 The vascular niche surrounds E-selectin-expressing endothelial cells that form bone marrow
453 sinusoids, and is made up of perivascular cells expressing high levels of CXCL-12 [called CXCL-12-
454 abundant reticular (CAR) cells], leptin receptor (Lepr)-expressing perivascular stromal cells, and
455 mesenchymal stem cells (MSCs) (301, 316). This vascular niche regulates HSC quiescence and the
456 supply of lineage-committed progenitors (301). Real-time *in vivo* microscopy of bone marrow sinusoids
457 in a breast cancer xenograft model has revealed opposing roles of E-selectin and CXCL-12 in tumor cell
458 trafficking (267). Whereas E-selectin interactions are critical for allowing breast cancer cell entry into the
459 bone marrow, CXCL-12/CXCR-4 interactions maintain breast cancer cells dormant in the vascular niche
460 (267). Additional mechanisms are involved in maintaining tumor cells dormant in the vascular niche. For
461 example, endothelium-derived extracellular matrix protein thrombospondin-1 (TSP-1) induces sustained
462 dormancy of breast cancer cells *in vivo* (119). Conversely, MSCs with both endothelial and pericytic cell
463 surface markers prevent the homing of breast and prostate cancer cells to the bone marrow (282). In
464 model systems, the tumor-suppressive nature of the vascular endothelium is lost when endothelial cells
465 start sprouting, which is characterized by reduced TSP-1 expression and enhanced expression of pro-
466 metastatic factors (periostin, tenascin, fibronectin) that promote tumor outgrowth (119). Interestingly,
467 immunohistochemical analysis of the bone marrow from breast cancer patients with micrometastatic
468 disease shows that dormant (Ki67-negative) breast cancer cells are preferentially localized in
469 perisinusoidal, CXCL12-rich vascular regions (267). By contrast, proliferative (Ki67-positive) breast
470 cancer cells in bone marrow biopsies from patients with macrometastatic disease are frequently

observed adjacent to the bone surface (267). This observation (267) is in agreement with the fact that calcium levels, which are high at the endosteal mineral surface, can promote breast cancer cell proliferation and bone metastasis formation in animals (354). Thus, it appears that the vascular niche provides a microenvironment supportive of dormancy at least in breast cancer.

As the name suggests, the endosteal niche is localized at the inner surface of the bone cavity in the endosteum, and is primarily made up of undifferentiated osteoblastic cells, such as spindle-shaped N-cadherin^{+}/CD45^{-} osteoblast (SNO) cells (138). Mature osteoblasts are short-lived cells and, as such, they are unlikely to be part of the endosteal niche (76, 256). Beside SNO cells, CAR cells are also present and are proposed to maintain the quiescent HSC pool through CXCL-12/CXCR-4 interactions (316). The disruption of this connection using CXCR4 antagonists, in mouse models, results in increased mobilization of HSCs from the bone marrow into the circulation (316). Osteoclasts are dispensable for HSC maintenance in the endosteal niche and may function as negative regulators in the hematopoietic system (231). With regard to the homing of tumor cells to the endosteal niche, ER-negative (but not ER-positive) breast cancer cells compete with HSC to interact with SNO cells through a specific Jagged-Notch2 interaction that mediates tumor cell dormancy both *in vitro* and *in vivo* (46). It must be pointed out however that these particular *in vivo* experiments were conducted using intratibial tumor cell inoculation, thereby bypassing the blood circulation, which impedes breast cancer cells from homing to the vascular niche. Other studies, using more clinically relevant mouse models in which tumor cells were disseminated into the bone via intra-arterial injection, reported that SNO cells support survival of ER-positive breast cancer cells through specific N-cadherin/E-cadherin interactions and connexin-43 (Cx43) gap junctions that trigger pro-survival mTOR signaling and calcium signaling pathways in tumor cells, respectively, hence promoting micrometastatic progression (354, 355). In addition, independently of the hormone receptor status or breast cancer subtype, CXCL-12 triggers activation of a Src-dependent AKT signaling pathway by binding to CXCR-4, enhancing the survival of breast cancer cells in the bone marrow (387). Of note, Werner-Klein *et al*. (364) performed single-cell

RNA-sequencing analysis of DTCs isolated from the bone marrow of non-metastatic breast cancer patients (n=30 DTCs; 21 patients) and found that mRNA expression of the IL-6 signal transducing unit gp130 (*IL6ST*) is strongly enriched in these cells, whereas the mRNA of the IL-6 binding receptor alpha chain CD126 (*IL6RA*) is absent. In the absence of CD126, the IL-6 signaling pathway can be activated in trans through the binding of IL-6 to the soluble form of CD126 (sIL6RA) prior to binding to gp130. Both IL-6 and sIL6RA are abundant in the bone marrow, and IL-6 trans-signaling through the PI3K/AKT pathway can be activated in tumor cells (364). However, the endosteal niche renders DTCs unresponsive to IL-6 trans-signaling (364). Interestingly, genetic analysis of DTCs revealed that only 4.4% (3/68) of nonmetastatic breast cancer patients harbored mutations in the gene for PI3K (*PIK3CA*), whereas 34.3% (23/67) of metastatic breast cancer patients displayed *PIK3CA* mutations, indicating that DTCs may undergo further selection to become more independent from their microenvironment during cancer progression. Overall, these results strongly indicate that the endosteal niche provides breast cancer cells with an environment supporting their survival, outgrowth and/or enabling tumor cells to acquire genetic alterations (e.g., *PIK3CA* mutation) that render them more autonomous (354, 355, 364, 387).

In prostate cancer, CXCR-4/CXCL-12 and Annexin 2 (ANXA2)/CXCL12 interactions also play a crucial role in the recruitment of tumor cells in the endosteal niche (167, 298, 318, 357). The targeting of CXCR4 in model systems results in increased numbers of prostate cancer cells in the circulation, supporting the notion that these tumor cells inhabit this endosteal niche (298, 357). The current hypothesis is that prostate cancer cells compete with HSCs for space in the endosteal niche (298). However, as opposed to breast cancer, it seems that prostate cancer cells homing in the endosteal niche may benefit from this supportive environment for maintenance of dormancy but not tumor outgrowth (42, 298, 320, 357). Notably, growth arrest-specific 6 (GAS6) is an osteoblast-derived ligand of the MER, TYRO3 and AXL tyrosine kinase receptors that has been shown to induce tumor dormancy in prostate cancer (320). When prostate cancer cells bind to osteoblastic cells in the endosteal niche,

they increase their expression level of AXL and consequently GAS6 inhibits tumor cell proliferation by binding to AXL (320). Similarly, high MER expression levels in prostate cancer cells are associated with tumor dormancy in the bone marrow (42). By contrast, when TYRO3 expression levels exceed AXL levels, prostate cancer cells exhibit rapid growth (320). Thus, a balance between expression levels of TYRO3 and AXL/MER may regulate prostate cancer cell dormancy in the endosteal niche. A similar role for AXL in promoting dormancy in models of multiple myeloma has been reported (173). Overall, the relative contribution of these niches/molecules to tumour cell dormancy in these various bone metastatic cancers has yet to be validated in clinical samples.

The bone microenvironment is also an immune privileged site, offering protection of HSCs from environmental insults and the resulting immune response. High resolution *in vivo* imaging shows co-localization of HSC and regulatory T cells (Treg) on endosteal surfaces in the trabecular bone marrow areas in mice (111). Treg cells are known to be potent immune suppressors. In addition to vascular and endosteal niches, it has been therefore proposed that Treg cells helped create an immune niche supporting stem cell function whilst providing sanctuary from immune attack (111). The bone marrow also contains very high numbers of myeloid-derived suppressor cells (MDSCs) (254, 370). MDSCs suppress anti-cancer immune activity by inhibiting NK and CD8+ T cells (254, 370). Thus, this type of protected environment would clearly also benefit resident tumor cells, preventing their elimination and promoting their survival in bone. In addition, bone marrow mesenchymal stem cells also promote tumor cell dormancy (247). *See section VII* for further discussion on the contribution of immune cells to tumor development.

2. Tumor cell dormancy

Tumor cell dormancy is defined as the arrest in the cell cycle (also known as mitotic or cellular dormancy). A second mode of dormancy refers to tumor mass dormancy of micrometastases where

545 there is a balance between cell proliferation and cell death, the latter is widely believed to be due to
546 immune surveillance and/or lack of blood supply (309). The signaling pathways through which tumor
547 mass dormancy is controlled are largely unknown, mostly because of the lack of appropriate animal
548 models that reproduce tumor dormancy in bone. Thus, although these two modes of dormancy coexist
549 in the bone marrow, we have concentrated here on molecular mechanisms that regulate tumor cell
550 dormancy in laboratory models.

551 In breast cancer, tumor cell dormancy appears to be determined by a balance between the
552 activities of activated protein kinases ERK1/2 and p38, where a switch towards ERK1/2 phosphorylation
553 favors proliferation whereas activation of p38 leads to quiescence (309). Mitogen- and stress-activated
554 kinase 1 (MSK1) is a downstream effector of the p38 and ERK1/2 signaling pathways (117). Using
555 experimental models of ER-positive human breast cancer (T47D, ZR-75) in which tumor cells form
556 latent micrometastatic bone lesions *in vivo*, Gawrzak and colleagues (117) showed that p38 depletion in
557 ER-positive human breast cancer cells decreases MSK1 expression. In turn, MSK1 depletion increases
558 the capacity of poorly metastatic ER-positive breast cancer cells to form overt metastasis in animals
559 (117). Thus, MSK1 is a dormancy enforcer and a negative regulator of metastasis initiation.

560 Another signal that regulates breast cancer dormancy in the bone marrow is leukemia inhibitory
561 factor (LIF) (163). By binding to LIF receptor (LIFR), LIF negatively regulates *STAT3* (signal transducer
562 and activator 3) in breast cancer cells. The loss of LIFR or STAT3 enables otherwise quiescent human
563 MCF-7 breast cancer cells to proliferate and specifically metastasise to bone (163). Indeed, LIFR
564 expression levels in primary tumor of breast cancer patients who are predicted to relapse in bone are
565 significantly lower compared with those with a good prognosis (163), further supporting the observation
566 that LIFR signaling mediates tumor cell dormancy in animal models of bone metastasis.

567 In prostate cancer, bone morphogenetic protein (BMP)-7 secreted from bone marrow stromal cells
568 promotes dormancy of prostate cancer stem-like cells, and an inverse correlation between expression of
569 the BMP7 receptor BMPR2 and occurrence of bone metastasis is found in patients with prostate cancer

(177). By binding to BMPR2, BMP7 induces the quiescence of prostate cancer stem-like cells through p38 activation and increased expression of the cell cycle inhibitor p21 (177). BMP7 also inhibits breast cancer stem cell population and reduces bone metastasis formation in animals (41).

Bone-derived growth factors TGFβ1 and TGFβ2 exhibit competing functions on the behavior of tumor cells in the bone marrow (309). TGFβ2 promotes tumor cell dormancy, whereas TGFβ1 switches off dormancy, leading to rapid tumor growth *in vivo* (309). In a head and neck squamous cell carcinoma model of bone metastasis, TGFβ2 (but not TGFβ1) activates p38, which up-regulates the metastasis suppressor gene *DEC2* (31). In turn, DEC2 induces p27 and down-regulates cyclin-dependent kinase 4 (CDK4), leading to tumor cell quiescence (31). In model systems of prostate cancer bone metastasis, TGFβ2 induces dormancy through p38 activation and AXL/GAS-6 expression (381, 382).

Due to the diversity of the molecular mechanisms that regulate tumor cell dormancy in laboratory models, these processes are difficult to validate in clinical samples. However, future reserch will establish if targeting key drivers of dormancy can be used as a method of retaining tumor cells in this state indefinitely, thereby preventing metastatic outgrowth and symptomatic disease.

3. Dormant cell reactivation

Bone resorption likely creates an environment that promotes tumor cell reactivation. Intravital imaging of the bone microenvironment in murine models of multiple myeloma has shown that tumor cells colonizing endosteal niches are in a dormant state (189). However, these tumor cells are reactived and released from the endosteal niche upon treatment of tumor-bearing animals with a soluble form of RANKL that stimulates osteoclast-mediated resorption (189). By contrast, sRANKL treatment has no effect on tumor cells colonizing soft tissue sites (189). Androgen deprivation by orchidectomy stimulates bone turnover of castrated animals bearing disseminated hormone-insensitive prostate cancer cells in the bones, thereby also increasing the incidence of overt bone metastasis in these animals (251). A similar effect was reported in an animal model of breast cancer, where ovariectomy-induced bone loss

triggered growth of disseminated hormone-insensitive breast cancer cells in bone (249,250). Thus, osteoclast-mediated bone resorption plays an important role at an early stage in the establishment of bone metastasis. This contention is also supported by experiments conducted in a mouse model of indolent breast cancer bone metastasis, showing that VCAM-1 overexpression in tumor cells promotes the recruitment of osteoclast precursors by binding to osteoclast integrin α4β1, leading to osteoclast formation and osteoclast-mediated bone resorption (214). In turn, bone-derived growth factorsTGFβ1 released from resorbed bone switches off dormancy, leading to rapid tumor growth *in vivo* (309). Furthermore, PTHrP expressed by tumor cells can act in autocrine fashion by reducing pro-dormancy LIFR gene expression (163), suggesting that PTHrP also plays a role in promoting tumor cell exit from dormancy. Thus, changes to the bone environment in favor of bone resorption are sufficient to trigger dormant cell reactivation. This idea is supported by the fact that bisphosphonates, by decreasing bone resorption, improve elimination of DTCs in the bone marrow of breast cancer patients with a minimal residual disease (307), and reduce development of bone metastases when given as a neoadjuvant treatment (66, 123).

Niches, tumor cell dormancy and reactivation: current understandings & open questions

- Experimental and clinical studies support the notion that vascular and endosteal niches can be hijacked by arriving tumor cells to provide immediate shelter, thereby preventing their elimination and promoting their survival in the bone marrow. Other existing niches, such as the immune niche, may also support tumor cell survival in the bone marrow. However, many aspects of the interplay between these niches and tumor cells remain elusive. The use of clinically applicable imaging technologies such as PET and SPECT with niche-specific tracers and single cell-omics techniques will certainly help to understand the dynamics of tumor-niche interactions in the future.

- The diversity of the molecular mechanisms associated with tumor dormancy in the bone marrow niches represent both a challenge and an opportunity for therapeutic targeting. How to avoid unwanted effects on normal homeostasis while disrupting interactions that maintain tumor cells in these bone marrow niches remains an open question.

IV. FITTING IN - ADAPTATION OF TUMOR CELLS TO THE BONE MARROW MICROENVIRONMENT

During the time tumor cells are resident in the bone marrow, exiting and re-entering a dormant state, they rewire their biology to meet the demands of the tissue colonized, thus modifying their primary properties in order to adopt a genetic phenotype similar to bone cells that, in turn, facilitates their survival in the bone microenvironment (16, 291). This process is called osteomimicry (178).

Immunohistochemical analysis of human clinical samples in breast and prostate carcinomas clearly shows that cancer cells metastatic to the bone highly express bone proteins *in situ* (16, 55, 108, 196, 353). In particular, paired immunochemistry on human primary breast tumor samples and matched liver, lung or bone metastases showed that only bone metastatic tumor cells express bone proteins such as cathepsin K (CTSK), osteonectin, cadherin-11 (CDH-11), and Cx-43, which are normally expressed by osteoblasts or osteoclasts (16, 108, 196).

The functions of these osteomimicry genes have been studied in animal models of bone metastasis. CDH-11 mediates interactions of breast and prostate cancer cells with osteoblasts *in vitro*, and its silencing in tumor cells greatly reduces bone metastasis formation *in vivo* (55, 154, 323). Breast cancer cells can get calcium from the osteogenic niche through Cx43 gap junctions that facilitate calcium influx from osteogenic cells to breast cancer cells and, in turn, calcium promotes tumor cell proliferation (354). Similarly, Cx43 overexpression in human LNCaP prostate cancer cells enhanced their capability to induce bone destruction *in vivo* following intratibial tumor cell injection, and moderately augmented tumor cell proliferation *in vitro*, when tumor cells were cocultured with osteoblasts (184).

643 Another example of osteomimicry is the expression of transcription factor RUNX2 (a master regulator of
644 osteoblast differentiation) in osteotropic tumor cells. The disruption of *RUNX2* expression in breast
645 cancer cells abolishes their ability to form osteolytic lesions *in vivo* (266). *RUNX2* in osteotropic breast
646 cancer cells promotes expression of metastasis-related factors [MMP-9, MMP-13, VEGF, osteopontin,
647 bone sialoprotein (BSP), ITGA5] and bone-resorbing factors (PTH-rP, IL-8), thereby explaining why
648 *RUNX2* inhibition in tumor cells decreases skeletal tumor burden and osteolysis (200, 266). Forkhead
649 box F2 (FOXF2) is another example of master transcription factor that mediates epithelial-to-
650 osteomimicry transition, increasing the tendency for breast cancer cells to metastasise to bone. In
651 particular, the orthotopic implantation of murine 4T1 breast cancer cells overexpressing FOXF2 or the
652 intracardiac inoculation of FOXF2-overexpressing human MDA-MB-231 breast cancer cells enhances
653 the formation of osteolytic bone metastases in animals (358). Mechanistically, FOXF2 directly
654 upregulates *CTSK* that, in turn, increases breast cancer cell invasion (358). Interestingly, high
655 expression levels of transcription factors FOXF2 and RUNX2 in primary mammary carcinomas correlate
656 with bone-specific metastasis in patients with breast cancer (200, 358).

657 It is highly likely that miRNA dysregulation in tumor cells contributes to osteomimicry (36). For
658 instance, the downregulation of miR-30, miR-135, and miR-203 enhances abnormal expression of
659 osteoblast-specific genes (*CDH-11*, *RUNX2*, *SOST, ITGA5, BSP, OPN*), which endows tumor cells with
660 full competence for survival in the bone marrow (75, 320). Other genes associated with osteomimicry
661 (*DKK-1*), osteoclastogenesis (*IL-8*, *IL-11*) and tumour cell invasiveness (*CTGF*, *ITGA5*, *ITGB3*) are
662 direct targets for repression by miR-30 family members, these miR-30s being downregulated in
663 osteotropic breast cancer cells (75). Conversely, miR-218 is highly expressed in human MDA-MB-231
664 breast cancer cells and acts as a promoter of bone metastasis formation through stimulation of the
665 expression of metastasis-related genes (*CXCR-4*, *BSP* and *OP*) that are associated with osteomimicry
666 and production of the bone-resorbing factor PTH-rP (321).

667

668 Osteomimicry: current understandings & open questions

669 • *In situ* expression of bone proteins in tumor cells from human bone metastasis specimens
670 unequivocally establishes osteomimicry as a process occurring during the development of bone
671 metastases in patients with advanced breast or prostate cancer.
672 • Experimentally, RUNX2, FOXF2 and some miRNAs (miR-30, miR-135, miR-203, and miR-218)
673 function as master regulators of osteomimicry.
674 • The importance of osteotropic factors as potential biomarkers for the prediction of bone
675 metastasis risk and/or response to bone-targeted agents remains to be investigated.

676

677 V. DISRUPTING THE BALANCE - TUMOR-INDUCED BONE DESTRUCTION

678 The radiographic appearance of bone metastases ranges from typically destructive (osteolytic) to
679 mostly bone-forming (osteoblastic) with most tumors demonstrating a mixture of lesions (Figure 1).
680 There is always an imbalance between bone formation and bone resorption during the development of
681 bone metastases. Therefore, predominantly osteolytic lesions are associated with high osteoclast
682 activity and reduced osteoblast activity, whereas predominantly osteoblastic lesions have a high
683 osteoblast activity and variable, but also often increased, osteoclast activity (35, 67).

684 The different molecular mechanisms associated with the formation of osteolytic lesions are
685 described below (Figure 4), whereas tumor-derived factors governing the formation of osteoblastic
686 lesions are described in the next section.

687 A. Factors Promoting Osteoclast-Mediated Bone Resorption

688 Several factors secreted by tumor cells stimulate osteoclast activity and bone resorption (PTHrP,
689 lysophosphatidic acid, macrophage-stimulating protein, prostaglandin E2, IL-8, IL-11, MMP-1, CCN3,
690 granulocyte macrophage-colony stimulating factor) (18, 26, 126, 252, 329, 361, 362). Among them,

PTHrP was the first to be recognized as involved in malignant osteolysis (126, 265). Using immunohistochemistry in a retrospective series of 31 human breast cancer metastasis specimens, PTHrP has been shown to be expressed in 92% of bone metastases (12 out of 13 samples) and 17% of metastases to non-bone sites (3 out of 18 samples) (265). Early investigations showed that preventive treatment of animals with a neutralizing antibody against PTHrP reduced the development of osteolytic lesions caused by human MDA-MB-231 breast cancer cells (126). PTHrP binds to the type 1 parathyroid hormone receptor (PTHR1), a seven-transmembrane G protein-coupled receptor expressed by osteoblast, which stimulates the expression of RANKL. In turn, RANKL binds to its receptor RANK on osteoclast precursors, leading to the formation of new osteoclasts and therefore enhanced bone resorption (126, 329). Moreover, tumor-derived PTHrP inhibits OPG production, thus promoting bone metastasis (329). The production of PTHrP by tumor cells is induced by transcription factors RUNX2 and Gli2. *RUNX2* is upregulated in osteotropic breast cancer cells and directly activates the Indian Hedgehog (IHH) pathway characterized by the upregulation of the Gli family of zinc finger transcription factors (Gli1, Gli2 and Gli3) (266). TGFβ released from resorbed bone also induces Gli2 expression in tumor cells (3). In turn, Gli2 (but not Gli1 and Gli3) induces PTHrP expression in bone metastatic human breast cancer cells and osteolysis in tumor-bearing animals (314). As a result, the blockade of the RUNX2-IHH pathway in MDA-MB-231 breast cancer cells by Runx2 short hairpin RNA inhibition prevents the osteolytic disease in bone metastatic animals (266). Likewise, the transcription factor MAF mediates breast cancer bone metastasis through the control of many factors including PTHrP (259). Interestingly, MAF expression in primary mammary tumors has been shown to predict treatment outomes of the bisphosphonate zoledronic acid in reducing the incidence of bone metastases in early-stage breast cancer (64). See section IX for further discussion.

Hypoxia also induces PTHrP expression and secretion by tumor cells through a HIF-dependent mechanism (222). Although bone is highly vascularized, the absolute oxygen tension in the bone marrow is quite low, and there is a moderate oxygen gradient between the peri-sinusoidal regions,

716 which have the lowest levels of oxygen tension (9.9 mmHg), and the endosteal region (13.5 mmHg),
717 which is perfused with small arteries (313). Thus, tumor cells experience hypoxic conditions in the bone
718 marrow. Moreover, tumor cells are also susceptible to hypoxia as they grow in the bone marrow, which
719 is caused by reduced vascular supplies of oxygen and nutrients. The role of HIF-1α in bone metastasis
720 formation has been therefore tested experimentally (146). The extent of bone destruction and
721 vascularisation of bone metastases in animals injected with MDA-MB-231 cells overexpressing an
722 active form of HIF-1α was significantly increased compared to mock-transfected cells (146). HIF-1α
723 also directly regulates the expression of transcription factor *TWIST* in human breast cancer cells (374),
724 and *TWIST* overexpression in osteotropic breast cancer cells promotes bone metastasis formation
725 through a mechanism dependent of miR-10b, facilitating tumor cell invasion and cancer-induced bone
726 destruction (74).

727 Platelet-derived lysophosphatidic acid (LPA) supports progression of osteolytic bone metastases in
728 breast cancer (26,27). By binding to its receptor LPA1 at the tumor cell surface, LPA promotes tumor
729 cell proliferation through the stimulation of a Pi3K/ZEB1/miR-21-dependent pathway (284). LPA also
730 induces the production of interleukins IL-6 and IL-8 by human breast cancer cells, which then stimulate
731 osteoclast-mediated bone resorption (26,27). Pharmacological inhibition of LPA action on its receptor,
732 using a LPA1 antagonist, substantially reduces progression of osteolytic bone metastases caused by
733 MDA-MB-231/B02 breast cancer cells in immunodeficient animals (27). Likewise, the treatment of
734 immunocompetent animals with a LPA1 antagonist inhibits spontaneous dissemination of murine 4T1
735 breast cancer cells in distant organs (lungs, liver) with no effect on primary tumor size (225).

736 Macrophage-stimulating protein (MSP) is produced by tumor cells in breast cancer (362). It binds
737 to the RON receptor tyrosine kinase, which is expressed by osteoclasts but not osteoblasts, and
738 stimulates osteoclast survival and activity (but not osteoclast differentiation) through a RANK-
739 independent, Src phosphorylation-dependent pathway (4).The intratibial injection of MSP-expressing
740 breast cancer cells in syngeneic wild-type mice causes a profound osteolysis (4). Moreover, the

741 therapeutic targeting of RON with tyrosine kinase inhibitor BMS-777607/ASLAN002 inhibits the
742 formation of osteolytic lesions in tumor-bearing animals (4) and reduces bone resorption in
743 postmenopausal women with advanced cancer (phase-I trial; ClinicalTrials.gov identifier
744 NCT01721148).

745

746 B. Factors Suppressing Osteoblast-Mediated Bone Formation

747 Tumor cells not only stimulate osteoclast activity, but also inhibit osteoblast activity, thereby
748 worsening the imbalance between bone formation and bone resorption, and promoting bone destruction
749 (361). Main factors produced by tumor cells that have been shown to suppress osteoblast differentiation
750 include activin A, the BMP inhibitor noggin, dickkopf-1 (DKK-1), and sclerostin (SOST-1) (198, 293, 326,
751 330, 395, 396).

752 Activin A is a member of the TGF-β superfamily of growth factors. It binds to activin type IIA
753 (ActRIIA) or type IIB (ActRIIB) receptors and induces the recruitment and phosphorylation of an activin
754 type I receptor (ActRIB), which then phosphorylates Smad2 and Smad3 intracellular signaling proteins
755 (198). In multiple myeloma, it has been reported that activin A secreted by plasma cells inhibits
756 osteoblast differentiation *via* Smad2-dependent downregulation of DLX (distal-less homeobox)-5 (198).
757 In breast and prostate cancer, activin A might modulate, via Smad signaling, the expression of pro-
758 osteoclastic factors (IL-11, CTGF, MMP-1) (198). Interestingly, in animal models of breast cancer bone
759 metastasis and of multiple myeloma with osteolytic lesions, the treatment of mice with a soluble activin
760 receptor type IIA fusion protein (ActRIIA.muFc) blocks bone destruction (51). Specifically, ActRIIA.muFc
761 stimulates osteoblastogenesis and promotes bone formation in tumor-bearing animals, thereby
762 preventing cancer cell-induced suppression of bone formation (51).

763 Noggin is a BMP antagonist encoded by *NOG*. In breast cancer, *NOG* mRNA expression levels are
764 significantly upregulated in bone metastatic lesions, compared to that observed in brain, lung and liver

765 lesions (326). The silencing of *NOG* in osteotropic breast cancer cell lines substantially reduces bone
766 metastasis formation in animals (326). Similarly, *NOG* is expressed in human prostate cancer cells that
767 metastasize to bone and cause osteolytic lesions in animals (293). Tumor-derived noggin interferes with
768 physiologic bone coupling by inhibiting bone formation, which thereby prevents repair of osteolytic
769 lesions generated by an excess of osteoclast-mediated bone resorption (293).

770 DKK-1 and SOST-1 are two Wnt (Wingless/int) protein antagonists. WNT agonists promote
771 osteoblast proliferation by binding to a receptor complex consisting of a member of the Frizzled
772 transmembrane receptor family and either LRP (low-density lipoprotein receptor-related protein) 5 or
773 LRP 6 (8). Both DKK-1 and SOST-1 exhibit redundant functions by blocking LRP5/6 binding to WNTs,
774 thereby inhibiting WNT signaling (8). High circulating levels of DKK-1 were first reported in multiple
775 myeloma patients with osteolytic lesions (332). Multipe myeloma cells express DKK-1 and the blockade
776 of DKK-1 using neutralizing antibodies results in a decrease of both osteolysis and skeletal tumour
777 growth in murine models of multiple myeloma (76, 372). DKK-1 is also expressed in breast, lung and
778 prostate cancers (39, 76, 130, 131). DKK-1 knockdown in breast and prostate cancer cell lines
779 decreases bone metastasis formation, while DKK-1 overexpression increases bone metastasis and
780 bone destruction *in vivo* (330, 396). Mechanistically, tumor-derived DKK-1 promotes osteolysis in animal
781 models of multiple myeloma and breast cancer bone metastasis and decreases the formation of
782 osteoblastic lesions in a model of prostate cancer bone metastasis by silencing canonical WNT
783 signaling of osteoblasts (76, 330, 396).

784 With regard to SOST-1, this WNT inhibitor is expressed in human primary breast tumors and
785 breast cancer cell lines, especially those that are hormone unresponsive (143, 395). An anti-SOST
786 antibody was shown to decrease the extent of osteolytic lesions in mouse models of MDA-MB-231
787 breast cancer bone metastasis (143, 395). As previously reported for DKK-1 in breast cancer (396),
788 SOST-1 promotes cancer-induced bone destruction by silencing canonical WNT signaling of
789 osteoblasts (143, 395). Furthermore, a treatment with an anti-SOST antibody also protects tumor-

bearing animals from cancer-induced muscle weakness, which is a debilitating event that can be associated with bone metastases in breast cancer patients (143). Plasma cells in multiple myeloma do not express SOST-1 (227). SOST-1 is however produced by osteocytes and treatment of animals with an anti-SOST antibody reduces osteolytic lesions induced by multiple myeloma, thereby preventing bone destruction (227).

C. Osteocytes – Silent Partners with a Role to Play

Osteocytes are terminally differentiated osteoblast lineage cells that reside in lacunae within the mineralized bone matrix (6, 80). Osteocytes are by far the most abundant cells of the bone. They are stellate cells that communicate with their environment *via* cytoplasmic projections termed dendrites. Dendrites of osteocytes form Cx43-dependent gap junctions with dendrites of neighbouring osteocytes as well as osteoblasts on the bone surface and cells in the bone marrow and vascular space, which results in the formation of a communication network in the bone matrix (80). Osteocytes modulate bone turnover by regulating osteoblast and osteoclast functions through the secretion of RANKL, SOST and DKK-1, and control calcium homeostasis through remodeling of the osteocytic perilacunar matrix (6, 80). They act as mechanosensors to control responses to mechanical loading of the skeleton (6, 80). Moreover, osteocytes regulate phosphate homeostasis through secretion into the circulation of fibroblast growth factor (FGF)-23 (80).

The contribution of osteocytes to bone metastasis is only beginning to be uncovered. This may be explained by the fact that studying osteocytes remains very challenging due to their location within the mineralized bone matrix. Methods used to isolate osteocytes from the bone matrix remain difficult and the phenotype of isolated osteocytes is not necessarily maintained *in vitro* (80). For example, human primary osteocytic cells in 2D culture do not express SOST-1, DKK-1 and FGF-23 (54). Thus, *in vitro* methods that are used to isolate and culture osteocytes may be a limitation to the study of osteocyte

functions such as in bone metastasis. Despite the technical challenges, it has been shown that tumor growth in bone induces pressure due to the lack of expansible space, suggesting that physical forces might modulate mechanotransduction properties of osteocytes (310). Indeed, application of hydrostatic pressure to cultures of MLO-Y4 osteocytic cells stimulated the secretion of factors associated with enhanced survival and invasion of prostate cancer cells *in vitro* (310). Osteocyte-derived CCL-5 and MMPs were among these factors promoting prostate cancer cell invasion *in vitro* (310). Whether these osteocyte-derived factors promote tumor cell invasion *in vivo* remains to be determined. Tissue engineered 3D bone models formed by primary human osteocytes have facilitated investigation into osteocyte functions (54). For example, primary human osteocytes in a 3D-culture system produce FGF-23, SOST-1, and DKK-1 as opposed to osteocytes in 2D culture (54). Using a 3D model, it has been shown that primary human prostate cancer cells induce a significant increase in the expression of FGF-23, RANKL and, to a lower extent, DKK-1 in primary human osteocytes, whereas SOST-1 expression is drastically decreased when compared to that observed with osteocytes in the absence of tumor cells (54). The authors suggested that the greater decrease in SOST-1 could favor the formation of osteoblastic lesions (54). It is however unclear how SOST-1 expression in osteocytes is downregulated by prostate cancer cells. These experimental findings are in contrast with the observation that high circulating levels of SOST-1 are found in prostate cancer patients with osteoblastic bone metastases (6). Further studies are therefore required to better understand the contribution of osteocytic-derived SOST-1 in prostate cancer bone metastasis.

In an *in vivo* model of bone disease caused by human JJN3 multiple myeloma cells, it has been shown that osteocytic dendrites were in direct contact with JJN3 cells in the bone marrow, leading to increases in osteocyte apoptosis and osteocytic RANKL and SOST production (83). *In vitro* cocultures between osteocyte-like MLO-A5 cells and JJN3 myeloma cells showed that cell-to-cell contact activated bidirectional Notch signaling in osteocytes and multiple myeloma cells, which increased multiple myeloma cell proliferation and induced osteocyte apoptosis. In turn, the induction of apoptosis promoted

839 osteocytic RANKL secretion, which then stimulated osteoclast formation (83). Thus, interactions
840 between osteocytes and multiple myeloma cells generate a microenvironment supportive of increased
841 tumor growth and bone destruction (83). These findings are in agreement with the fact that treatment of
842 animals with an anti-SOST antibody prevents bone destruction in different preclinical models of multiple
843 myeloma bone disease (5TGM1, 5T2MM, and MM1.5) (227).

844 In a breast cancer animal model, Cx43 hemichannels in osteocytes have been shown to play a
845 critical role in the suppression of bone metastasis (393). Specifically, Cx43 osteocyte-specific knockout
846 mice and osteocyte-specific Δ130-136 transgenic mice with impaired Cx43 gap junctions and
847 hemichannels showed increased tumor growth after intra-tibial injection of Py8119 mouse mammary
848 carcinoma cells (393). Additionally, R76W transgenic mice with functional hemichannels but not gap
849 junctions in osteocytes did not display a significant difference (393). Cx43 gap junctions mediate
850 communication between adjacent cells, whereas Cx43 hemichannels serve as a portal for the exit of
851 molecules in the extracellular microenvironment (80). Zhou and colleagues (393) established a specific
852 role for osteocytic Cx43 hemichannels in suppressing breast cancer growth and bone metastasis,
853 whereas osteocytic Cx43 gap junctions did not play such a role. In agreement with this observation
854 (393), ATP is released from osteocytes through Cx43 hemichannels and exerts inhibitory effects on
855 breast cancer cell migration *in vitro* and tumor growth *in vivo* (392). However, these findings are
856 contrary to the pro-metastatic role of Cx43 gap junctions between osteoblasts and breast cancer cells,
857 which promote progression of osteolytic lesions in animals (354).

858 Overall, these findings strongly suggest that osteocytes have a role to play in the development of
859 bone metastases. However, a lot of uncertainties remain as to whether osteocytes have bone
860 metastasis suppressor or promoter activities, and whether this activity depends on the cancer cell type
861 that metastasizes to bone. Further studies are therefore warranted to investigate the contribution of
862 osteocytes in bone metastasis formation.

863

D. The Fertile Soil - Contribution of the Bone Matrix

Bone is a riche source of growth factors, including TGFβ, IGFs and PDGF (platelet-derived growth factor) (361). For example, while there is no difference in bone marrow TGF-β levels between healthy controls and castration-resistant prostate cancer patients without bone metastases, patients with bone metastases have aberrantly high levels of TGF-β (161). Indeed, when released from the resorbed bone matrix, TGFβ acts on tumor cells, via SMAD- and COX2-dependent signaling pathways, and stimulates the expression of factors such as Gli2, PTHrP, the Notch ligand Jagged-1, IL-11 and PGE2 (3, 168, 295, 361). Jagged-expressing tumor cells are capable of directly activating osteoclasts by activating the Notch signaling pathway and the therapeutic targeting of Jagged-1 with a monoclonal antibody inhibits bone metastasis formation in animals (295, 390). In addition, TGFβ released from the bone matrix during bone destruction contributes to muscle weakness by decreasing Ca^{2+}-induced muscle force production (359). Bone-derived IGF-I stimulates growth of breast cancer cells *via* activation of the IGF type I receptor (IGF-IR)/Akt/NFkB pathway, and IGFR-IR was found to be elevated in 13/15 cases of bone metastases obtained from patients with a range of tumor types, supporting a role for the IGF axis in development of human disease (147). Similarly, bone-derived IGF-II stimulates skeletal outgrowth of prostate cancer cells *in vivo* (174). Bone-derived PDGF activates the Akt/PKB survival pathway in osteotropic breast and prostate cancer cells, as PDGF receptors in tumor cells growing in bone are highly expressed compared to nonmetastatic cancer cells (89, 199). Thus, evidence from both model systems and clinical samples support that bone-derived growth factors contribute to bone metastasis formation by promoting skeletal tumor outgrowth. To date, therapeutic targeting of growth factors has so far not resulted in patient benefit however trials including anti-growth factor agents as part of combination therapy for patients with bone metastases are ongoing. For example, the XENERA-1 trial (ClinicalTrials.gov Identifier: NCT03659136) aims to assess the antitumor activity of xentuzamab, a monoclonal antibody that binds both IGF-I and IGF-II and inhibits the binding

888 of these ligands to IGF-R, in patients with ER+/HER2- advanced or metastatic breast cancer and bone
889 metastases.

890 Bone is a mineralized tissue, rich in calcium. We previously discussed the contribution of calcium
891 from the osteogenic niche that facilitates tumor cell proliferation through Cx43 gap junctions (354).
892 However, calcium is also released from bone during osteoclastic resorption. It binds on tumor cells
893 (breast, prostate, renal cell carcinoma) *via* a calcium-sensing receptor (CaSR) and promotes tumor cell
894 proliferation and migration (29, 110, 354). Additionally, calcium stimulates the secretion of PTHrP and
895 epiregulin by tumor cells (29, 361). PTH-rP promotes osteoclast-mediated bone resorption and
896 epiregulin decreases *OPG* expression in osteoblasts, thereby both contributing to the progression of
897 osteolytic lesions (29, 361).

898

899 Osteolysis: current understandings & open questions

900 • Understanding the cancer-associated mechanisms that stimulate osteoclast-mediated bone
901 resorption has led to the development of anti-resorptive pharmaceutical agents that have
902 become established as a valuable additional approach to the treatment of bone metastases in
903 patients with advanced cancer.
904 • The observation that tumor cells not only stimulate osteoclast activity, but also inhibit osteoblast
905 activity, suggests that stimulating osteoblastic bone formation to promote bone repair could be
906 a novel alternative approach to treat malignant skeletal lesions.
907 • The contribution of osteocytes to bone metastasis is only beginning to be uncovered. A better
908 understanding of the interplay between osteocytes and tumor cells will represent an opportunity
909 for therapeutic targeting.

910

VI. TOO MUCH OF A GOOD THING - TUMOR-DERIVED FACTORS REGULATING OSTEOSCLEROSIS

A number of molecular mechanisms responsible for the formation of osteoblastic lesions have been identified (Figure 5), described in the following sections.

A. Factors Promoting Osteoblast-Mediated Bone Formation

Tumor cells in the bone environment secrete factors that activate osteoblasts, leading to the formation of skeletal lesions with extensive new bone deposition(osteosclerosis) (210, 248). Among them, endothelin-1 (ET-1) was recognized as a major mediator of osteosclerosis; it stimulates osteoblast proliferation and inhibits osteoclast activity and motility (2, 53, 210, 378). In this respect, prostate cancer patients with bone metastases have far higher circulating levels of ET-1, compared to those with localized cancer (276). Interestingly, *TMPRSS2-ERG* is the most frequent fusion gene expressed in prostate cancer, it is associated with cancer progression, and its expression in PC3c-T1E4 prostate cancer cells has been demonstrated to promote *ET-1* expression and formation of osteoblastic lesions in animals (84). Human ZR-75-1 breast cancer cells that produce ET-1 stimulate new bone formation and osteoblast proliferation in organ cultures, and osteoblastic metastases in animals (378). Stimulatory effects of ET-1 on osteoblasts are mediated by two receptors, ETAR and ETBR, which activate similar signaling pathways and down-regulate expression of the Wnt signaling inhibitor DKK-1 (276). Osteoblast proliferation and bone metastasis are both inhibited by ETAR antagonists atrasentan and zibotentan, as well as by the dual ETAR and ETBR antagonist bosentan, highlighting the prominent role played by ET-1 in the formation of osteoblastic lesions in preclinical settings (276, 378). Despite this, both atrasentan and zibotentan have failed to show benefit in CRPC patients with bone metastases (47, 240).

Breast and prostate cancer cells can produce BMPs, such as BMP-2, BMP-4 or BMP-6, which facilitate the development of osteoblastic bone metastates by stimulating tumor growth and osteogenesis (77, 78, 171, 195). In this respect, prostate cancer cell-derived BMP-4 mediates

conversion of endothelial cells into osteoblasts, thereby promoting aberrant bone formation (204). Analyses of human samples of prostate cancer bone metastases confirmed the presence of cells co-expressing endothelial and osteoblastic markers (Tie-2 and osteocalcin, respectively), which together with the detection of increased expression of BMP-4 in bone metastases compared to that of primary prostate tumors, support the hypothesis that endothelial-to-osteoblast conversion could also take place in human disease (204). Overall, these findings (77, 78, 171, 195, 204) illustrate how the dysregulation of BMPs can have deleterious effects on the bone microenvironment. Furthermore, the BMP inhibitor noggin is also secreted by tumor cells and it is the balance between BMPs and noggin that determines, at least in part, the phenotype of breast and prostate cancer bone metastases (293).

Prostate cancer cells secrete multiple WNT agonists, including canonical WNTs 3A, 7B and 10B, which, by binding to LRP5/6, are known mediators of osteoblast differentiation and mineralization (129, 202, 210, 237). However, the WNT antagonist DKK-1 is also secreted by prostate cancer cells and, as aforementioned for BMPs and noggin (293), it is the relative expression levels of WNT agonists and DKK-1 that determine the phenotype of skeletal lesions (130). For example, C4-2B prostate cancer cells express the WNT agonists WNT7A and WNT8B, but not DKK-1, and they induce mixed osteoblastic/osteolytic lesions in animals (130). DKK-1 overexpression in C4-2B cells antagonizes WNT functions, which leads to the suppression of WNT signaling in osteoblasts and results in the formation of highly osteolytic lesions in animals (130). Among the many proteins downstream of WNT, autocrine WNTs induce BMP-4 and BMP-6 expression in prostate cancer cells that, in turn, promotes osteoblast differentiation (77, 195). *WNT* expression in tumor cells is itself regulated by many factors. For example, T-box family transcription factor TBX2 is overexpressed in human prostate cancer specimens and bone metastases from xenograft mouse models of human prostate cancer (239). It promotes transcription of *WNT3A* in prostate cancer cells and the blockade of WNT3A with neutralizing antibodies dramatically reduces experimental bone metastasis formation (239). Similarly, *WNT5A* and *WNT7B* are targets for the transcription factor ERRα ("Estrogen Receptor Related Receptor alpha") and the androgen receptor

(AR), respectively, which are both highly expressed in castration-resistant prostate cancer cells, and they promote tumor growth and development of osteoblastic lesions in animals (108, 391). Thus, there is evidence that WNT signaling is central to osteoblast-stimulatory activity of metastatic prostate cancer, however therapeutic targeting of this pathway is in its infancy (237).

PTHrP can be actively involved in the progression of osteoblastic lesions in prostate cancer by enhancing proliferation of bone marrow stromal cells and early osteoblast differentiation (203). Moreover, prostate-specific antigen (PSA), a serine protease expressed by prostate cancer cells and a well-known marker of cancer progresssion, can cleave IGF binding protein (IGFBP)-5, rendering IGF-I available to bind to its receptor and stimulate osteoblast proliferation (219). PSA also enhances the bioavailability of TGF-β in the bone microenvironment (379). Like PSA, production of urokinase-type plasminogen activator (uPA) by prostate cancer cells can increase IGF-I and TGF-β bioavailibility to the bone microenvironment (113).

Several other osteoblast-regulatory factors expressed by tumor cells have been identified. These include growth factors [PDGF BB (377), FGFs (FGF-8, FGF-9) (201, 342) and VEGF (176)], adrenomedullin (299), TGFβ-regulated gene PMEPA1 (107) and prostatic acid phosphatase (PAP) (188). Prostate cancer cells can also secrete neuropeptides, such as substance P (124) and Sema3A (112), which stimulate osteoblast differentiation.

B. Factors Suppressing Osteoclast-Mediated Bone Resorption

Tumor cells that induce osteoblastic lesions not only stimulate osteoblast activity but may sometimes also inhibit osteoclast activity. Among the osteoclast inhibitors produced by cancer cells are ET-1 and OPG (210). Patients with metastatic prostate cancer have high circulating levels of ET-1 and OPG (90, 210, 383). Tumor-derived ET-1 directly inhibits osteoclast-mediated bone resorption by binding to the surface of osteoclasts *via* membrane receptors ETA and ETB (2, 53). Tumor-derived

OPG inhibits osteoclast differentiation by binding to RANKL, thereby preventing its interaction with RANK (183). The overexpression of OPG in human C4-2 prostate cancer cells protects these cells from TRAIL (tumor necrosis factor-related apoptosis-inducing ligand)-induced apoptosis, decreases osteoclast formation and promotes the formation of osteoblastic lesions in animals (70). OPG expression in prostate cancer cells is regulated by factors such as PAP, a tumor-derived acid phosphatase that promotes osteoblast differentiation and bone mineralization (175, 188). PAP knockdown in pro-osteoblastic VCAP prostate cancer cells decreases OPG while increasing RANK/RANKL expression (175). Conversely, PAP overexpression in pro-osteolytic PC3M prostate cancer cells has the inverse effect, increasing OPG while decreasing RANK/RANKL expression (175). The transcription factor ERRα induces OPG expression in MDA-MB-231-B02 breast cancer cells, thereby inhibiting osteoclast differentiation (109). Tumor-derived PSA also stimulates OPG production and inhibits RANKL expression in osteoblasts (379). BMP-2 enhances Wnt/beta-catenin-dependent transcriptional activation of the OPG promoter in osteoblasts (287). Thus, tumor-derived ET-1 and OPG produced by both tumor cells and osteoblasts, by means of their ability to inhibit osteoclast activity, contribute substantially to the formation of osteosclerostic bone metastases in model systems. When it comes to human disease, evidence that tumor-derived OPG contributes to the pathology of bone lesions is limited, hampered by the lack of bone metastases available for research. A meta-analysis found that in studies of prostate cancer, patients with bone metastases had higher levels of serum OPG compared to patients with metastases in other sites or healthy control (383). However, as this study did not include any information about the lesion types of the patients (sclerotic/lytic/mixed) it was not possible to link elevated serum OPG levels to decreased osteoclast activity and a shift in the balance towards osteoblastic bone lesions.

Osteosclerosis: current understandings & open questions

- Despite elevated levels of the osteoblast stimulating factor ET-1 in patients with bone metastases and supportive data from a number of *in vitro* and *in vivo* model systems, drugs targeting ET receptors have failed to provide benefit in trials of CRPC.
- WNT signalling regulates osteoblast differentiation and is activated in human prostate cancer, however the precise role of WNT family members in development and progression of the disease remains to be established.
- Studies that combine detailed characterisation of bone lesions with paired measurements of factors modifying bone turnover in serum and/or bone marrow are required to provide evidence of which molecules are the most promising therapeutic targets in metastatic prostate cancer.

VII. CONTRIBUTION OF BONE MARROW CELLS TO TUMOR DEVELOPMENT – MULTIPLE INTERACTIONS BEYOND THE VICIOUS CYCLE

In addition to the main cell types responsible for bone remodeling described above (osteoblasts, osteocytes and osteoclasts), the bone microenvironment includes a myriad of interconnected cell populations, including a rich vascular network, immune cells, adipocytes, nerve cells, and megakaryocytes. Tumor cells arriving in this environment are proposed to utilize the mechanisms that regulate normal physiological processes in order to avoid immune surveillance and establish cellular interactions that support their expansion to overt metastases. As described in earlier sections, endothelial cells contribute in tumor cell extravasation, tumor cell dormancy and formation of osteoblastic lesions (119, 204, 267, 273). The role of platelets in stimulating bone metastasis formation has also been described above (26, 27, 190, 191). With regard to megakaryocytes, the platelet-producing cells, little is known about their role in bone metastasis, with both promoting and inhibitory roles having been reported (191, 223). In the following sections we chose to cover some of the key discoveries linking immune cells, nerve cells and adipocytes to the development of bone metastases in solid tumors.

A. The Immune Cells of the Bone Microenvironment

It has long been clear that the immune system plays an integral part of both normal bone homeostasis, as well as in a number of pathologies associated with bone loss, mainly through the link with inflammation. Combining the bone biology and immunology research fields to increase our understanding of their close connection has resulted in the new discipline of "osteoimmunology" (245). Initially focused on the bone-destructive effects of immune infiltrates through stimulation of osteoclasts by pro-inflammatory cytokines, research is expanding to other areas, including bone metastasis (253, 370). Although the inflammatory response undoubtedly contributes to the extent and severity of cancer-induced bone disease, as covered in the following sections, evidence from model systems support that immune cells may also affect tumor cell colonization and progression in bone (Figure 6).

1. Immune cells inhibiting local tumor growth in the bone microenvironment.

CD8$^+$ T cells

CD8+ T cells are central players in controlling infections and cancer, recognised as one of the most important immune cells associated with tumour destruction (253). By cross-presenting tumor antigens, dendritic cells (DCs) activate CD8$^+$ T cells. In turn, tumor-specific cytotoxic CD8$^+$ T cells participate in the killing of antigen-positive tumor cells (45). The anti-tumor effects of tumor-specific cytotoxic CD8$^+$ T cells is dependent on their ability to produce interferon (IFN)-γ. Pioneering work by the Faccio group has demonstrated that activation of CD8$^+$ T cells reduces bone metastasis formation in animals, whereas depletion of CD8$^+$ T cells enhances it (386). Specifically, using phospholipase C gamma (PLCγ) 2$^{-/-}$ mice, which have broadly compromised immune responses and are osteopetrotic due to reduced osteoclast number and functionality, Zhang and colleagues (386) reported an unexpected increased tumor growth in bone despite osteoclast dysfunction. This was found to be due to a defective anti-tumor T cell response in tumor-bearing PLCγ2$^{-/-}$ mice. Similar experiments were then conducted in Lyn$^{-/-}$ mice, which have enhanced T-cell responses and decreased bone mass due to high number of osteoclasts.

1059 Lyn$^{-/-}$ mice had a reduced bone tumor burden despite osteolysis (386). Importantly, injection of antigen-
1060 specific wild-type cytotoxic CD8^{+} T cells in PLCγ2$^{-/-}$ mice or depletion of CD8^{+} T cells in Lyn$^{-/-}$ mice
1061 normalized tumor growth in bone, regardless of osteoclast activity (386). This study is important in that it
1062 used both genetic and pharmacological approaches to demonstrate that the extent of tumor growth in
1063 bone is not only linked to the level of osteoclast activity as stipulated by the vicious cycle of cancer-
1064 induced bone destruction. In addition, these findings demonstrate that CD8^{+} T cells have the potential to
1065 act as regulators of tumor growth in bone. This contention is supported by the observation that
1066 transcription factor ERRα in murine 4T1 breast cancer cells inhibits the progression of bone metastases
1067 by increasing the recruitment of CD8^{+} T cells in the bone marrow (28). However, this remains to be
1068 established for human cancers where our capacity to identify T cell subsets in bone metastatic foci is
1069 limited.
1070
1071 Natural killer (NK) cells
1072 Mature NK cells represent 1% of the lymphocyte population in bone, which is the primary site of murine
1073 NK cell development (253). In contrast, human NK cells are shown to differentiate from precursors and
1074 located in the secondary lymphoid organs like spleen and lymph nodes, and single cell RNA sequencing
1075 of NK cells isolated from both blood and bone marrow of healthy donors has revealed the presence of
1076 multiple heterogenous subsets with potentially different functions (373).
1077 NK cells are involved in the nonspecific elimination of tumor cells through the production of IFNγ,
1078 release of cytolytic granules or TRAIL/FASL-induced apoptosis (253). Pathways of IFN induction are
1079 regulated by IFN regulatory factors (IRF3, IRF5 and IRF7) and NFκB (20). Bidwell and colleagues (20)
1080 found that *irf7* expression was suppressed in murine 4T1.2 mouse breast cancer cells isolated from
1081 bone metastases, compared to those of matched primary mammary tumors. Enforced expression of Irf7
1082 in bone metastatic 4T1.2 cells restored an antimetastatic immune response in immunocompetent tumor-
1083 bearing animals (20). Conversely, the inoculation of Irf7-overexpressing 4T1.2 cells to mice deficient in

NK and $CD8^+$ T cell responses led to accelerated development of bone metastases, compared to immunocompetent mice (20). Similarly, the impairment of NK-cell-mediated anti-tumour immunity with a JAK/STAT inhibitor enhanced skeletal tumor burden in preclinical models of breast cancer metastasis (25). Taken together, these data indicated that NK cells (and $CD8^+$ T cells), through the production of IFN-γ, contributed to the suppression of bone metastasis and that NK cells are potential therapeutic targets in this setting. The clinical relevance of these findings was confirmed in over 800 patients in whom high expression of Irf7-regulated genes in primary tumors was associated with prolonged bone metastasis-free survival (20). A comprehensive review describing how NK cells may control metastasis points out that NK cells appear to have a particular role in reducing metastatic dissemination and speculates that this may be due to their ability to eliminate tumor cells that escape the immunosuppressive microenvironment of the primary tumor (211). It also includes an overview of clinical trials with immunotherapy agents boosting NK cell effector functions, the outcomes of which will provide important information about the impact of NK cells in metastatic disease, including in skeletal metastasis.

2. Immunosuppressive cells promoting local tumor growth in the bone microenvironment

Myeloid Derived Suppressor Cells (MDSCs)

MDSCs describe a heterogeneous collective of immature progenitor populations for the myeloid cells, for which a variety of roles in tumor progression have been reported (172). Bone marrow accumulation of MDSCs is found in many cancer types, indicating pathological disruption of myeloid cell maturation (114). An important role of MDSCs in the metastatic process is their immune-suppressive functions, which include induction of oxidative stress, interference with lymphocyte trafficking and expansion of Treg cells (172). MDSCs are proposed to increase the number of Treg cells and modify tumor growth in bone independent of osteoclast activation through modification of T cell responses (45). For instance, $PLC\gamma2^{-/-}$ mice are osteopetrotic due to reduced osteoclast number and functionality (45). Interestingly,

1111 despite osteoclast dysfunction, tumor growth in bone of PLC$\gamma 2^{-/-}$ mice was significantly higher than that
1112 observed in their wild-type counterparts due to an aberrant increased percentage of MDSCs in the bone
1113 marrow that, in turn, inhibited anti-tumor T cell response in tumor-bearing PLC$\gamma 2^{-/-}$ mice (45, 386).
1114
1115 Few studies have investigated the potential role of MDSCs in human bone metastases, but a recent
1116 report compared polymorphonuclear (PN-) MDSC distribution in primary prostate tumours (n=90) and
1117 their corresponding lymph node metastases (n=37) to that of bone metastases (n=35) (363). PN-
1118 MDSCs were found to mainly infiltrate the stroma (rather than the epithelial areas), and that this was
1119 more prominent in the metastases compared to the primary tumour. The authors propose that this
1120 stromal location would facilitate better suppression of infiltrating T cells by the PN-MDSCs and that the
1121 high levels of CXCL5 in bone may drive MDSC infiltration and ultimately metastatic progression (363).
1122
1123 A combination of the CXCR4 antagonist AMD3465 and the IDO1 inhibitor D1MT has been shown to
1124 delay the progression of breast cancer bone metastases in mice through activation of CD8+ T-cells and
1125 inhibition of Treg cells and MDSCs, supporting that suppression of MDSCs could potentially reduce
1126 metastatic progression in bone (385).
1127
1128 MDSCs isolated from tumor bearing mice have been also shown to be able to differentiate into
1129 functional osteoclasts *in vitro* and *in vivo* (81, 288). Interestingly, only MDSCs from mice with confirmed
1130 tumor growth in bone had osteoclastic potential, whereas those isolated from mice with peripheral
1131 tumors or control mice did not (288). The increased understanding of the inter-connectivity between
1132 cells residing in the bone marrow has resulted in studies exploring the effects of anti-resorptive agents
1133 beyond their traditional osteoclast targets. For example, in mouse models, a single, clinically relevant
1134 dose of the osteoclast inhibitor zoledronic acid has widespread effects on a number of cell types in the
1135 bone marrow, including hematopoietic stem cells, myeloid-biased progenitor cells and lymphoid-biased

cells (340). Importantly, bone marrow cells isolated from zoledronic acid treated animals, but not from control, were able to suppress tumor growth *in vivo* when co-injected with tumor cells, supporting the finding that anti-resorptive agents could support the generation of tumor-suppressing myeloid cells (340). In follow-up studies, these findings were confirmed, demonstrating that even a single dose of zoledronic acid skews myeloid progenitor cells to enter the macrophage, rather than the osteoclast lineage (339). This exemplifies the potential for unexpected (both beneficial and harmful) effects of anti-cancer therapies on bone marrow cell populations with implications for tumour progression, generally not considered when assessing the clinical benefits of cancer treatment. As the anti-resorptive bisphosphonates are increasingly used as adjuvant therapies in post-menopausal breast cancer without the precise mechanism conveying their positive effects on survival (66), it will be interesting to see if additional patient benefit could be linked to effects of these agents on a range of bone marrow cell populations.

Macrophages

Macrophages develop from circulating monocytes within tissues and are heterogeneous and highly plastic cells, which can polarize into pro- or anti-inflammatory sub-types (M1 and M2, respectively) depending on signaling cues (45, 118). However, there are many other discrete sub-populations across the M1/M2 spectrum determined by the location and activation status of macrophages (45, 118). Macrophages are consistently found in bone metastases from patients with prostate cancer (369). In breast cancer, tumor-associated macrophages are also significantly increased in bone metastases compared to matched primary mammary tumors (394). Experimentally, tumor-associated macrophages were found to promote breast and lung cancer bone metastasis formation (102, 148). Additionally, a population of specialist osteal tissue macrophages termed ‘osteomacs’, whose normal function is to regulate osteoblast differentiation (50), were found to facilitate formation of osteoblastic lesions in an animal model of prostate cancer (369). Nonetheless, their specific role in bone metastasis has proven

elusive, in part because of ablation techniques that remove a number of myeloid related populations, including the closely related osteoclast precursors that are established as major drivers of bone metastasis. For example, treatment of animals with clodronate-encapsulated liposomes markedly reduced the number of monocytes in peripheral blood, and the formation of bone metastasis when HARA-B lung cancer cells were injected intracardiacally to mice (148). However, in this study, clodronate-encapsulated liposomes not only reduced macrophages within tumors, but also osteoclasts in metastastic bone lesions, thereby explaining the reduction of bone destruction (148). Similarly, the use of an anti-mouse CD115 monoclonal antibody, which specifically targets monocytic cells, inhibited breast cancer bone metastasis formation in animals by blocking osteoclast activity (102). Beside the use of techniques that can directly interfere with osteoclast function (102, 148), tumor cells can recruit macrophages and osteoclasts through the same mechanism of action. For example, breast cancer-derived CCL2, which is a ligand for the chemokine receptor CCR2 expressed by myelomonocytic progenitors such as macrophages and osteoclast precursors, can stimulate through the same molecular mechanism the migration of macrophages in lung parenchyma and the differentiation of osteoclasts in the bone marrow, which ultimately aids metastasis to lungs and bone (214). Similar findings were reported with CCL2 produced by human prostate cancer cells, which promoted recruitment of macrophages within subcutaneous tumor xenografts and osteoclast-mediated bone destruction in animals bearing bone metastases (232). Thus, despite some suggestions that macrophages and osteomacs can contribute to bone metastasis (45, 369), further studies, including of samples human bone metastases, are needed to establish the precise mechanisms that could regulate these highly adaptable cells in the context of tumour growth in bone.

Dendritic cells

Dendritic cells are specialised antigen-presenting cells that are derived from hematopoietic bone marrow progenitor cells that differentiate into 2 subsets: conventional or myeloid dendritic cells (mDCs, similar to monocytes, produce IL-12) and plasmacytoid dendritic cells (pDCs, resembling plasma cells, produce IFN-α). These cells are responsible for presentation of antigens on their surface to induce T cell activation and prime $CD8^+$ T cells (253). However, DCs in tumors can have limited antigen-presenting function, thereby affecting the generation of anti-tumor immune responses (218). Additionally, DCs in cancer may exhibit immunosuppressive properties under certain circumstances (218). For example, using different syngeneic breast cancer models, Sawant and colleagues (289) observed an increased number of pDCs with increased bone metastasis in animals. Conversely, depletion of pDCs following treatment of animals with PDCA1 antibody prevented breast cancer bone metastasis formation (289). Furthermore, isolated $CD8^+$ T cells from pDC-depleted mice exhibited enhanced cytotoxic activity compared to those from untreated animals, indicating that in bone-metastatic disease pDCs exhibit immunosuppressive properties on $CD8^+$ T cells (289). Thus, there is some evidence to support that pDCs may be critical regulators of bone metastasis, however this is one of the least investigated immune cell types in this context, with a paucity of informative clinical studies to provide solid data to allow a conclusion regarding their importance to be drawn at this point.

Regulatory T (Treg) cells)

Treg cells are potent immune suppressors, impairing $CD8^+$ cell proliferation (45). The role of Treg cells in bone remodeling has not been extensively studied, with a few examples of inhibition of osteoclast maturation by Treg cells due to their production of IL-10, IL-4 and TGFβ (30). In prostate cancer, Treg cells are significantly increased in the bone marrow of patients with bone metastasis compared to those without (389). Furthermore, the intravenous injection of activated Treg cells to immunodeficient NOD/SCID mice bearing human PC3 prostate cancer skeletal lesions leads to a reduction of bone destruction, due to the osteoclast-inhibitory effect of Treg cells (389).

A study by Jiao *et al* (161) has shed light on why a combination of anti-CTLA-4 (ipilimumab) and anti-PD-1 (nivolumab) checkpoint inhibitors that reduce primary prostate tumour growth in patients are largely ineffective in reducing bone metastatic disease. The study compared levels of TGFβ in bone from patients with and without bone metastases and mapped T cell subsets in primary tumors and bone metastases from patients treated with ipilimumab. Results showed that the increased levels of TGFβ released during cancer-induced bone resorption caused helper T cells to polarise into Th17 CD4 cells instead of the Th1 CD4 effector cells required to trigger an anti-tumour immune response (161). Combining anti-TGFβ and anti-CTLA-4 therapy in a mouse model resulted in reduced bone metastasis, a strategy that the researchers now will take forward in clinical trials of patients with metastatic prostate cancer (161). This study (161) is an example of 'reverse translation', where a clinical observation is explored in model systems to identify mechanisms responsible for the observed effects and how they can be overcome. It also highlights that the specific immune tumor environment in bone presents a particular challenge when considering immunotherapy approaches in patients with bone metastases.

Immune cells and bone metastasis: current understandings & open questions

- Depletion of CD8+ T cells results in increased bone metastasis in model systems, but direct evidence for a role of CD8+ T cells in development of human skeletal metastases is missing.
- The anti-metastatic functions of NK cells are not yet established in human disease, but evidence from models of bone metastasis support their role in suppressing tumour growth.
- Myeloid Derived Suppressor Cells (MDSCs) have been identified in bone metastases in model systems and in human samples, where they are proposed to increase Tregs and inhibit immune elimination of tumour cells.
- Macrophages are highly plastic cells with context-specific functions, their role in the different stages of human bone metastasis remains to be defined.

- Plasmacytoid dendritic cells (pDCs) may be critical regulators of bone metastasis in model systems, however there is a paucity of informative clinical studies to draw any conclusion at this point.
- The specific immune tumour environment in bone presents a particular challenge when considering immunotherapy approaches in patients with bone metastases. Differences between murine and human immune cell populations must be considered when translating findings from in vivo model systems to human disease.

B. Nerve cells

Bone is highly innervated, and a recent extensive review describes how bone homeostasis is influenced by sympathetic, parasympathetic, and sensory nerves (95). How bone remodelling is regulated by nerve cells is illustrated by the example of norepinephrine, released by sympathetic nerves, which activates β2-adrenergic receptors expressed by osteoblasts, stimulating synthesis of RANKL that in turn modifies both osteoblast and osteoclast activity (95).

As a cancer diagnosis is associated with increased levels of stress and depression, a number of studies have investigated whether stress-induced activation of the sympathetic nervous system (SNS) results in increased bone metastasis. In a model of learned helplessness (chronic immobilization stress), intracardiac injection of MDA-MB-231 breast cancer cells in mice that had undergone 2 weeks of SNS activation resulted in increased number of bone metastatic foci associated with larger lytic bone lesions, compared to control (44). Similar results were obtained when injecting MDA-MB-231 breast cancer cells in mice pre-treated for 3 weeks with isoproterenol (ISO), which is used as a surrogate of SNS activation through stimulation of the β2-adrenergic receptor (44, 234). Importantly, these effects observed in mice under chronic stress or treated with ISO were inhibited by administration of the β2-blocker isopropranolol (44, 234), supporting that sympathetic nerve activity mediates the 'pro-metastatic' effect of chronic stress. Furthermore, breast cancer bone metastasis in animals that are under chronic

1259 stress is inhibited by knocking down RANK in MDA-MB-231 cells (44). Mechanistically, it is proposed
1260 that stress-induced activation of the β2-adrenergic receptor in osteoblasts stimulates RANKL production
1261 that, in turn, promotes MDA-MB-231 cell migration to bone in a RANK-dependent manner (44). ISO pre-
1262 treatment of bone marrow stromal cells also induce the expression of pro-inflammatory cytokines (IL-1β,
1263 IL-6) that increase the expression of E- and P-selectins by endothelial cells and the subsequent
1264 adhesion of MDA-MB-231 breast cancer cells to these cells under static and dynamic conditions *in vitro*
1265 (56). It has also been suggested that DTCs residing in endosteal niches could be affected by
1266 norepinephrine (82). Specifically, Decker *et al.* (82) found that the binding of norepinephrine to β2-
1267 adrenergic receptors affects osteoblasts *in vitro* through downregulation of the dormancy-inducing
1268 molecule GAS6, thereby re-activating proliferation of dormant disseminated prostate cancer cells that
1269 interacted with these osteoblasts. Taken together, these experimental data (44, 56, 82) suggest that
1270 stress-induced activation of the SNS prior to tumor cells arriving in bone alters the microenvironment to
1271 become more supportive of tumor outgrowth. Whether this also applies in human disease is difficult to
1272 investigate. A study including over 100,000 women in UK did not find any evidence of increased risk of
1273 breast cancer in those who reported high levels of stress (292). The current experimental evidence for
1274 neuronal involvement therefore relates to progression of established disease, and great care should be
1275 taken not to suggest that patients are in any way responsible for their disease progression through their
1276 ability to manage the inevitable stress associated with a cancer diagnosis and treatment.

1277 Once bone metastases are established and progressing, their interaction with the nervous system
1278 is obvious. Pain is one of the most common and difficult to treat complications associated with skeletal
1279 metastases (229). Tumor cells, their associated stromal cells and osteoclasts can generate pain by
1280 releasing algogenic substances including protons (create acidosis), bradykinin, endothelins,
1281 prostaglandins, proteases, and tyrosine kinase activators such as nerve growth factor (NGF) (100).
1282 Sensory fibers in the bone marrow express acid-sensing nociceptor TRPV1 (transient receptor potential
1283 vanilloid 1) and the NGF tyrosine kinase receptor type 1 (TrkA) (23, 351). In murine models of intra-

femoral or intra-tibial injection of NGF-expressing breast, prostate or Lewis lung cancer cells, tumour growth in bone is associated with induced sprouting of sensory nerve fibers and lytic bone lesions (23, 162, 226, 351). SNS activation and bone pain (as judged by hyperalgesia and flinching) caused by Lewis lung cancer cells are substantially reduced in TRPV1$^{-/-}$ mice compared to wild-type animals with comparable tumor burden (351). Similarly, systemic administration of a neutralizing anti-NGF antibody to animals bearing breast or prostate cancer bone metastasis reduces ectopic nerve fiber sprouting and attenuates nociceptive behaviors (spontaneous guarding and flinching) (23,162, 226). Overall these findings suggest that targeting NGF and/or TRPV1 are potential strategies to treat bone pain.

Nerve cells and bone metastasis: current understandings & open questions

- Bone homeostasis is influenced by sympathetic, parasympathetic, and sensory nerves. These interactions are affected by numerous factors released by tumor cells.
- In model systems, stress-induced activation of the sympathetic nervous system alters the bone microenvironment to become more supportive of tumor outgrowth. However, there is no evidence that it also applies in human disease.

C. Adipocytes

The adipose content of the bone marrow increases with age, obesity levels and metabolic conditions (134, 230). A large body of research underpins the current view that bone marrow fat is a hormone-sensitive endocrine tissue with the capacity to modify bone mass, and hence could contribute to skeletal tumour growth through a range of mechanisms, including provision of energy and pro-survival factors for tumor cells (134). However, the majority of these studies are from model systems, often involving injection of large numbers of tumor cells directly into bone of immunocompromised mice fed a high fat diet. Due to suitable clinical material being difficult to obtain, the relevance of the

information generated in model systems described in this section remains to be validated in studies of human samples.

Adipocytes can be drivers of chronic inflammation, resulting in immune cell infiltration and release of high levels of pro-inflammatory cytokines, including CXCL-1, CXCL-2, IL-1β, IL-6 and TNFα, molecules known to stimulate bone resorption and bone metastasis (134, 135, 140, 151). Using an *in vivo* diet-induced obesity model, Herroon *et al.* (142) have demonstrated a direct link between adipocytes and prostate cancer growth in bone. Specifically, the intratibial injection of PC3 prostate cancer cells into high-fat-diet-fed mice led to larger tumors than those observed in mice on normal diet (142). *In vitro*, prostate cancer cells exposed to lipids supplied by bone marrow adipocytes displayed increased invasive and proliferative capacity compared to control, which was associated with induction of lipid chaperone FABP4 (fatty acid binding protein 4) and IL-1β in tumor cells (142). Although FABP4 is known for its expression in adipocytes, it was also expressed by PC3 cells co-cultured with adipocytes, and its inhibition with a selective inhibitor (BMS309403) blocked PC3 cell invasion *in vitro* (142). Immunohistochemical staining showed that in the small number of human prostate cancer bone metastasis samples analysed (n=5), FABP4 positivity was more pronounced compared to benign prostate lesions and primary tumor tissues (142). The authors acknowledge that further studies are however required to establish whether FABP4 acts as a mediator between adipocytes and tumor cells to stimulate tumor growth in human bone metastases.

In addition to FABP4, the expression of oxidative stress enzyme HO-1 (heme oxygenase 1) was also found to be significantly upregulated in prostate cancer bone metastases from high-fat-diet-fed mice (141). *In vitro*, bone marrow adipocytes induced the upregulation of HO-1 in prostate cancer cells, whereas, *in vivo*, HO-1 overexpression in human prostate cancer cells promoted skeletal tumor growth and osteolysis (141). Importantly, a link to human prostate cancer was established by analysis of five datasets of patients with metastatic disease (n=89), showing significant upregulation of HO-1 in metastatic foci (including in bone) compared to primary tumors. HO-1 expression was identified by

immunohistochemical staining in two samples from prostate cancer bone metastasis biopsies, providing some limited support that HO-1 is associated with tumor progression in bone, which requires validation in a larger sample set.

Bone marrow adipocytes also promote metabolic reprogramming of prostate cancer cells in bone towards a glycolytic phenotype (87) (*see section VII for further discussion*). Additional studies utilizing these diet-induced models of increased bone marrow adiposity demonstrated that the adipose-derived chemokines CXCL1 and CXCL2 cause accelerated osteoclastogenesis *in vitro,* leading to enhanced prostate cancer-associated bone degradation *in vivo* (135) (Table 1).

Adipocytes have also been linked to bone metastasis in a number of cancer types other than prostate cancer. In multiple myeloma, bone marrow adipocytes support tumor cell proliferation and migration *in vitro* through mechanisms that are, at least in part, mediated by leptin (43). Furthermore, myeloma cells promote MSC differentiation into adipocytes at the expense of osteoblasts by inhibiting expression of the ubiquitin ligase MURF1, thereby suppressing osteoblast-mediated bone formation in tumor-bearing animals and in cells from patients with myeloma (208). In a model of breast cancer bone metastasis, using conditioned medium generated from cultured adult human bone fragments, migration of MDA-MB-231 cells was found to correlate with increasing levels of the adipokine leptin and IL-1β (328). Direct co-cultures demonstrated high numbers of breast cancer cells associated with the marrow adipose tissue within the bone fragments, supporting that tumour cells colonise areas of bone with high adiposity (328). Similar findings were reported from studies of melanoma models of bone metastasis, showing that melanoma cells were located in close proximity to adipocytes when colonising bone (52, 356). Furthermore, the intra-tibial injection of B16F10 melanoma cells resulted in larger tumors and increased osteoclast numbers in mice fed a high-fat diet, compared to that observed in mice fed a normal diet (52). In agreement with these findings, melanoma cell-adipocyte co-culture experiments showed an increase in pro-inflammatory and pro-osteoclastic production of cytokines and chemokines (IL-6, IL-1β, CXCL-1, CXCL-2, and CXCL-5) by melanoma cells (52). Wang and colleagues (356)

reported that following intra-cardiac injection of B16F10 melanoma cells into mice, regardless of diet, there was a transient, highly significant increase in bone marrow adipocity and serum leptin levels compared to that of age-matched controls. *In vitro*, conditioned medium from melanoma cells promoted differentiation of adipocytes; conversely melanoma cell proliferation was stimulated by exposure to adipocyte-conditioned medium. The authors conclude that adipocytes may contribute to the lytic bone disease caused by melanoma cells (356). However, as tumours grow and induce lytic bone disease the number of adipocytes is rapidly reduced (356). This decrease in the number of adipocytes may result from a lipolysis that fuels tumor cells with adipocyte-derived fatty acids, thereby promoting tumor growth (140).

Collectively, the experimental studies described above represent the increasing volume of data providing a strong link between bone marrow adipocytes and the progression of bone metastasis, however evidence from human disease to support this remains surprisingly limited. A relatively small retrospective study of 2,731 patients with early breast cancer found no significant link between body mass index and the subsequent pattern of metastasis, but in agreement with other reports, obese and owerweight patients had significantly shorter survival compared to the normal weight group (375). As levels of obesity are rising, including in young people, there are concerns that we are facing an increase in almost all cancers, as well as the potential for more aggressive metastatic disease, including in bone, driven by some of the mechanisms described in this section.

<u>Adipocytes and bone metastasis: current understandings & open questions</u>

- Bone marrow fat is a hormone-sensitive endocrine tissue with the potential to modify bone mass and to influence skeletal tumour growth through provision of energy and tumour cell survival factors, as well as through generation of a pro-inflammatory environment.

- Solid evidence from clinical studies for a link between obesity and bone metastasis is lacking, this important area should be the focus of retrospective analyses of large datasets that include detailed information about patient BMI and metastatic sites.

VIII. FUELING EXPANSION - REPROGRAMMING ENERGY METABOLISM TO FACILITATE BONE METASTASIS PROGRESSION

Metastatic tumor cells colonizing distant organs must rewire their biology in order to grow in the colonized organ (1,133, 271, 291). Most cancer cells use glycolysis (an oxygen-independent metabolic pathway) for glucose metabolism even when oxygen is sufficient (133). This phenomenon is called "the Warburg effect" or "aerobic glycolysis". Consequently, glucose is utilized for ATP generation through lactate production and *via* the pentose phosphate pathway (PPP) for nucleotide synthesis that is essential for cell proliferation (Figure 7). Not only does the Warburg effect allow cells to maintain ATP levels but also reduce oxidative stress and generation of ROS, enabling cancer cells to survive at the metastatic site (291). The existence of these metabolic adaptation mechanisms in cancer cells has been observed *in situ* in patients with bone metastasis (262), as visualized by ^{18}F-FDG-PET scanning of breast cancer bone metastases (Figure 8).

In order to increase glucose uptake, cancer cells upregulate glucose transporters, notably glucose transporter 1 (GLUT1), phosphoglycerate kinase and lactate dehydrogenase A (133). These enzymes of the glycolytic pathway, including phosphoglycerate kinase and PPP-associated proteins, such as 6-phosphogluconolactonase, were observed to be highly expressed in osteotropic breast cancer cells and bone metastases from patients with breast cancer (48). Moreover, osteotropic MDA-MB-231 breast cancer cells produce large amounts of lactate, compared to non-osteotropic ones (197). Lactate is released from MDA-MB-231 cells by monocarboxylate transporter 4 (MCT4) and uptaken by osteoclasts through the transporter MCT1, which then fuels their oxidative metabolism and promotes osteoclast-mediated bone resorption (197) (Figure 7). These experimental findings are supported by immunohistochemical analysis of a small number of human breast cancer bone metastasis specimens

(n = 4) showing a strong staining for MCT4 in tumor cells at the bone metastatic site (197). In prostate cancer bone metastasis, bone marrow adipocytes promote aerobic glycolysis in tumor cells *in vitro* and *in vivo* by up-regulating *HIF-1*α (87). In turn, HIF-1α stimulates the expression of proteins involved in glucose uptake, such as GLUT1, and glycolytic genes, such as phosphoglycerate kinase and lactate dehydrogenase A (87). Thus, aerobic glycolysis seems to be key in supporting skeletal tumor growth and osteolysis, at least experimentally. This metabolic adaptation system of cancer cells in bone also occurs in breast cancer patients with bone metastasis (262), as judged by FDG-PET (Figure 8). Although some clinical studies suggest that FDG has a higher sensitivity for detecting bone metastasis than primary lesions in prostate cancer, FDG remains however of limited use in this cancer type when compared to imaging agent ^{18}F-fluorocholine (FCH), thereby suggesting prostate cancer cells also use nonglucose metabolic pathways to thrive in colonized organs (352).

Beside aerobic glycolysis, cancer cells in bone can use additional sources of energy. In the previous section we have discussed the contribution of bone marrow adipocytes, which can provide free fatty acids as an energy source for tumor cell survival and growth. In addition, the cell membrane phospholipid choline can be abnormally metabolized and internalized in tumor cells overexpressing choline kinase (352). This abnormal regulation of the phospholipid metabolism has been observed *in situ* in cancer cells metastatic to bone (290, 348), as visualized by FCH-PET scanning of prostate cancer bone metastases (Figure 8). Autophagy could be another potential source of energy for tumor cells in bone (233). For example, the small-GTPase Rab5a and Runx2 stimulate autophagosome trafficking in human osteotropic metastatic breast cancer cells (224, 324). However, further studies are clearly needed to understand how autophagy facilitates bone metastasis formation. Of note, cancer cells transport a significant portion of glucose-derived pyruvate into mitochondria where it serves as an anaplerotic substrate to replenish tricarboxylic acid (TCA) cycle intermediates used for the biosynthesis of fatty acids and cholesterol as well as protein acetylation (1) (Figure 7). In this respect, there is experimental evidence that this mitochondrial metabolism can be used as a source of energy for

prostate cancer cells in bone (331). High levels of cholesterol in human prostate cancer bone metastasis specimens were observed, compared to normal bone (331). In addition, immunohistochemistry shows intense staining of the low-density lipoprotein receptor and variable levels of the scavenger receptor class B type 1 and 3-hydroxy-3-methylglutaryl-coenzyme reductase in prostate cancer cells that are metastatic to bone, thereby indicating possibilities for influx and *de novo* synthesis of cholesterol (331).

Taken together, these studies provide evidence that aerobic glycolysis and/or abnormal phospholipid and mitochondrial metabolism in tumor cells may contribute to skeletal tumor burden and bone destruction *in vivo*.

Reprogramming energy metabolism: facts & open questions

- There is substantial evidence from model systems and human studies that energy metabolism is disrupted to favour glycolysis in breast cancer bone metastases. However, further work is required to validate the importance of aerobic glycolysis in other model systems of cancer and bone metastasis, and in patients with advanced cancer and bone metastasis other than breast cancer.
- The importance of other mechanisms (autophagy, increased cholesterol synthesis) and the potential for therapeutic targeting of energy metabolism to inhibit bone metastasis needs to be established in model systems in order to determine the implications for human disease.

IX. BLOCKING BONE DECONSTRUCTION - CURRENT THERAPIES FOR THE TREATMENT OF BONE METASTASIS

In general, the treatment of bone metastases is aimed at palliating morbidity associated with skeletal lesions. It cures only rarely (*e.g.*, in lymphoma) and treatment varies depending on the tumor type. The treatment of bone metastasis includes external beam radiotherapy, systemic therapy with

cytotoxic antineoplastic drugs (chemotherapy) and endocrine agents, targeted therapies and targeted radionucleotide therapy. In addition, orthopedic intervention may be necessary for impending pathological fractures. Optimal management of skeletal metastases requires a multimodality approach that involves the combined expertise of medical and radiation oncologists, interventional radiologists, nuclear medicine and orthopedic oncologists, general physicians, palliative medicine specialists and the symptom control team (69, 72). Treatment decisions depend on whether the bone disease is localized or widespread, the presence or absence of extra-skeletal metastases, and the nature of the underlying malignancy. Systemic therapy for bone metastases can be directed against the tumor cell to reduce both cell proliferation and, in consequence, the production of cytokines and growth factors influencing bone cell function. Alternatively, systemic treatment is directed toward blocking the effect of these substances on host cells. Chemotherapy, biologically targeted agents, and endocrine treatments have direct antitumor effects, whereas bone targeted agents (BTA) such as the bisphosphonates and denosumab are effective by preventing host cells (primarily osteoclasts) from reacting to tumor products.

In the past two decades BTA have become established as a valuable additional approach to the range of current treatments (60, 345). Biochemical data indicate that osteoclast-mediated bone resorption is of importance not only in osteolytic bone metastases such as in breast and lung cancer but also in prostate cancer osteoblastic lesions, with values of resorption markers in the latter at least as high as those seen in breast cancer and other solid tumors (*see section X for further discussion*). As a result, the osteoclast is a key therapeutic target for skeletal metastases irrespective of the tissue of origin.

BTA provide an additional treatment approach for the relief of bone pain across a range of tumor types, with effects that seem to be independent of the nature of the underlying tumor or radiographic appearance of metastases (367).

Approved BTA for use in oncology include the bisphosphonates, the RANK ligand inhibitor denosumab and bone seeking radionuclides including radium-223, strontium-89 and samarium-153.

As our understanding of the signaling mechanisms between bone cells and tumor cells increases, several new, targeted agents have entered clinical development. These agents include inhibitors of cathepsin K and Src kinase (both key regulators of osteoclast function), mammalian target of rapamycin (mTOR) inhibitors such as everolimus, endothelin antagonists such as atrasentan, several agents targeting TGF beta and various anabolic agents including inhibitors of the WNT signaling pathway.

Below, we describe the progress and future directions of existing bone-targeted therapies and report emerging therapies that have arisen from advances in our understanding of the biology of bone metastases (Figure 9).

A. Inhibiting Bone Resorption by Targeting Osteoclasts

1. Bisphosphonates

The bisphosphonates are pyrophosphate analogs, characterized by a P-C-P–containing central structure rather than the P-O-P of pyrophosphate and a variable R' chain that determines the relative potency, adverse effects, and precise mechanism of action (58). The P-C-P backbone renders bisphosphonates resistant to phosphatase activity and promotes binding to the mineralized bone matrix. The absorption of oral bisphosphonates from the gut is poor, variable, and dramatically inhibited by food intake. After intravenous administration of a bisphosphonate, the kidney rapidly excretes approximately 25% to 40% of the absorbed dose and the remainder binds avidly to exposed bone around resorbing osteoclasts, leading to high local concentrations of bisphosphonate in the resorption lacunae.

During bone resorption, bisphosphonates are internalized by the osteoclast, where they cause disruption of several biochemical processes involved in osteoclast function, ultimately leading to apoptotic cell death. Nitrogen-containing bisphosphonates inhibit farnesyl pyrophosphate (FPP) synthase within the mevalonate pathway that is responsible for events that catalyse post-translational

modification of a number of proteins, including the small guanosine triphosphatases such as Ras and Rho (58). Bisphosphonates that do not contain nitrogen, such as clodronate, induce osteoclast apoptosis through the generation of cytotoxic adenosine triphosphate analogs. The biological half-life of bisphosphonates is long with effects after a single dose still detectable several years later (34, 58).

Based on the results of large randomized controlled trials (see below), BTA have become the standard of care for the treatment and prevention of skeletal complications associated with bone metastases in patients with solid tumors (60, 345). The primary end point of these studies was the influence of bone-targeted treatment on the number of patients experiencing SREs, as well as the time to the first SRE and the rate of SREs as determined by either a simple annual rate or more complex multiple event analysis techniques.

Bisphosphonates for metastatic bone disease

The greatest experience with BTAs from the use of bisphosphonates in the management of bone metastases from breast cancer, where the value of the agents is undisputed. Placebo controlled randomized trials have shown that both the oral agents, clodronate and ibandronate, and the intravenous formulations of pamidronate, ibandronate and zoledronic acid all have useful clinical efficacy.

Pamidronate was the first intravenous bisphosphonate to be systematically evaluated and has clinically important efficacy on skeletal morbidity, quality of life, pain and analgesic use in patients with breast cancer (153, 207). Zoledronic acid is the most potent bisphosphonate available and has been the bisphosphonate of choice in most clinical settings and health care systems around the world for more than a decade. In a placebo-controlled trial of zoledronic acid, the percentage of patients with at least one SRE after one year was significantly reduced from 50% in the placebo group to 30% with zoledronic acid (P = .003) (179). In comparison with placebo, zoledronic acid also significantly delayed the time to first SRE and reduced the overall risk of SREs by 41% (179). Zoledronic acid is somewhat more

effective than pamidronate and oral ibandronate in preventing SREs (9, 278). In a multiple event analysis, zoledronic acid reduced the risk of developing skeletal complications by an additional 20% compared with pamidronate (P = .025) (277). In a randomized comparison of oral ibandronate and intravenous zoledronic acid, the two agents had broadly similar activity although ibandronate did not meet the strict statistical criteria for non-inferiority defined in the study protocol (9).

Bisphosphonates have been shown to reduce bone pain and biochemical markers of bone resorption in patients with osteoblastic bone lesions that are associated with advanced prostate cancer. Despite somewhat disappointing preliminary results with other bisphosphonates, zoledronic acid was investigated in patients with CRPC and bone metastases and showed that the increased potency of this compound translated into improved clinical benefit (283). Treatment with zoledronic acid reduced the overall risk of skeletal complications by 36% and extended the time to first skeletal complication by more than 4 months. Bone pain was also reduced at all time points (283).

The pathophysiology of bone metastases is broadly similar in all tumor types, and BTA could thus be expected to be of value in preventing skeletal morbidity across the range of tumors involving the skeleton, especially if metastatic bone disease was a patient's dominant site of disease. As part of the development program for zoledronic acid, a phase-III randomized, placebo-controlled trial was performed in patients with bone metastases from a wide range of solid tumours other than breast or prostate cancer; more than half of the persons recruited had lung cancer (279, 280). Zoledronic acid significantly reduced the proportion of patients with at least one SRE, almost doubled the time to the first SRE compared with placebo and reduced the overall risk for SRE(s) by about 30% compared with placebo.

There remains uncertainty regarding the most appropriate duration and schedule of treatment. Bone targeted therapy should certainly not be stopped following the development of a first skeletal related event whilst on treatment; this should not be considered a failure of treatment, as the trials demonstrate a significant reduction in second and subsequent complications with continued treatment.

Several trials have investigated the schedule of bisphosphonate treatment and suggested that the efficacy of 3 monthly and monthly administration of zoledronic acid is similar. For example, the CALGB 70604 (Alliance) trial, randomized patients with bone metastases from a range of different primary tumor types to zoledronic acid on a monthly or three-monthly schedule from the outset of treatment for two years (145). This study demonstrated non-inferiority of less frequent administration; in both arms, 29% of patients developed ≥1 SRE and suggests that three monthly administration of zoledronic acid is a reasonable choice for most patients (145).

Bisphosphonates for prevention of cancer treatment induced bone loss

There are now increasing numbers of long-term survivors from cancer who have received combination chemotherapy, radiotherapy, and hormonal cancer treatments. Many of these survivors are at increased risk of osteoporosis, largely because of the endocrine changes induced by treatment. Cancer treatment–induced bone loss (CTIBL) is a particularly important long-term problem for women with breast cancer and men receiving androgen deprivation therapy (ADT). For example, the fracture incidence in women with breast cancer on an aromatase inhibitor was found to be around 18–20% after 5 years follow-up suggesting that about one in five women on an aromatase inhibitor without a bone protective agent will sustain a fracture (121).

More than half of cases of prostate cancer occur in men over the age of 70 and thus many men with prostate cancer are at risk of osteoporosis, exacerbated by the ADT many will receive as treatment for their cancer. ADT reduces serum concentrations of testosterone to less than 5% of normal level and estrogen to less than 20% of normal level with consequent adverse effects on bone turnover and an increase in fracture rate, as clearly demonstrated by large retrospective epidemiological studies (296). In addition, ADT affects muscle mass, making falls more likely.

The effects of both bisphosphonates and denosumab (*see next section*) on CTIBL have been

studied in multiple randomized clinical trials. These studies have used dosing regimens that are similar, but not necessarily identical to those used for the treatment of age-related osteoporosis. In breast cancer, zoledronic acid is the most comprehensively studied bisphosphonate. In premenopausal women, zoledronic acid (4 mg IV every 6 months) prevented the bone loss associated with ovarian function suppression (OFS) and tamoxifen or an aromatase inhibitor whereas in the control group reductions in BMD at 3 years were around 5% and 10% with OFS plus tamoxifen and anastrazole respectively (123). In postmenopausal women, three companion trials (Z-FAST, ZO-FAST, E-ZO-FAST) compared the efficacy of a similar dosing schedule of zoledronic acid given either in conjunction with initiation of an aromatase inhibitor (immediate group), or if required due to a decline in BMD during adjuvant aromatase inhibitor therapy to a T-score<–2.0 at any site or a non-traumatic fracture (delayed group). At 5 years all three trials reported similar results with 7-9% and 4-6% differences in lumbar spine and hip BMD respectively between the two treatment arms in favor of zoledronic acid (38, 62, 209). None of these studies were designed to show a significant difference in fracture incidence between the treatment arms. Nevertheless, the BMD effects are similar to those seen in trials performed in postmenopausal osteoporosis in which bisphosphonates confer a relative risk reduction (RRR) of 45% for vertebral fractures and approximately 16% RRR for non-vertebral fractures (94).

Several other randomized clinical trials have investigated the efficacy of oral bisphosphonates for preventing CTIBL in breast cancer (127). The numbers of patients included in each study is somewhat less than for the zoledronic acid studies and thus, unlike for other forms of osteoporosis, the evidence for efficacy of oral bisphosphonates in this specific setting is less robust. Indirect cross trial comparisons suggest the increases in BMD with oral regimens are somewhat less than with zoledronic acid or denosumab, especially in younger women receiving ovarian function suppression (OFS) or experiencing chemotherapy induced menopause. Again, none of the trials with oral agents were designed to assess reliably the impact of oral bisphosphonates on fracture risk.

Alendronate, risedronate, pamidronate, and zoledronic acid have all been shown to prevent loss

in BMD in patients with prostate cancer but the studies have been small and, while the preservation of BMD would argue for a favorable effect on fractures, the magnitude of effect cannot be reliably assessed (144).

Overall, current guidelines for preventing CTIBL suggest that patients having adjuvant endocrine treatment should be managed according to risk of fracture (60, 127). Patients with a T-score of greater than -2 and no additional risk factors for fracture are advised to exercise and receive calcium and vitamin D, with risks and BMD monitored every one-two years. If the T-score is less than -2, or there are two or more risk factors for fracture, patients should receive the same advice and supplements plus a bisphosphonate or denosumab (*see next section*). Guidelines recommend continuing anti-resorptive therapy for as long as the patient is receiving endocrine treatment.

Disease modifying effects of bisphosphonate treatments

The potential benefits of bone-targeted treatments on the clinical course of breast cancer in terms of prevention of recurrence and death from breast cancer have been an area of intense study over the past 20 years. In breast cancer patients with no sign of distant metastases, but having a minimal residual disease in the bone marrow, CTIBL leads to the release of bone-derived growth factors from resorbed bone that, in turn, may activate DTCs from a dormant to a proliferative state and trigger bone relapses. Of note, adjuvant zoledronic acid treatment of patients with early breast cancer improves elimination of DTCs (307). Bisphosphonates exert, at least experimentally, a variety of direct and indirect anticancer activities (58, 312). Moreover, bisphosphonates, by decreasing bone resorption, may also make the bone microenvironment less hospitable for tumor cells, thereby explaining the elimination of DTCs. These findings suggested a greater role for the use of bisphosphonates than has previously been considered. Individual trials provided varying results that suggested benefits were restricted to women who had low levels of reproductive hormones due to either natural age-related menopause or ovarian function suppression. This hypothesis was confirmed by the Early Breast Cancer Trialists'

Collaborative Group (EBCTCG) meta-analysis of individual patient data from >18,000 breast cancer patients included in randomized trials of adjuvant bisphosphonates. The meta-analysis showed that adjuvant bisphosphonates (intravenous zoledronic acid, oral clodronate and oral ibandronate) only reduced breast cancer recurrences and breast cancer deaths in postmenopausal women (93). Overall, across all age and menopausal groups, despite a reduction in bone metastases, adjuvant use of a bisphosphonate had no significant effect on breast cancer recurrence (rate ratio (RR)=0.94) and the effect on breast cancer mortality, though statistically significant, was small (RR=0.91) (93). However, in postmenopausal women or those receiving ovarian suppression with goserelin, clinically important benefits were seen with statistically significant improvements in overall breast cancer recurrence (RR=0.86), distant recurrence at any site (RR=0.82), bone recurrence (RR=0.72) and breast cancer-specific mortality (RR=0.82) (123). This equates to prevention of more that 1 in 6 breast cancer deaths at 10 years. Several international guidelines now recommend the use of adjuvant bisphosphonates in postmenopausal early breast cancer, especially for those at moderate to high for recurrence (86, 128).

Understanding why the benefits of adjuvant bisphosphonates appear restricted to postmenopausal women is a priority area for further research. There does not appear to be a link between bone resorption rates and treatment efficacy (33). On the other hand, more detailed evaluation on the primary tumor may help identify patients who will benefit from an adjuvant bisphosphonate. For example, a study demonstrated that patients with a MAF negative tumour (79% of all patients), evaluated using a FISH assay for the transcription factor, had significantly improved survival at 10 years and a lower relapse rate with the use of adjuvant zoledronic acid (64). On the other hand, in the 21% of women with tumors that over-express MAF no benefits were seen in this subset of patients treated with an adjuvant bisphosphonate and, in younger patients, disease outcomes were significantly worse (64).

For reasons that remain unclear, the disease modifying effects of bisphosphonates in postmenopausal breast cancer have not been seen in men with prostate cancer treated with ADT. In the randomized controlled STAMPEDE trial, the addition of zoledronic acid alone or in combination with

docetaxel chemotherapy to ADT for men with advanced prostate cancer did not improve survival, despite extending the time to first skeletal complication (157). By contrast, docetaxel showed evidence of survival improvement when combined to ADT (157).

2. Anti-RANKL antibody: Denosumab

Therapeutic candidates to inhibit RANK/RANKL interaction were developed. Fusion proteins were initially engineered. These are recombinant proteins comprising the Fc portion of human IgG1 fused with the N-terminal ligand binding cysteine-rich domain (CRD) of OPG (OPG-Fc/AMGN-007 and Fc-OPG) or the four extracellular CRDs of RANK (RANK-Fc) (183). Both Fc-OPG and RANK-Fc potently inhibit bone resorption in preclinical models of osteoporosis and of cancer and bone metastasis (183, 312). However, following repeat dosing of human RANK-Fc in primates, autoantibodies were detected (183). This highlighted the potential risk of an immune response against endogeneous RANK or OPG in patients, when using RANK-Fc or AMGN-007, respectively. An anti-RANKL antibody approach was therefore preferred, which led to the development of denosumab.

Denosumab is a fully human, synthetic antibody that binds to RANKL with high affinity, thereby preventing its interaction with RANK in a way similar to that of OPG (183). Denosumab is administered by subcutaneous injection. The biological half-life of denosumab is only weeks compared to the months or years seen with bisphosphonates (34). Rebound osteolysis may occur following discontinuation of denosumab with accelerated bone loss and, in a few patients, an increased incidence of vertebral fractures 12-36 months after treatment cessation (334).

Denosumab for metastatic bone disease

Denosumab has been shown to be superior to zoledronic acid for the prevention of SREs from breast cancer. 2046 patients were randomly assigned to receive four weekly subcutaneous injections of denosumab (120 mg) or intravenous zoledronic acid (4 mg), with supplements of calcium and vitamin D.

1681 Denosumab was statistically superior to zoledronic acid in delaying the first SRE (315). Overall,
1682 denosumab treatment delayed the occurrence of all types of SREs. However, no differences in survival
1683 or investigator-reported disease progression were found between the two treatment groups.
1684 Denosumab was investigated in patients with CRPC and bone metastases and showed that in a
1685 randomized trial versus zoledronic acid, this compound was statistically superior to the bisphosphonate
1686 in delaying the first SRE and the overall risk of SREs (105).
1687 Denosumab has also been studied in advanced solid tumors other than breast and prostate
1688 cancers. Non-inferiority to zoledronic acid was demonstrated with a trend to better control of skeletal
1689 morbidity (139).
1690
1691 *Denosumab for prevention of cancer treatment induced bone loss*
1692 Denosumab is the only agent to have a specific license for CTIBL following large randomised
1693 trials in postmenopausal women with breast cancer receiving an aromatase inhibitor and in men with
1694 prostate cancer receiving ADT (121, 305). In both studies, fracture incidence was the primary endpoint.
1695 The ABCSG-18 trial compared adjuvant denosumab (60 mg by subcutaneous injection given twice a
1696 year) with placebo (both with calcium and Vitamin D supplements) in 3425 postmenopausal women
1697 receiving adjuvant aromatase inhibitor treatment (121). Women treated with denosumab had a 50%
1698 (95% CI 39–65%, $p<0.0001$) risk reduction for any clinical fracture. The fracture risk reduction appeared
1699 to be irrespective of age and baseline BMD. Furthermore, the disease-free survival (secondary
1700 endpoint) was significantly improved in the denosumab group (HR=0.82, 95% CI 0.69 – 0.98, $p = 0.026$)
1701 compared to placebo group (122). In a placebo-controlled trial of denosumab in 1468 men receiving
1702 ADT for non-metastatic prostate cancer, 36 months of denosumab treatment was associated with a
1703 significantly reduced incidence of new vertebral fractures (1.5% with denosumab *vs*. 3.9% with placebo;
1704 relative risk [RR] 0.38; 95% CI 0.19-0.78). BMD increased from baseline at all sites in the denosumab
1705 group, whereas it declined in the placebo group (305).

As aforementioned for bisphosphonates, current guidelines for preventing CTIBL recommend continuing anti-resorptive therapy for as long as the patient is receiving endocrine treatment. Patients treated with denosumab may need additional bone protection with a bisphosphonate when denosumab is discontinued to prevent rebound osteolysis and the increased risk of multiple vertebral fractures associated with treatment withdrawal (334).

Disease modifying effects of denosumab treatment

The disease modifying effects of denosumab have also been assessed in early breast cancer but this agent, at least when given in the intensive schedule selected in the adjuvant D-CARE study, had no effect on disease recurrence in either pre- or postmenopausal women (63). The osteoporosis schedule of denosumab has a beneficial effect on the underlying disease, as observed in the ABCSG-18 study (121,122). However, no survival benefits have been yet seen and the agent is therefore only recommended for fracture prevention (122). Of note, the presence of RANK-positive CTCs in the bloodstream of metastatic breast cancer patients (n = 20/42) is associated with a better response to denosumab therapy with respect to time to first SRE [HR=0.25 (0.1 – 10.62), P = 0.0012)], compared to metastatic patients with RANK-negative CTCs (n = 22/42) (254). It would be interesting to determine if RANK expression in CTCs could help identify high-risk early-stage breast cancer patients who might benefit from adjuvant denosumab. Similarly, a *post-hoc* analysis of the D-CARE trial will be conducted to determine if the expression of transcription factor MAF in primary tumors will help clarify the potential anticancer mechanism of denosumab (122) (*see section X-C for further discussion*).

Denosumab may have some disease modifying effects in prostate cancer: 1432 men with non-metastatic CRPC who were at high risk for bone metastasis by virtue of either a PSA of ≥8.0 ng/mL and/or PSA doubling time ≤10.0 months were randomized to receive monthly denosumab, 120 mg, or placebo in addition to continuation of ADT. Denosumab significantly increased bone metastasis-free survival by a median of 4.2 months compared with placebo and delayed the time to symptomatic first

bone metastases (306). However, this effect on the disease process did not translate into an improvement in overall survival and, in light of the relatively high cumulative incidence of ONJ (4% at 3 years), the marginal benefits were not considered sufficient to change clinical practice.

The RANK/RANKL pathway has been shown to play a crucial role in the initiation and progression of inherited breast cancer caused by mutation in the tumor-suppressor gene breast cancer 1 (*BRCA1*) (269). *BRCA1* mutation carriers have a greater propensity to generate cancer stem cells, whose expansion is RANKL/RANK dependent, and denosumab inhibits the expansion of these cancer stem cells *in vitro* (269). Therefore, these findings strongly suggest that targeting the RANK/RANKL pathway could be beneficial for the prevention of breast cancer in *BRCA1* mutation carriers. Two pilot studies are currently evaluating the biological effects of denosumab on Ki67 proliferation index (primary endpoint) in normal breast and fallopian tube fimbrial tissues from *BRCA1* and *BRCA2* mutation carriers (ACTRN12614000694617 and ClinicalTrials.gov NCT03382574 studies). Furthermore, a randomized, double-blind, placebo-controlled, multi-center, international phase 3 study will determine if denosumab can prevent breast cancer development in women carrying a BRCA1 germline mutation (ABCSG-50, EudraCT number 2017-002505-35; estimated number of subjects to be enrolled in the study: 2,918).

3. Novel antiresorptive agents

LGR4/RANKL and small-molecule RANKL inhibitors

The leucine-rich repeat-containing G-protein-coupled receptor 4 (LGR4) has recently been identified as a RANKL receptor that negatively regulates osteoclast differentiation (215). LGR4 competes with RANK to bind RANKL and suppresses canonical RANK signaling during osteoclast differentiation (215). A soluble LGR4 extracellular domain (ECD), which binds to RANKL, was examined in animal models of osteoporosis. LGR4-ECD notably increased bone mass and inhibits osteoclast differentiation *in vivo* (215). Interestingly, LGR4-ECD had little physiological effect on osteoclast

differentiation in normal mice, which suggests that LGR4-ECD could antagonize excessive RANKL in benign and malignant osteoclast-related diseases with few side effects.

The efficacy of a small-molecule RANKL inhibitor (AS2676293) has been tested in an animal model of bone metastasis (238). Oral administration of AS2676293 to animals inhibits formation of osteolytic lesions caused by MDA-MB-231 breast cancer cells. *In vitro*, AS2676293 inhibits osteoclastogenesis.

These antiresorptive agents are still at a preclinical stage of development.

Cathepsin K inhibitors

Cathepsin K is a lysosomal cysteine protease highly expressed in osteoclasts, which degrades collagen during bone resorption. Cathepsin K inhibitors (AFG-495, L-235) reduce bone destruction and skeletal tumor burden in animal models of breast cancer bone metastasis (92, 196), providing a direct proof for causal role of cathepsin K in bone metastasis formation. Additionally, metastatic tumor cells in bone express cathepsin K and AFG-495 dose-dependently inhibits breast cancer cell invasion *in vitro*, but not tumor growth *in vivo*. These results suggest that cathepsin K inhibitors in the treatment of bone metastasis could potentially have a dual effect, inhibiting both osteoclast-mediated bone resorption and, to a less extent, tumor burden (196). Interestingly, a phase II trial in women with breast cancer and bone metastases showed that cathepsin K inhibitor odanacatib (which is structurally related to L-235) successfully reduced circulating levels of bone resorption markers after 4 weeks of treatment (160). Similarly, a phase-II trial in postmenopausal women with osteoporosis showed that odanacatib therapy was effective at inhibiting bone resorption and increasing bone mineral density (186). A large phase III trial, Long-Term Odanacatib Fracture Trial (LOFT), enrolling 16,713 participants with osteoporosis from 387 centres was therefore conducted (24). However, development of the agent has been discontinued due to possible cardiovascular adverse events. A phase III trial assessing the efficacy of odanacatib in

reducing the risk of bone metastasis in women with breast cancer (ClinicalTrials.gov identifier NCT00692458) has also been withdrawn for undisclosed commercial reasons.

Mammalian target of rapamycin (mTOR) inhibitors

In bone physiology, RANKL and M-CSF promote osteoclast survival by signalling through mTOR (19). Rapamycin and everolimus (a rapamycin analog) are both mTOR inhibitors that block osteoclast differentiation *in vitro* and suppress bone resorption in an animal model of bone loss caused by ovariectomy (19, 36). In addition to their antiresorptive effects, mTOR inhibitors exhibit anti-cancer effects. Everolimus and temsirolimus were the first mTOR inhibitors to be approved in the treatment of advanced breast and renal cell cancers. Interestingly, everolimus and temsirolimus inhibit skeletal tumor burden and osteolysis in animal models of bone metastasis caused by breast and renal cell carcinoma, respectively (36, 343). Everolimus combined with aromatase inhibitor exemestane has been approved for patients with advanced hormone receptor-positive/HER2-negative breast cancer who progress on prior nonsteroidal aromatase inhibitor therapy with either letrozole or anastrozole (BOLERO-2 study). In this study, the progression-free survival was significantly improved in the everolimus plus exemestane arm, compared to the placebo plus exemestane arm (11). Because exemestane is known to increase bone turnover, an exploratory analysis in the BOLERO-2 study has been conducted in patients with or without bone-only disease (120, 152). Compared to placebo plus exemestane, everolimus plus exemestane increased the median progression-free survival (5.3 and 12.9 months, respectively), regardless of bisphosphonate use and presence of bone metastases at baseline, indicating a 67% reduction in the risk of progression (120, 152). In addition, bone marker levels increased with exemestane monotherapy, but decreased when used in combination therapy with everolimus (120). Overall, these results suggest that everolimus plus exemestane decreases disease progression in the bone, probably by suppressing increased bone turnover observed with exemestane monotherapy in addition to the greater antitumor effects of the combination.

Src non-receptor tyrosine kinase inhibitors

Src is one of eleven members of a family of non-receptor tyrosine kinases that interact with several protein-tyrosine kinase receptors, G-protein-coupled receptors and integrins, which are expressed at the plasma membrane (281). c-Src plays multiple roles in regulating cell proliferation, survival, adhesion, migration, invasion, metastasis, and angiogenesis (281). Although Src is ubiquitously expressed in vertebrate cells, much higher protein levels are found in osteoclasts, platelets and neurons than most other cells. Of note, the most noticeable phenotype of Src knock-out mice is osteopetrosis (enhanced bone mass) as a result of osteoclast dysfunction (281). In fact, following integrin $\alpha v\beta 3$ activation, Src phosphorylation regulates the organization of osteoclast's actin cytoskeleton, which enables the osteoclast to attach and spread to bone and optimally resorb bone. Additionally, following RANK/RANKL interaction, Src activation triggers signaling through the PI3K/AKT/mTOR pathway and promotes osteoclast survival (312). As exemplified in experimental bone metastasis, the injection of cancer cells in Src knock-out mice shows that these animals are protected from tumor-associated bone destruction because Src-defective osteoclasts do not resorb bone (361). Thus, Src plays a central role in osteoclast function. In addition, elevated expression and activity of c-Src have been reported in a variety of cancers. Three Src inhibitors (dasatinib, saracatinib and bosutinib) underwent clinical studies in patients with cancer and (bone) metastases (312). To date clinical development has yielded disappointing results in the setting of solid tumors and bone metastases.

RON receptor tyrosine kinase inhibitor.

RON is a receptor tyrosine kinase receptor expressed by osteoclasts (4). Tumor-derived MSP promotes the formation of osteolytic lesions in an animal model of breast cancer bone metastasis, whose extent is inhibited upon treatment of metastatic animals with RON tyrosine kinase inhibitor BMS-777607/ASLAN002 (4). A phase-I trial in postmenopausal women with advanced cancer shows that

BMS-777607/ASLAN002 reduced bone resorption after 4 weeks of treatment (ClinicalTrials.gov identifier NCT01721148).

Dual c-MET and VEGFR2 receptor tyrosine kinase inhibitor (cabozantinib)

Receptor tyrosine kinases c-MET and vascular endothelial growth factor (VEGF) receptor 2 (VEGFR2) and their respective ligands, hepatocyte growth factor (HGF) and VEGF, are expressed by both osteoblasts and osteoclasts, enabling the activation of autocrine and paracrine HGF/c-MET and VEGF/VEGFR2 signaling pathways for the regulation of osteoblast and osteoclast activities (194). Additionally, c-MET and VEGFR signaling facilitates tumor progression through the activation of multiple pathways (PI3K/AKT/mTOR, SRC, MAPKinases) (194). Dual tyrosine kinase inhibitors that target both VEGFR2 and c-MET (cabozantinib and TAS-115) were therefore developed and investigated in pre-clinical and clinical settings (194).

Cabozantinib inhibits human osteoclast differentiation and osteoclast-mediated bone resorption and increases OPG production by human osteoblasts *in vitro* (104). In animal models of prostate cancer, cabozantinib decreased skeletal tumor burden and formation of osteoblastic lesions, indicating that this compound suppresses bone metastasis formation, at least in part, through inhibition of bone remodeling (79, 192). Similarly, TAS-115 inhibits human PC-3 prostate cancer bone metastasis formation and suppresses bone destruction (360). The effect of cabozantinib was therefore investigated in the treatment of prostate cancer patients with bone metastasis.

In phase-II studies, cabozantinib treatment of metastatic CRPC patients with bone metastases showed a remarkable 68% rate of normalization of bone scans and suggested disease benefits with prolongation of progression-free survival (194). However in the phase-III trial COMET-1, comparing cabozantinib with prednisone in patients with metastatic CRPC and bone metastases following prior treatment with docetaxel and either abiraterone or enzalutamide, although cabozantinib improved progression-free survival and time to first SRE, it failed to improve overall survival, the primary end point of the trial (304). In the light of these data, further development of cabozantinib in prostate cancer has

been halted and a second trial (COMET-2) comparing cabozantinib with mitoxantrone plus prednisone in a similar patient population as COMET-1 closed prematurely. It is likely that the dramatic effects on bone scan appearances seen in the initial studies reflected the direct effects of cabozantinib on bone cell function and skeletal blood flow rather than effects on the underlying malignant disease.

Cabozantinib is approved for the treatment of patients with advanced renal cell carcinoma after previous antiangiogenic therapy on the basis of significant improvements in progression-free survival and overall survival when compared with everolimus (phase-3 METEOR trial). Pre-specified analyses of progression-free survival and overall survival were conducted in a sub-group of patients with bone metastasis from the METEOR trial (99). Compared to everolimus, cabozantinib treatment was associated with a significant improvement of progression-free survival and overall survival in patients with bone metastases, indicating it is a good treatment option for these patients.

miR-34a mimic (MRX34)

MiR-34a is a critical suppressor of osteoclastogenesis and bone resorption by directly targeting the pro-osteoclastic factor Tgif2 (transforming growth factor-β-induced factor 2) (181). Its expression is therefore downregulated during osteoclast differentiation. The pharmacological administration of a miR-34a mimic delivered in nanoparticles (whose aim is to replenish the lost miRNA expression) can attenuate bone metastases in animals bearing breast or skin tumours (181). A phase I, open-label, multicenter, dose-escalation study investigated the safety, pharmacokinetics and pharmacodynamics of a miR-34a mimic (MRX34) encapsulated in lipid nanoparticles, in patients with unresectable primary liver cancer or advanced or metastatic cancer with or without liver involvement or hematologic malignancies (15). However, the clinical trial was terminated prematurely due to cases of immune-related serious adverse events (ClinicalTrials.gov identifier NCT01829971).

BET inhibitor

The bromodomain and extraterminal (BET) protein family (BRD2, BRD3, BRD4 and BRDT) is an important class of chromatin readers, regulating chromatin accessibility to transcription factors and RNA polymerase. JQ1, a thienotriazolo-1,4-diazapine that binds selectively to BET bromodomain proteins, inhibits osteoclast differentiation by interfering with BRD4-dependent RANKL activation of *NFATC1* transcription (185). Moreover, JQ1 inhibits bone resorption in experimental models of malignant osteolytic lesions and osteoporosis (12, 185). JQ1 is still at a preclinical stage of development.

Dock5 inhibitor

Dock5 (Dedicator of cytokinesis 5), a guanine nucleotide exchange factor for the small GTPase Rac, participates to the formation of the sealing zone in osteoclasts. C21, a chemical inhibitor of Dock5, reduces osteoclast-mediated bone resorption *in vitro* and blocks bone destruction in a melanoma model of bone metastasis *in vivo* (349). C21 is still at a preclinical stage of development.

Jagged/Notch inhibitor

In bone metastasis, tumor-derived Jagged1 activate Notch signaling in osteoclast precursors, promoting osteoclast differentiation and bone resorption (295). Tumor-derived Jaddged1 also engages Notch signaling in osteoblasts, stimulating IL-6 production. In turn, IL-6 secreted from osteoblasts stimulates tumor growth (295). Therefore, a fully human monoclonal antibody (15D11) against Jagged-1 has been developed. 15D11 inhibits bone metastasis formation in animals and sensitizes bone metastases to chemotherapy (390). 15D11 is still at a preclinical stage of development.

B. Promoting Bone Formation by Targeting Osteoblasts

1. Agents blocking WNT inhibitors

Anti-DKK1 (BHQ880 and DKN-01) and anti-SOST (blosozumab, BPS804 and romosozumab) antibodies have been developed to block the inhibitory effect of Wnt antagonists DKK-1 and SOST on osteoblast-mediated bone formation (71, 170, 227, 395). BHQ880 and DKN-01 are in phase I/II clinical

trials for patients with multiple myeloma and other solid tumors, such as cholangiocarcinoma, esophageal cancer and gastric cancer, whereas romosozumab, blosozumab and BPS804 are in phase II/III clinical trials for osteoporosis (170). In a phase III trial romosozumab decreased the risk of vertebral fractures in postmenopausal women with osteoporosis (36% lower risk with romosozumab than placebo) (71). Experimentally, an anti-SOST antibody decreased the extent of osteolytic lesions in a mouse model of breast cancer bone metastasis and multiple myeloma (227, 395). However, up to know, there is no clinical study investigating the effect of anti-SOST antibodies in cancer-induced bone diseases.

Of note, the inhibition of one of these two WNT inhibitors (DKK1 and SOST) may engender a compensatory response in order to return to a steady state (106). By contrast, a bispecific antibody targeting SOST and DKK-1 (Hetero-DS) leads to synergistic bone formation in rodents and non-human primates (106). Thus, Hetero-DS could have a valuable role in increasing bone mass and improving healing of lytic bone lesions associated with bone metastasis.

2. Endothelin-1 receptor inhibitors

Preclinical studies have uncovered a prominent role for ET-1 in the formation of osteosclerostic lesions (276, 378). However, phase 3 trials of the inhibitors, atrasentan and zibotentan in combination with docetaxel failed to improve overall survival in patients with metastatic castration-resistant prostate cancer compared with docetaxel alone and this treatment approach seems unlikely to reach the clinic (47, 240).

3. Androgen Inhibitors

Abiraterone acetate is an orally administered selective androgen biosynthesis inhibitor derived from the structure of pregnenolone. It is an irreversible inhibitor of cytochrome CYP17A, resulting in virtually undetectable serum and intratumoral androgen levels (244). Abiraterone was evaluated in chemotherapy-naïve and chemotherapy-treated men with metastatic castration-resistant prostate cancer (COU-AA-301 and COU-AA-302 trials). The data from these two phase-III trials showed that

abiraterone treatment significantly improves overall survival and skeletal outcomes (delay of symptomatic progression and reduction of time to first SRE) (116). These benefits of abiraterone treatment on metastatic bone disease may not only be related to a systemic control of the disease but also associated with a direct effect in the bone. Indeed, abiraterone exhibits direct bone anabolic and anti-resorptive effects (156).

Another promising inhibitor is the androgen receptor antagonist enzalutamide. Enzalutamide was evaluated in chemotherapy-naïve and chemotherapy-treated men with metastatic castration-resistant prostate cancer patients (13, 116). In these two phase-III trials (AFFIRM and PREVAIL), enzalutamide significantly decreased the risk of death and improved skeletal outcomes (time to first SREs and radiographic progression, respectively).

However, despite approval of abiraterone and enzalutamide in metastatic castration-resistant prostate cancer, virtually all patients eventually acquire secondary resistance. One plausible explanation for resistance may involve the presence of the androgen-receptor isoform encoded by splice variant 7 (AR.V7), which lacks the ligand-binding domain, but remains constitutively active as a transcription factor (5).

4. *Activin A inhibitors*

Activin A binds to activin type IIA (ActRIIA) or type IIB (ActRIIB) receptors and induces the recruitment and phosphorylation of an activin type I receptor (ActRIB), which then phosphorylates Smad2 and Smad3 intracellular signaling proteins (312). The treatment of non-human primates with a soluble chimeric protein composed of the extracellular domain of ActRIIA fused to human IgG-Fc (sotatercept, formerly called ACE-011) increased bone volume by decreasing bone resorption and increasing bone formation (212). In animal models of multiple myeloma with osteolytic lesions, the treatment of mice with a soluble activin receptor type IIA fusion protein (ActRIIA.muFc) blocked bone destruction (51). Specifically, ActRIIA.muFc treatment significantly stimulated osteoblastogenesis, prevented myeloma-induced suppression of bone formation, blocked the development of osteolytic

bone lesions and increased survival (51). In the clinic, sotatercept improved bone mineral density and bone formation in multiple myeloma patients (312). These pre-clinical and clinical findings suggest that stimulating osteoblastic bone formation to facilitate bone repair might be an alternative or additional therapeutic approach to the use of antiresorptive agents to treat osteolytic lesions. Although higher serum levels of activin A were reported in breast or prostate cancer patients with bone metastases, compared with those of patients without bone metastases, there are currently no ongoing trials in breast or prostate cancer with bone metastases (312).

C. Targeting the Bone Matrix and the Microenvironment

1. Bone targeted radiopharmaceuticals

The therapeutic use of radioactive-labeled tracer molecules is currently an area of considerable interest and research. Targeted radiotherapy has potential advantages over external beam radiotherapy in that the radiation dose may be delivered more specifically to the tumor and normal tissues may partially be spared unnecessary irradiation (242). Theoretically, it should also be possible to administer high doses of radiation to the tumor on a recurrent basis if necessary.

The α-emitting radiopharmaceutical ^{223}radium dichloride (radium-223, a calcium-mimetic radioisotope) and the β-emitting radiopharmaceuticals ^{89}strontium (strontium-89, a calcium-mimetic radioisotope) and ethylene diamine tetramethylene phosphonate-^{153}samarium (samarium-153, a bisphosphonate-conjugated radioisotope) bind to bone mineral and preferentially to newly formed bone matrix, such as areas of osteosclerostic bone metastatic lesions (59, 312). These radiopharmaceuticals emit radiation causing DNA damage and cell death. Radium-223 almost exclusively produces alpha particles that produce a high-linear energy transfer (LET) with ultra-short penetration (< 100 μm; 2-10 cell diameters) resulting in a highly localized antitumor effect on adjacent bone metastases while limiting damage to the surrounding normal tissue (242). In contrast to radium-223, strontium-89 and samarium-

153 have a low-LET with a penetration range of 3 to 8 millimetres, which results in considerably more dose to normal tissues, notably the bone marrow and this limits the use of these agents and the ability to combine with other treatments (59, 312).

Strontium-89 and samarium-153 are approved for palliation of bone pain (103), but only occasionally used and it is the bone-seeking, alpha particle emitting, radium-223 that is of most relevance to current practice. Radium-223 is now approved for the treatment of bone metastases from CRPC following a placebo-controlled randomized phase-III trial (ALSYMPCA) in which radium-223 increased the survival of patients by 3.6 months and further reduced skeletal morbidity over and above a bisphosphonate (258). Treatment was well tolerated and improved quality of life with no significant long-term toxicities identified (258, 348). Radium-223 has subsequently been studied earlier in the course of metastatic prostate cancer and in combination with other therapies. However, a double-blinded, placebo-controlled randomized phase-III trial (ERA 223; NCT02043678) investigating the efficacy and safety of radium-223 and abiraterone *versus* abiraterone alone in chemotherapy-naïve CRPC patients with bone metastases showed that the combination of radium-223 and abiraterone did not improve either disease or skeletal outcomes compared with abiraterone alone. Furthermore, more bone fractures were observed in the combined treatment arm, particularly in patients not receiving concomitant antiresorptive agents (zoledronic acid, denosumab). In breast cancer, experimental findings showed that radium-223 inhibits skeletal tumor burden and bone destruction in a mouse model of breast cancer bone metastasis (319). A phase IIa study was conducted in breast cancer patients with bone-dominant disease. Radium-223 induced metabolic changes, as judged by a 25% decrease of ^{18}F-fluorodeoxyglucose uptake in osteosclerostic lesions using positron emission tomography and computed tomography (59).

2. Agents targeting nerve- or bone-derived growth factors.

The release of algogenic factors by cancer/stromal cells and osteoclasts can induce sensitization and activation of sensory fibers that innervate the bone. Bisphosphonates and denosumab have been

approved for the treatment of bone pain (100). A phase II study evaluated the safety and efficacy of the anti-NGF antibody tanezumab in patients with painful bone metastases taking daily opioids (308). The data are encouraging and suggest that tanezumab treatment results in sustained analgesic improvements.

The TGFβ signaling pathway plays a critical and dual role in cancer progression. Several inhibitors of TGFβ signaling, such as neutralizing antibodies, antisense oligonucleotides, and receptor kinase inhibitors, have been developed and shown to have inhibitory effects on bone metastases in animal models (40, 101).

X. THE VALUE OF BONE TURNOVER BIOMARKERS IN BONE METASTASIS

As previously discussed in *section I-C*, clinical presentations of bone metastases are highly diverse, and many locations remain asymptomatic. Nowadays, plain radiography is insufficient to correctly identify bone metastases since more than 50% of an affected bone is required to be detected. As a consequence, bone metastases are often diagnosed at the time symptoms occur, increasing the risk of developing SREs, which significantly impair patients' quality of life (64).

In adults, the bone mass is maintained by a continuous bone remodeling, which is a balance between osteoclast-mediated bone resorption and osteoblast-mediated bone formation (76). The turnover between resorption and formation leads to the release of bone-derived molecules that are amenable to measurement in blood and urine (350). Some of these molecules have been used as biochemical biomarkers of bone turnover, reflecting ongoing rates of bone formation or bone resorption (Table 2). Since cancer cells cause a distortion of bone turnover, several clinical studies examined whether variations in the expression levels of these bone biomarkers in blood or urine were associated with malignant bone disease progression (61, 90). However, at present, the high inter- and intra-individual variability represents a limitation to the routine use of these biomarkers (90). Clinical

applications of these biomarkers for the detection and monitoring of bone metastasis and response to antiresorptive therapies have recently been reviewed (90) and are outlined in the following sections.

A. Bone Formation Markers

Biochemical markers of bone formation include bone alkaline phosphatase (BALP), which is an enzyme localized at the plasma membrane of osteoblasts, and procollagen I carboxyl-terminal and amino-terminal propeptides (PICP and PINP, respectively), which are cleaved during the processing of type I collagen (Table 2) (350). Their clinical applications in the management of malignant bone diseases are summarized below.

Diagnosis of bone metastasis

In breast and prostate cancer, serum concentrations of PINP were found significantly increased in patients with bone metastases (90). In a retrospective analysis, PINP was measured in the serum of prostate cancer patients with different clinical outcomes (N0/M0: no metastases, N1/M0: lymph node metastases only, and M1: bone metastases) (180). Increased PINP levels in the M1 group were detectable 8 months before the first positive bone scintigraph (180). PICP and BALP were also investigated in the prostate cancer setting. In particular, serum BALP concentrations significantly correlated with the extent of bone involvement (90). Furthermore, a meta-analysis including 19 trials and 3,628 patients with solid tumors showed that serum BALP levels in patients with bone metastases were 2.9-fold higher ($P < 0.05$) than in patients without bone lesions (91). Another meta-analysis in lung cancer including 16 trials and 1,720 patients with or without bone metastases showed that high concentrations of BALP were also associated with bone metastasis (155).

Prognosis of bone metastasis

BALP levels were assessed in patients with bone metastases from breast cancer (n = 1,648), castration-resistant prostate cancer (n = 643) and lung cancer and other solid tumors (n = 773) treated with a bisphosphonate (zoledronic acid or pamidronate). High serum levels of BALP at baseline and on-study were associated with increased risks of SREs, disease progression and death in patients who did not receive bisphosphonate therapy (61, 90). Similar findings were reported in a retrospective analysis involving 5,543 patients who received zoledronic acid or denosumab for bone metastasis treatment (206). Furthermore, after 3 months of treatment with either denosumab or zoledronic acid, patients with serum BALP levels ≥ median at month 3 had significantly reduced overall survival compared with those who had serum BALP levels < median (HR = 2.44; $P < 0.0001$) (206).

The prognosis value of PINP for bone metastasis was investigated in breast cancer (33). Specifically, PINP was measured at baseline in the serum from 872 patients from a large randomized trial of adjuvant zoledronic acid (AZURE) in early breast cancer (33). High baseline PINP was prognostic for future bone recurrence at any time ($P < 0.006$), but was not predictive for distant metastasis taken as a whole, demonstrating the bone metastasis specificity of PINP (33).

Response to bone-targeted therapies

The predictive value of BALP and PICP has been evaluated in castration-resistant prostate cancer patients with bone metastases (n = 778) treated on a placebo-controlled phase III trial of docetaxel with or without atrasentan (SWOG S0421) (187). High baseline serum levels of BALP and PICP were associated with poor overall survival (HR = 1.23; $P < 0.001$ and HR = 1.38; $P < 0.001$, respectively). Increasing BALP and PICP levels by week 9 of therapy were associated with a significantly increased risk of death (HR = 1.28; $P < 0.001$ and HR = 1.35; $P < 0.001$, respectively). For patients with the highest biomarker levels (upper 25th percentile), improved survival was observed in the atrasentan arm compared with placebo arm (HR = 0.65; $P < 0.04$ and HR = 0.61; $P < 0.02$ for BALP and PICP, respectively).

B. Bone Resorption Markers

Biochemical markers of bone resorption include (***1***) the carboxyterminal telopeptide of type I collagen (ICTP), which is a degradation product of mature type I collagen cleaved by MMP, (***2***) C- and N-telopeptides (CTX and NTX, respectively), which are proteolytic fragments generated by cathepsin K cleavage of type I collagen, (***3***) pyridinoline (PYD) and deoxypyridinoline (DPD), which are nonreducible pyridinium cross-links present in the mature form of type I collagen, and (***4***) tartrate resistant acid phosphatase 5b (TRACP), which is an osteoclast-derived enzyme (Table 2) (350). Additional potential bone resorption markers are RANKL and OPG, the RANK-L/OPG ratio being used to estimate the osteolysis rate, and miRNAs (Table 2), the latter being significantly upregulated in the serum of patients with osteoporotic fractures and breast cancer patients with osteolytic bone metastases (96, 294). BSP and osteopontin, which are osteoblast-derived bone matrix components, have been also investigated as potential bone markers associated with osteolysis (165). ICTP, TRACP, serum CTX, and urinary NTX are the most common resorption markers used in clinical practice. Clinical applications for these bone resorption markers in the management of malignant bone diseases are summarized below.

Diagnosis of bone metastasis

Serum concentrations of TRACP were found increased in patients with bone metastases from breast cancer, but not lung cancer (90, 155). In prostate cancer, high NTX and CTX levels were associated with bone metastases (35). In lung cancer, a meta-analysis including 16 trials and 1,720 patients with or without bone metastasis showed that high concentrations of NTX and ICTP (but not CTX) were associated with bone metastasis (155).

Increased serum levels of BSP and OPN have been associated with bone metastases from breast, lung and prostate cancer (165). However, these proteins are also expressed by tumor cells, suggesting they can be considered tumor markers rather than bone biomarkers (165).

2094 Increased serum levels of RANKL and/or OPG have been associated with bone metastases from
2095 prostate cancer (165). Similarly, the RANKL/OPG ratio is increased in severe osteolysis associated with
2096 primary bone tumors and bone metastasis from lung, renal and breast cancer (125). However, the low
2097 sensitivity of the assays to measure circulating levels of RANKL and OPG and the observation that
2098 RANK, RANKL and OPG are expressed in a wide variety of different cell types including tumors cells
2099 have so far limited the routine measurement of these molecules (165, 183).

2100 Circulating miRNAs originating from tissues, being remarkably stable in blood, may be able to
2101 serve as biomarkers. For example, Seeliger *et al*. (294) identified 5 miRNAs (miR-21, miR-23a, miR-25,
2102 miR-100 and miR-125b) that were upregulated in both the serum and the bone tissue of osteoporotic
2103 patients with bone fractures. Although these miRNAs are not bone tissue-specific, they have been
2104 reported to play a role in bone remodeling when they are expressed by osteoblasts (294). Similarly, Ell
2105 *et al*. (96) identified a series of 4 miRNAs (miR-16, miR-211, miR-378 and Let-7a) that were specifically
2106 upregulated during osteoclast differentiation. The authors then thought to investigate this series of
2107 miRNAs as potential biomarkers for osteolytic bone metastases. They found that miR-16 and miR-378
2108 were consistently increased in the serum from breast cancer patients with bone metastases (n = 38),
2109 compared to healthy female donors (n = 21) (96).

2110 *Prognosis of bone metastasis*

2111 NTX levels were assessed in patients with bone metastases from breast cancer (n = 1,648),
2112 castration-resistant prostate cancer (n = 643) and lung cancer and other solid tumors (n = 773) treated
2113 with a bisphosphonate (zoledronic acid or pamidronate). High serum levels of NTX at baseline and on-
2114 study were associated with increased risks of SREs, disease progression and death in patients who did
2115 not receive bisphosphonate therapy (61, 90). Similar findings were reported in a retrospective analysis
2116 involving 5,543 patients who received zoledronic acid or anti-RANKL antibody denosumab for bone
2117 metastasis treatment (206). Furthermore, after 3 months of treatment with either denosumab or

2118 zoledronic acid, patients with urinary NTX levels ≥ median at month 3 had significantly reduced overall
2119 survival compared with those who had urinary NTX levels < median (HR = 1.85; *P* < 0.0001) (206).

2120 The prognosis value of CTX and ICTP for bone metastasis was investigated in breast cancer (33).
2121 These bone resorption markers were measured at baseline in the serum from 872 patients from a large
2122 randomized trial of adjuvant zoledronic acid (AZURE) in early breast cancer (33). High baseline CTX or
2123 ICTP was prognostic for future bone recurrence at any time (*P* < 0.009 and 0.008, respectively), but
2124 were not predictive for overall distant recurrence, demonstrating the bone metastasis specificity of CTX
2125 and ICTP (33).

2126 *Response to bone-targeted therapies*

2127 The value of measuring NTX levels to assess response to bisphosphonate therapy was
2128 investigated by exploring databases from phase III trials of zoledronic acid in solid tumors and multiple
2129 myeloma. The analysis revealed that patients with high NTX levels at baseline that normalize during
2130 zoledronic acid treatment have improved survival as compared to patients with persistent elevated NTX
2131 levels (61).

2132 The predictive value of NTX and PYD has been evaluated in castration-resistant prostate cancer
2133 patients with bone metastases (n = 778) treated on a placebo-controlled phase III trial of docetaxel with
2134 or without atrasentan (SWOG S0421) (187). As for bone formation markers BALP and PICP, high
2135 baseline levels of NTX and PYD were associated with poor overall survival (HR = 1.40; *P* < 0.001 and
2136 HR = 1.52; *P* < 0.001, respectively). Increasing bone resorption marker levels by week 9 of therapy
2137 were associated with a significantly increased risk of death (HR = 1.36; *P* = 0.002 and HR = 1.36; *P* =
2138 0.002 for NTX and PYD, respectively). In contrast to what was observed for patients with the highest
2139 bone formation marker levels, there was however no survival benefit from atrasentan when using NTX
2140 or PYD in the upper 25th percentile. Nonetheless, when combining all four biomarkers in the highest
2141 quartile, there was clear evidence that patients had a survival benefit from atrasentan (HR = 0.33;
2142 median survival = 13 [atrasentan] vs 5 months [placebo]; *P* = .005) (187).

C. Insights from markers not associated with bone turnover

Westbrook and colleagues (365) have identified two proteins [macrophage-capping protein (CAPG) and PDZ domain-containing protein (GIPC1)] from proteomic analysis of osteotropic human breast cancer cell lines whose expression in tumor cells was subsequently validated by immunohistochemistry using tumor tissue microarrays (TMAs) from breast cancer patients (n = 364) of the AZURE trial. Clinical validation of these two markers showed that patients who did not receive a bisphosphonate therapy were more likely to develop a first distant recurrence in bone (HR = 4.5; P <0.001) and die (HR = 1.8; P = 0.045) if CAPG and GIPC1 were highly expressed in the primary tumor. Moreover, patients with high expression of CAPG and GIPC1 had a 10-fold increase in treatment benefit, compared with patients on standard therapy (365).

Dedicator of cytokinesis protein 4 (DOCK4) is another protein specifically expressed in osteotropic tumor cells (366). DOCK4 expression in primary tumors was validated by immunohistochemistry, using TMAs from breast cancer patients (n = 345) of the AZURE trial (366). Adjusted Cox regression analyses showed that patients who did not receive a bisphosphonate therapy were more likely to develop a first distant recurrence in bone (HR = 2.13; P = 0.034) if DOCK4 was highly expressed in the primary tumor (366).

MAF is a transcription factor of the AP-1 family shown to mediate breast cancer bone metastasis (260). The value of MAF expression in primary tumors to predict the treatment outcomes of adjuvant zoledronic acid in breast cancer patients from the AZURE trial (n = 1,739) has been investigated (64). In patients with MAF-negative tumors (79% of all patients), there was a lower relapse rate with the use of zoledronic acid (HR = 0.74), but not in patients who had MAF-positive tumors (64). Additionally, MAF positivity was associated with increased extraskeletal recurrence in the zoledronic acid group (HR = 6.92) (64). Data from ABCSG-18 trial are also being used in a *post-hoc* analysis addressing MAF to help clarify the anticancer mechanism of denosumab (122).

XI. CONCLUSION

Bone is one of the most common sites for metastasis, especially from breast, prostate and lung cancer. These skeletal metastases contribute substantially to morbidity and mortality in patients with advanced cancer. It is therefore essential to better understand the pathophysiology of bone metastasis in order to improve therapies for the treatment and prevention of bone metastasis and predict the risk of disease relapse. In this review, we described the importance of the systemic effect of primary tumors in preparing a pre-metastatic niche to facilitate the arrival of tumor cells in the bone marrow and we highlighted the prominence of metastatic niches in mediating dormancy of tumor cells. We also discussed the key role of the environment for reactivation of dormant tumor cells, which then undergo further selection to acquire a full complement of metastasis-colonization functions that dormant tumor cells did not express before. We also explained how, at a later stage, tumor cells induce osteolytic or osteoblastic lesions. These findings provide the rationale for the use of bone-targeted agents such as the bisphosphonates, the RANK ligand inhibitor denosumab and bone seeking radiopharmaceuticals. However, as our understanding of the signaling mechanisms between tumor cells and cells in the bone marrow microenvironment increases, several new, targeted agents have entered clinical development. They could be used in combination with anti-resorptive agents to efficiently block the development of skeletal lesions. Another attractive avenue of research would be to reconstruct bone lesions by restoring osteoblast anabolic functions. Finally, we showed that bone markers potentially provide important insight for predicting the risk of disease relapse in patients with cancer and evaluating a patient's risk of worsening skeletal health.

2189 ACKNOWLEDGEMENT

2190 The authors gratefully acknowledge Jean Paul Roux (INSERM UMR1033, Lyon, France) for providing
2191 photographs of bone metastases. Figures were created using adapted images from Servier Medical Art
2192 by Servier, licensed under a Creative Commons Attribution 3.0 unported license (available at
2193 http://smart.servier.com).

2194

2195 GRANTS

2196 P. Clézardin is funded by Inserm, Inserm Cancer, the University of Lyon, the French National Cancer
2197 Institute (INCa; grant n°: PLBIO14-164 and PLBIO14-213), “Appel à Projets LIA/LEA 2016” (grant n°:
2198 ASC17018CSA), Weston Park Cancer Charity (grant n°: CA163) and the LabEX DEVweCAN (ANR-10-
2199 LABX-61) from Université de Lyon, within the program "Investissements d'Avenir" (ANR-11-IDEX-0007)
2200 operated by the French National Research Agency (ANR). P.D. Ottewell is funded by Research Council
2201 UK (grant nº MR/P000096/1) and Weston Park Cancer Charity (grant nºs CA 142 and CA175).

2202

XII. REFERENCES

1. Ahn CS, Metallo CM. Mitochondria as biosynthetic factories for cancer proliferation. *Cancer Metab*. 3:1, 2015. doi: 10.1186/s40170-015-0128-2.

2. Alam AS, Gallagher A, Shankar V, Ghatei MA, Datta HK, Huang CL, Moonga BS, Chambers TJ, Bloom SR, Zaidi M. Endothelin inhibits osteoclastic bone resorption by a direct effect on cell motility: implications for the vascular control of bone resorption. *Endocrinology*. 130:3617-3624, 1992.

3. Alexaki VI, Javelaud D, Van Kempen LC, Mohammad KS, Dennler S, Luciani F, Hoek KS, Juàrez P, Goydos JS, Fournier PJ, Sibon C, Bertolotto C, Verrecchia F, Saule S, Delmas V, Ballotti R, Larue L, Saiag P, Guise TA, Mauviel A. GLI2-mediated melanoma invasion and metastasis. *J Natl Cancer Inst.* 102:1148-1159, 2010. doi: 10.1093/jnci/djq257.

4. Andrade K, Fornetti J, Zhao L, Miller SC, Randall RL, Anderson N, Waltz SE, McHale M, Welm AL. RON kinase: A target for treatment of cancer-induced bone destruction and osteoporosis. *Sci Transl Med*. 9. pii: eaai9338, 2017. doi: 10.1126/scitranslmed.aai9338.

5. Antonarakis ES, Lu C, Luber B, Wang H, Chen Y, Zhu Y, Silberstein JL, Taylor MN, Maughan BL, Denmeade SR, Pienta KJ, Paller CJ, Carducci MA, Eisenberger MA, Luo J. Clinical Significance of Androgen Receptor Splice Variant-7 mRNA Detection in Circulating Tumor Cells of Men With Metastatic Castration-Resistant Prostate Cancer Treated With First- and Second-Line Abiraterone and Enzalutamide. *J Clin Oncol*. 35:2149-2156, 2017. doi: 10.1200/JCO.2016.70.1961.

6. Atkinson EG, Delgado-Calle J. The Emerging Role of Osteocytes in Cancer in Bone. *JBMR Plus* 3:e10186, 2019. doi: 10.1002/jbm4.10186.

7. Barker HE, Cox TR, Erler JT. The rationale for targeting the LOX family in cancer. *Nat Rev Cancer* 12: 540-552, 2012. doi: 10.1038/nrc3319.

8. Baron R, Kneissel M. WNT signaling in bone homeostasis and disease: from human mutations to treatments. *Nat Med*. 19:179-192, 2013. doi: 10.1038/nm.3074.

9. Barrett-Lee P, Casbard A, Abraham J, Hood K, Coleman R, Simmonds P, Timmins H, Wheatley D, Grieve R, Griffiths G, Murray N. Oral ibandronic acid versus intravenous zoledronic acid in treatment of bone metastases from breast cancer: a randomised, open label, non-inferiority phase 3 trial. *Lancet Oncol*. 15:114-122, 2014. doi: 10.1016/S1470-2045(13)70539-4.

10. Barry-Hamilton V, Spangler R, Marshall D, McCauley S, Rodriguez HM, Oyasu M, Mikels A, Vaysberg M, Ghermazien H, Wai C, Garcia CA, Velayo AC, Jorgensen B, Biermann D, Tsai D, Green J, Zaffryar-Eilot S, Holzer A, Ogg S, Thai D, Neufeld G, Van Vlasselaer P, Smith V. Allosteric inhibition of lysyl oxidase-like-2 impedes the development of a pathologic microenvironment. *Nat Med* 16: 1009-1017, 2010. doi: 10.1038/nm.2208.

11. Baselga J, Campone M, Piccart M, Burris HA 3rd, Rugo HS, Sahmoud T, Noguchi S, Gnant M, Pritchard KI, Lebrun F, Beck JT, Ito Y, Yardley D, Deleu I, Perez A, Bachelot T, Vittori L, Xu Z, Mukhopadhyay P, Lebwohl D, Hortobagyi GN. Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. *N Engl J Med*. 366:520-529, 2012. doi: 10.1056/NEJMoa1109653.

12. Baud'huin M, Lamoureux F, Jacques C, Rodriguez Calleja L, Quillard T, Charrier C, Amiaud J, Berreur M, Brounais-LeRoyer B, Owen R, Reilly GC, Bradner JE, Heymann D, Ory B. Inhibition of BET proteins and epigenetic signaling as a potential treatment for osteoporosis. *Bone*. 94:10-21, 2017. doi: 10.1016/j.bone.2016.09.020.

13. Beer TM, Armstrong AJ, Rathkopf DE, Loriot Y, Sternberg CN, Higano CS, Iversen P, Bhattacharya S, Carles J, Chowdhury S, Davis ID, de Bono JS, Evans CP, Fizazi K, Joshua AM, Kim CS, Kimura G, Mainwaring P, Mansbach H, Miller K, Noonberg SB, Perabo F, Phung D, Saad F, Scher HI, Taplin ME, Venner PM, Tombal B; PREVAIL Investigators. Enzalutamide

in metastatic prostate cancer before chemotherapy. *N Engl J Med.* 371:424-433, 2014. doi: 10.1056/NEJMoa1405095.

14. Beermann J, Piccoli MT, Viereck J, Thum T. Non-coding RNAs in development and disease: background, mechanisms, and therapeutic approaches. *Physiol Rev* 96: 1297-1325, 2016. doi: 10.1152/physrev.00041.2015.

15. Beg MS, Brenner AJ, Sachdev J, Borad M, Kang YK, Stoudemire J, Smith S, Bader AG, Kim S, Hong DS. Phase I study of MRX34, a liposomal miR-34a mimic, administered twice weekly in patients with advanced solid tumors. *Invest New Drugs.* 35:180-188, 2017. doi: 10.1007/s10637-016-0407-y.

16. Bellahcène A, Bachelier R, Detry C, Lidereau R, Clézardin P, Castronovo V. Transcriptome analysis reveals an osteoblast-like phenotype for human osteotropic breast cancer cells. *Breast Cancer Res Treat* 101: 135-148, 2007. doi: 10.1007/s10549-006-9279-8.

17. Bellahcène A, Castronovo V, Ogbureke KU, Fisher LW, Fedarko NS. Small integrin-binding ligand N-linked glycoproteins (SIBLINGs): multifunctional proteins in cancer. *Nat Rev Cancer* 8: 212-226, 2008. doi: 10.1038/nrc2345.

18. Bendre MS, Gaddy-Kurten D, Mon-Foote T, Akel NS, Skinner RA, Nicholas RW, Suva LJ. Expression of interleukin 8 and not parathyroid hormone-related protein by human breast cancer cells correlates with bone metastasis in vivo. *Cancer Res*. 62: 5571-5579, 2002.

19. Bertoldo F, Silvestris F, Ibrahim T, Cognetti F, Generali D, Ripamonti CI, Amadori D, Colleoni MA, Conte P, Del Mastro L, De Placido S, Ortega C, Santini D. Targeting bone metastatic cancer: Role of the mTOR pathway. *Biochim Biophys Acta* 1845:248-254, 2014. doi: 10.1016/j.bbcan.2014.01.009.

20. Bidwell BN, Slaney CY, Withana NP, Forster S, Cao Y, Loi S, Andrews D, Mikeska T, Mangan NE, Samarajiwa SA, de Weerd NA, Gould J, Argani P, Möller A, Smyth MJ, Anderson RL,

Hertzog PJ, Parker BS. Silencing of Irf7 pathways in breast cancer cells promotes bone metastasis through immune escape. *Nat Med*. 18:1224-1231, 2012. doi: 10.1038/nm.2830.

21. Blake ML, Tometsko M, Miller R, Jones JC, Dougall WC. RANK expression on breast cancer cells promotes skeletal metastasis. *Clin Exp Metastasis* 31:233-245, 2014. doi: 10.1007/s10585-013-9624-3.

22. Bliss SA, Sinha G, Sandiford OA, Williams LM, Engelberth DJ, Guiro K, Isenalumhe LL, Greco SJ, Ayer S, Bryan M, Kumar R, Ponzio NM, Rameshwar P. Mesenchymal Stem Cell-Derived Exosomes Stimulate Cycling Quiescence and Early Breast Cancer Dormancy in Bone Marrow. *Cancer Res* 76: 5832-5844, 2016. doi: 10.1158/0008-5472.CAN-16-1092.

23. Bloom AP, Jimenez-Andrade JM, Taylor RN, Castañeda-Corral G, Kaczmarska MJ, Freeman KT, Coughlin KA, Ghilardi JR, Kuskowski MA, Mantyh PW. Breast cancer-induced bone remodeling, skeletal pain, and sprouting of sensory nerve fibers. *J Pain.* 12:698-711, 2011. doi: 10.1016/j.jpain.2010.12.016.

24. Bone HG, Dempster DW, Eisman JA, Greenspan SL, McClung MR, Nakamura T, Papapoulos S, Shih WJ, Rybak-Feiglin A, Santora AC, Verbruggen N, Leung AT, Lombardi A. Odanacatib for the treatment of postmenopausal osteoporosis: development history and design and participant characteristics of LOFT, the Long-Term Odanacatib Fracture Trial. *Osteoporos Int.* 26:699-712, 2015. doi: 10.1007/s00198-014-2944-6.

25. Bottos A, Gotthardt D, Gill JW, Gattelli A, Frei A, Tzankov A, Sexl V, Wodnar-Filipowicz A, Hynes NE. Decreased NK-cell tumour immunosurveillance consequent to JAK inhibition enhances metastasis in breast cancer models. *Nat Commun*. 7:12258, 2016. doi: 10.1038/ncomms12258.

26. Boucharaba A, Serre CM, Grès S, Saulnier-Blache JS, Bordet JC, Guglielmi J, Clézardin P, Peyruchaud O. Platelet-derived lysophosphatidic acid supports the progression of osteolytic bone metastases in breast cancer. *J Clin Invest* 114; 1714-1725, 2004. doi: 10.1172/JCI22123.

27. Boucharaba A, Serre CM, Guglielmi J, Bordet JC, Clézardin P, Peyruchaud O. The type 1 lysophosphatidic acid receptor is a target for therapy in bone metastases. *Proc Natl Acad Sci USA* 103; 9643-9648, 2006. doi: 10.1073/pnas.0600979103.

28. Bouchet M, Lainé A, Boyault C, Proponnet-Guerault M, Meugnier E, Bouazza L, Kan CWS, Geraci S, El-Moghrabi S, Hernandez-Vargas H, Benetollo C, Yoshiko Y, Duterque-Coquillaud M, Clézardin P, Marie JC, Bonnelye E. ERRα Expression in Bone Metastases Leads to an Exacerbated Antitumor Immune Response. *Cancer Res* 80; 2914-2926, 2020. doi: 10.1158/0008-5472.CAN-19-3584.

29. Boudot C, Hénaut L, Thiem U, Geraci S, Galante M, Saldanha P, Saidak Z, Six I, Clézardin P, Kamel S, Mentaverri R. Overexpression of a functional calcium-sensing receptor dramatically increases osteolytic potential of MDA-MB-231 cells in a mouse model of bone metastasis through epiregulin-mediated osteoprotegerin downregulation. *Oncotarget* 8:56460-56472, 2017. doi: 10.18632/oncotarget.16999.

30. Bozec A, Zaiss MM. T regulatory cells in bone remodelling. *Curr Osteoporos Rep* 15:121-125, 2017. doi: 10.1007/s11914-017-0356-1.

31. Bragado P, Estrada Y, Parikh F, Krause S, Capobianco C, Farina HG, Schewe DM, Aguirre-Ghiso JA. TGF-β2 dictates disseminated tumour cell fate in target organs through TGF-β-RIII and p38α/β signalling. *Nat Cell Biol* 15: 1351-1361, 2013. doi: 10.1038/ncb2861.

32. Braun S, Vogl FD, Naume B, Janni W, Osborne MP, Coombes RC, Schlimok G, Diel IJ, Gerber B, Gebauer G, Pierga JY, Marth C, Oruzio D, Wiedswang G, Solomayer EF, Kundt G, Strobl B, Fehm T, Wong GY, Bliss J, Vincent-Salomon A, Pantel K. A pooled analysis of bone marrow micrometastasis in breast cancer. *N Engl J Med.* 353:793-802, 2005. doi:10.1056/NEJMoa050434.

33. Brown J, Rathbone E, Hinsley S, Gregory W, Gossiel F, Marshall H, Burkinshaw R, Shulver H, Thandar H, Bertelli G, Maccon K, Bowman A, Hanby A, Bell R, Cameron D, Coleman R.

Associations Between Serum Bone Biomarkers in Early Breast Cancer and Development of Bone Metastasis: Results From the AZURE (BIG01/04) Trial. *J Natl Cancer Inst*. 110:871-879, 2018. doi: 10.1093/jnci/djx280.

34. Brown JE, Coleman RE. Denosumab in patients with cancer-a surgical strike against the osteoclast. *Nat Rev Clin Oncol*. 9:110-118, 2012. doi: 10.1038/nrclinonc.2011.197.

35. Brown JE, Cook RJ, Major P, Lipton A, Saad F, Smith M, Lee KA, Zheng M, Hei YJ, Coleman RE. Bone turnover markers as predictors of skeletal complications in prostate cancer, lung cancer, and other solid tumors. *J Natl Cancer Inst.* 97:59-69, 2005. DOI: 10.1093/jnci/dji002.

36. Browne AJ, Kubasch ML, Göbel A, Hadji P, Chen D, Rauner M, Stölzel F, Hofbauer LC, Rachner TD. Concurrent antitumor and bone-protective effects of everolimus in osteotropic breast cancer. *Breast Cancer Res*. 19:92, 2017. doi: 10.1186/s13058-017-0885-7.

37. Browne G, Taipaleenmäki H, Stein GS, Stein JL, Lian JB. MicroRNAs in the control of metastatic bone disease. *Trends Endocrinol Metab* 25: 320-327, 2014. doi: 10.1016/j.tem.2014.03.014.

38. Brufsky AM, Harker WG, Beck JT, Bosserman L, Vogel C, Seidler C, Jin L, Warsi G, Argonza-Aviles E, Hohneker J, Ericson SG, Perez EA. Final 5-year results of Z-FAST trial: adjuvant zoledronic acid maintains bone mass in postmenopausal breast cancer patients receiving letrozole. *Cancer*. 118:1192-1201, 2012. doi: 10.1002/cncr.26313.

39. Bu G, Lu W, Liu CC, Selander K, Yoneda T, Hall C, Keller ET, Li Y. Breast cancer-derived Dickkopf1 inhibits osteoblast differentiation and osteoprotegerin expression: implication for breast cancer osteolytic bone metastases. *Int J Cancer*. 123:1034-1042, 2008. doi: 10.1002/ijc.23625.

40. Buijs JT, Stayrook KR, Guise TA. The role of TGF-β in bone metastasis: novel therapeutic perspectives. *Bonekey Rep*. 1:96, 2012. doi: 10.1038/bonekey.2012.96.

41. Buijs JT, van der Horst G, van den Hoogen C, Cheung H, de Rooij B, Kroon J, Petersen M, van Overveld PG, Pelger RC, van der Pluijm G. The BMP2/7 heterodimer inhibits the human breast cancer stem cell subpopulation and bone metastases formation. *Oncogene* 31: 2164-2174, 2012. doi: 10.1038/onc.2011.400.

42. Cackowski FC, Eber MR, Rhee J, Decker AM, Yumoto K, Berry JE, Lee E, Shiozawa Y, Jung Y, Aguirre-Ghiso JA, Taichman RS. Mer Tyrosine Kinase Regulates Disseminated Prostate Cancer Cellular Dormancy. *J Cell Biochem* 118: 891-902, 2017. doi: 10.1002/jcb.25768.

43. Caers J, Deleu S, Belaid Z, De Raeve H, Van Valckenborgh E, De Bruyne E, Defresne MP, Van Riet I, Van Camp B, Vanderkerken K. Neighboring adipocytes participate in the bone marrow microenvironment of multiple myeloma cells. *Leukemia*. 21:1580-1584, 2007. doi: 10.1038/sj.leu.2404658

44. Campbell JP, Karolak MR, Ma Y, Perrien DS, Masood-Campbell SK, Penner NL, Munoz SA, Zijlstra A, Yang X, Sterling JA, Elefteriou F. Stimulation of host bone marrow stromal cells by sympathetic nerves promotes breast cancer bone metastasis in mice. *PLoS Biol.* 10:e1001363, 2012. doi: 10.1371/journal.pbio.1001363.

45. Capietto AH, Faccio R. Immune regulation of bone metastasis. *Bonekey Rep*. 3:600, 2014. doi: 10.1038/bonekey.2014.95.

46. Capulli M, Hristova D, Valbret Z, Carys K, Arjan R, Maurizi A, Masedu F, Cappariello A, Rucci N, Teti A. Notch2 pathway mediates breast cancer cellular dormancy and mobilisation in bone and contributes to haematopoietic stem cell mimicry. *Br J Cancer*. 121:157-171, 2019. doi: 10.1038/s41416-019-0501-y.

47. Carducci MA, Saad F, Abrahamsson PA, Dearnaley DP, Schulman CC, North SA, Sleep DJ, Isaacson JD, Nelson JB. A phase 3 randomized controlled trial of the efficacy and safety of atrasentan in men with metastatic hormone-refractory prostate cancer.; Atrasentan Phase III Study Group Institutions. *Cancer.* 110:1959-66, 2007. doi: 10.1002/cncr.22996.

48. Cha YJ, Jung WH, Koo JS. Differential Site-Based Expression of Pentose Phosphate Pathway-Related Proteins among Breast Cancer Metastases. *Dis Markers* 2017:7062517, 2017. doi: 10.1155/2017/7062517.

49. Chaffer CL, San Juan BP, Lim E, Weinberg RA. EMT, cell plasticity and metastasis. *Cancer Metastasis Rev* 35: 645-654, 2016. doi: 10.1007/s10555-016-9648-7.

50. Chang MK, Raggatt LJ, Alexander KA, Kuliwaba JS, Fazzalari NL, Schroder K, Maylin ER, Ripoll VM, Hume DA, Pettit AR. Osteal tissue macrophages are intercalated throughout human and mouse bone lining tissues and regulate osteoblast function in vitro and in vivo. *J Immunol*.181:1232-1244, 2008.

51. Chantry AD, Heath D, Mulivor AW, Pearsall S, Baud'huin M, Coulton L, Evans H, Abdul N, Werner ED, Bouxsein ML, Key ML, Seehra J, Arnett TR, Vanderkerken K, Croucher P. Inhibiting activin-A signaling stimulates bone formation and prevents cancer-induced bone destruction in vivo. *J Bone Miner Res.* 25:2633-2646, 2010. doi: 10.1002/jbmr.142.

52. Chen GL, Luo Y, Eriksson D, Meng X, Qian C, Bäuerle T, Chen XX, Schett G, Bozec A. High fat diet increases melanoma cell growth in the bone marrow by inducing osteopontin and interleukin 6. *Oncotarget*. 7:26653-26669, 2016. doi: 10.18632/oncotarget.8474.

53. Chiao JW, Moonga BS, Yang YM, Kancherla R, Mittelman A, Wu-Wong JR, Ahmed T. Endothelin-1 from prostate cancer cells is enhanced by bone contact which blocks osteoclastic bone resorption. *Br J Cancer* 83:360-365, 2000. doi: 10.1054/bjoc.2000.1261.

54. Choudhary S, Ramasundaram P, Dziopa E, Mannion C, Kissin Y, Tricoli L, Albanese C, Lee W, Zilberberg J. Human ex vivo 3D bone model recapitulates osteocyte response to metastatic prostate cancer. *Sci Rep*. 8:17975, 2018. doi: 10.1038/s41598-018-36424-x.

55. Chu K, Cheng CJ, Ye X, Lee YC, Zurita AJ, Chen DT, Yu-Lee LY, Zhang S, Yeh ET, Hu MC, Logothetis CJ, Lin SH. Cadherin-11 promotes the metastasis of prostate cancer cells to bone. Mol Cancer Res 6:1259-67, 2008. doi: 10.1158/1541-7786.MCR-08-0077.

56. Clément-Demange L, Mulcrone PL, Tabarestani TQ, Sterling JA, Elefteriou F. β2ARs stimulation in osteoblasts promotes breast cancer cell adhesion to bone marrow endothelial cells in an IL-1β and selectin-dependent manner. *J Bone Oncol.* 13:1-10, 2018. doi: 10.1016/j.jbo.2018.09.002.

57. Clézardin P. Integrins in bone metastasis formation and potential therapeutic implications. *Curr Cancer Drug Targets* 9: 801-806, 2009. DOI: 10.2174/156800909789760348.

58. Clézardin P, Benzaïd I, Croucher PI. Bisphosphonates in preclinical bone oncology. *Bone*. 49:66-70, 2011. doi: 10.1016/j.bone.2010.11.017.

59. Coleman R. Treatment of Metastatic Bone Disease and the Emerging Role of Radium-223. *Semin Nucl Med*. 46:99-104, 2016. doi: 10.1053/j.semnuclmed.2015.10.012.

60. Coleman R, Body JJ, Aapro M, Hadji P, Herrstedt J. Bone health in cancer patients: ESMO Clinical Practice Guidelines. *Ann Oncol*. 25 Suppl 3, iii124-iii137, 2014.

61. Coleman R, Costa L, Saad F, Cook R, Hadji P, Terpos E, Garnero P, Brown J, Body JJ, Smith M, Lee KA, Major P, Dimopoulos M, Lipton A. Consensus on the utility of bone markers in the malignant bone disease setting. *Crit Rev Oncol Hematol*. 80:411-432, 2011. doi: 10.1016/j.critrevonc.2011.02.005.

62. Coleman R, de Boer R, Eidtmann H, Llombart A, Davidson N, Neven P, von Minckwitz G, Sleeboom HP, Forbes J, Barrios C, Frassoldati A, Campbell I, Paija O, Martin N, Modi A, Bundred N. Zoledronic acid (zoledronate) for postmenopausal women with early breast cancer receiving adjuvant letrozole (ZO-FAST study): final 60-month results. *Ann Oncol.* 24:398-405, 2013. doi: 10.1093/annonc/mds277.

63. Coleman R, Finkelstein DM, Barrios C, Martin M, Iwata H, Hegg R, Glaspy J, Periañez AM, Tonkin K, Deleu I, Sohn J, Crown J, Delaloge S, Dai T, Zhou Y, Jandial D, Chan A. Adjuvant denosumab in early breast cancer (D-CARE): an internatinal, multicentre, randomised, controlled, phase 3 trial. *Lancet Oncol*. 21:60-72, 2020. doi: 10.1016/S1470-2045(19)30687-4.

64. Coleman R, Hall A, Albanell J, Hanby A, Bell R, Cameron D, Dodwell D, Marshall H, Jean-Mairet J, Tercero JC, Rojo F, Gregory W, Gomis RR. Effect of MAF amplification on treatment outcomes with adjuvant zoledronic acid in early breast cancer: a secondary analysis of the international, open-label, randomised, controlled, phase 3 AZURE (BIG 01/04) trial. *Lancet Oncol.* 18:1543-1552, 2017. doi: 10.1016/S1470-2045(17)30603-4.

65. Coleman RE. Clinical features of metastatic bone disease and risk of skeletal morbidity. *Clin Cancer Res*. 12:6243s-6249s, 2006. doi:10.1158/1078-0432.CCR-06-0931.

66. Coleman RE, Collinson M, Gregory W, Marshall H, Bell R, Dodwell D, Keane M, Gil M, Barrett-Lee P, Ritchie D, Bowman A, Liversedge V, De Boer RH, Passos-Coelho JL, O'Reilly S, Bertelli G, Joffe J, Brown JE, Wilson C, Tercero JC, Jean-Mairet J, Gomis R, Cameron D. Benefits and risks of adjuvant treatment with zoledronic acid in stage II/III breast cancer. 10 years follow-up of the AZURE randomized clinical trial (BIG 01/04). *J Bone Oncol.* 13:123-135, 2018. doi: 10.1016/j.jbo.2018.09.008.

67. Coleman RE, Major P, Lipton A, Brown JE, Lee KA, Smith M, Saad F, Zheng M, Hei YJ, Seaman J, Cook R. Predictive value of bone resorption and formation markers in cancer patients with bone metastases receiving the bisphosphonate zoledronic acid. J Clin Oncol. 23:4925-4935, 2005. doi: 10.1200/JCO.2005.06.091.

68. Coleman RE, Rubens RD. The clinical course of bone metastases from breast cancer. Br J Cancer. 55:61–66, 1987. doi: 10.1038/bjc.1987.13.

69. Confavreux CB, Pialat JB, Bellière A, Brevet M, Decroisette C, Tescaru A, Wegrzyn J, Barrey C, Mornex F, Souquet PJ, Girard N. Bone metastases from lung cancer: A paradigm for multidisciplinary onco-rheumatology management. *Joint Bone Spine*. pii: S1297-319X(18)30048-4, 2018. doi: 10.1016/j.jbspin.2018.03.005.

70. Corey E, Brown LG, Kiefer JA, Quinn JE, Pitts TE, Blair JM, Vessella RL. Osteoprotegerin in prostate cancer bone metastasis. *Cancer Res*. 65:1710-1718, 2005. doi: 10.1158/0008-5472.CAN-04-2033.

71. Cosman F, Crittenden DB, Grauer A. Romosozumab Treatment in Postmenopausal Osteoporosis. *N Engl J Med*. 376:396-397, 2017. doi: 10.1056/NEJMc1615367.

72. Costa L, Badia X, Chow E, Lipton A, Wardley A. Impact of skeletal complications on patients' quality of life, mobility, and functional independence. *Support Care Cancer* 16:879–889, 2008. doi:10.1007/s00520-008-0418-0.

73. Cox TR, Rumney RMH, Schoof EM, Perryman L, Høye AM, Agrawal A, Bird D, Latif NA, Forrest H, Evans HR, Huggins ID, Lang G, Linding R, Gartland A, Erler JT. The hypoxic cancer secretome induces pre-metastatic bone lesions through lysyl oxidase. *Nature* 522: 106-110, 2015. doi: 10.1038/nature14492.

74. Croset M, Goehrig D, Frackowiak A, Bonnelye E, Ansieau S, Puisieux A, Clézardin P. TWIST1 expression in breast cancer cells facilitates bone metastasis formation. J Bone Miner Res. 29:1886-1899, 2014. doi: 10.1002/jbmr.2215.

75. Croset M, Pantano F, Kan CWS, Bonnelye E, Descotes F, Alix-Panabières C, Lecellier CH, Bachelier R, Allioli N, Hong SS, Bartkowiak K, Pantel K, Clézardin P. MicroRNA-30 family members inhibit breast cancer invasion, osteomimicry, and bone destruction by directly targeting multiple bone metastasis-associated genes. *Cancer Res* 78: 5260-5273, 2018. doi: 10.1158/0008-5472.CAN-17-3058.

76. Croucher PI, McDonald MM, Martin TJ. Bone metastasis: the importance of the neighbourhood. *Nat Rev Cancer* 16: 373-386, 2016. doi: 10.1038/nrc.2016.44.

77. Dai J, Hall CL, Escara-Wilke J, Mizokami A, Keller JM, Keller ET. Prostate cancer induces bone metastasis through Wnt-induced bone morphogenetic protein-dependent and

independent mechanisms. *Cancer Res* 68: 5785-5794, 2008. doi: 10.1158/0008-5472.CAN-07-6541.

78. Dai J, Keller J, Zhang J, Lu Y, Yao Z, Keller ET. Bone morphogenetic protein-6 promotes osteoblastic prostate cancer bone metastases through a dual mechanism. *Cancer Res* 65:8274-8285, 2005. doi: 10.1158/0008-5472.CAN-05-1891.

79. Dai J, Zhang H, Karatsinides A, Keller JM, Kozloff KM, Aftab DT, Schimmoller F, Keller ET. Cabozantinib inhibits prostate cancer growth and prevents tumor-induced bone lesions. *Clin Cancer Res.* 20:617-630, 2014. doi: 10.1158/1078-0432.CCR-13-0839.

80. Dallas SL, Prideaux M, Bonewald LF. The osteocyte: an endocrine cell ... and more. *Endocr Rev*. 34:658-690, 2013. doi: 10.1210/er.2012-1026.

81. Danilin S, Merkel AR, Johnson JR, Johnson RW, Edwards JR, Sterling JA. Myeloid-derived suppressor cells expand during breast cancer progression and promote tumor-induced bone destruction. *Oncoimmunology*. 1:1484-1494, 2012. doi: 10.4161/onci.21990.

82. Decker AM, Jung Y, Cackowski FC, Yumoto K, Wang J, Taichman RS. Sympathetic signaling re-activates proliferation of dormant disseminated prostate cancer cells in the bone marrow. *Mol Cancer Res*. 15:1644-1655, 2017. doi: 10.1158/1541-7786.MCR-17-0132.

83. Delgado-Calle J, Anderson J, Cregor MD, Hiasa M, Chirgwin JM, Carlesso N, Yoneda T, Mohammad KS, Plotkin LI, Roodman GD, Bellido T. Bidirectional Notch Signaling and Osteocyte-Derived Factors in the Bone Marrow Microenvironment Promote Tumor Cell Proliferation and Bone Destruction in Multiple Myeloma. *Cancer Res*. 76:1089-1100, 2016. doi: 10.1158/0008-5472.CAN-15-1703.

84. Delliaux C, Tian TV, Bouchet M, Fradet A, Vanpouille N, Flourens A, Deplus R, Villers A, Leroy X, Clézardin P, de Launoit Y, Bonnelye E, Duterque-Coquillaud M. *TMPRSS2:ERG* gene fusion expression regulates bone markers and enhances the osteoblastic phenotype of

prostate cancer bone metastases. *Cancer Lett*. 438:32-43, 2018. doi: 10.1016/j.canlet.2018.08.027.

85. Devignes CS, Aslan Y, Brenot A, Devillers A, Schepers K, Fabre S, Chou J, Casbon AJ, Werb Z, Provot S. HIF signaling in osteoblast-lineage cells promotes systemic breast cancer growth and metastasis in mice. *Proc Natl Acad Sci U S A* 115: E992-E1001, 2018. doi: 10.1073/pnas.1718009115.

86. Dhesy-Thind S, Fletcher GG, Blanchette PS, Clemons MJ, Dillmon MS, Frank ES, Gandhi S, Gupta R, Mates M, Moy B, Vandenberg T, Van Poznak CH. Use of Adjuvant Bisphosphonates and Other Bone-Modifying Agents in Breast Cancer: A Cancer Care Ontario and American Society of Clinical Oncology Clinical Practice Guideline. *J Clin Oncol*. 35:2062-2081, 2017. doi: 10.1200/JCO.2016.70.7257.

87. Diedrich JD, Rajagurubandara E, Herroon MK, Mahapatra G, Hüttemann M, Podgorski I. Bone marrow adipocytes promote the Warburg phenotype in metastatic prostate tumors via HIF-1α activation. *Oncotarget*. 7:64854-64877, 2016. doi: 10.18632/oncotarget.11712.

88. Dietterle J, Frydrychowicz C, Müller W, Hoffmann KT, Jähne K, Meixensberger J. Tumor-to-Tumor Metastasis of Multiple Meningiomas and Clear Cell Renal Cell Carcinoma Metastasis as First Clinical Appearance of Kidney Cancer: A Case Report and Analysis. *J Neurol Surg Rep*. 81(1):e10-e14, 2020. doi: 10.1055/s-0040-1708846.

89. Dolloff NG, Shulby SS, Nelson AV, Stearns ME, Johannes GJ, Thomas JD, Meucci O, Fatatis A. Bone-metastatic potential of human prostate cancer cells correlates with Akt/PKB activation by alpha platelet-derived growth factor receptor. *Oncogene*. 24:6848-6854, 2015. doi: 10.1038/sj.onc.1208815.

90. D'Oronzo S, Brown J, Coleman R. The role of biomarkers in the management of bone-homing malignancies. *J Bone Oncol*. 9:1-9, 2017. doi: 10.1016/j.jbo.2017.09.001.

2520 91. Du WX, Duan SF, Chen JJ, Huang JF, Yin LM, Tong PJ. Serum bone-specific alkaline
2521 phosphatase as a biomarker for osseous metastases in patients with malignant carcinomas: a
2522 systematic review and meta-analysis. *J Cancer Res Ther*. 10 Suppl:C140-133, 2014. doi:
2523 10.4103/0973-1482.145842.
2524 92. Duong LT, Wesolowski GA, Leung P, Oballa R, Pickarski M. Efficacy of a cathepsin K inhibitor
2525 in a preclinical model for prevention and treatment of breast cancer bone metastasis. Mol
2526 Cancer Ther. 13:2898-2909, 2014. doi: 10.1158/1535-7163.MCT-14-0253.
2527 93. Early Breast Cancer Trialists Cooperative Group. Adjuvant bisphosphonate treatment in early
2528 breast cancer: meta-analyses of individual patient data from randomized trials. *Lancet*. 386:
2529 1353-1361, 2015. doi: 10.1016/S0140-6736(15)60908-4.
2530 94. Eastell R, O'Neill TW, Hofbauer LC, Langdahl B, Reid IR, Gold DT, Cummings SR.
2531 Postmenopausal osteoporosis. *Nat Rev Dis Primers*. 2:16069, 2016. doi:
2532 10.1038/nrdp.2016.69.
2533 95. Elefteriou F. Impact of the Autonomic Nervous System on the Skeleton. *Physiol Rev*. 98: 1083-
2534 1112, 2018. doi: 10.1152/physrev.00014.2017.
2535 96. Ell B, Mercatali L, Ibrahim T, Campbell N, Schwarzenbach H, Pantel K, Amadori D, Kang Y.
2536 Tumor-induced osteoclast miRNA changes as regulators and biomarkers of osteolytic bone
2537 metastasis. *Cancer Cell.* 24: 542-556, 2013. doi: 10.1016/j.ccr.2013.09.008.
2538 97. Engblom C, Pfirschke C, Zilionis R, Da Silva Martins J, Bos SA, Courties G, Rickelt S, Severe
2539 N, Baryawno N, Faget J, Savova V, Zemmour D, Kline J, Siwicki M, Garris C, Pucci F, Liao
2540 HW, Lin YJ, Newton A, Yaghi OK, Iwamoto Y, Tricot B, Wojtkiewicz GR, Nahrendorf M,
2541 Cortez-Retamozo V, Meylan E, Hynes RO, Demay M, Klein A, Bredella MA, Scadden DT,
2542 Weissleder R, Pittet MJ. Osteoblasts remotely supply lung tumors with cancer-promoting
2543 SiglecFhigh neutrophils. *Science* 358, 2017. pii: eaal5081. doi: 10.1126/science.aal5081.

98. Erler JT, Bennewith KL, Cox TR, Lang G, Bird D, Koong A, Le QT, Giaccia AJ. Hypoxia-induced lysyl oxidase is a critical mediator of bone marrow cell recruitment to form the premetastatic niche. *Cancer Cell.* 15: 35-44, 2009. doi: 10.1016/j.ccr.2008.11.012.

99. Escudier B, Powles T, Motzer RJ, Olencki T, Arén Frontera O, Oudard S, Rolland F, Tomczak P, Castellano D, Appleman LJ, Drabkin H, Vaena D, Milwee S, Youkstetter J, Lougheed JC, Bracarda S, Choueiri TK. Cabozantinib, a New Standard of Care for Patients With Advanced Renal Cell Carcinoma and Bone Metastases? Subgroup Analysis of the METEOR Trial. *J Clin Oncol.* 36:765-772, 2018. doi: 10.1200/JCO.2017.74.7352.

100. Falk S, Bannister K, Dickenson AH. Cancer pain physiology. *Br J Pain*. 8:154-162, 2014. doi: 10.1177/2049463714545136.

101. Fang Y, Chen Y, Yu L, Zheng C, Qi Y, Li Z, Yang Z, Zhang Y, Shi T, Luo J, Liu M. Inhibition of breast cancer metastases by a novel inhibitor of TGFbeta receptor 1. *J Natl Cancer Inst* 105: 47-58, 2013. doi: 10.1093/jnci/djs485.

102. Fend L, Accart N, Kintz J, Cochin S, Reymann C, Le Pogam F, Marchand JB, Menguy T, Slos P, Rooke R, Fournel S, Bonnefoy JY, Préville X, Haegel H. Therapeutic effects of anti-CD115 monoclonal antibody in mouse cancer models through dual inhibition of tumor-associated macrophages and osteoclasts. *PLoS One*. 8:e73310, 2013. doi: 10.1371/journal.pone.0073310.

103. Finlay IG, Mason MD, Shelley M. Radioisotopes for the palliation of metastatic bone cancer: a systematic review. *Lancet Oncol*. 6: 392-400, 2005. doi: 10.1016/S1470-2045(05)70206-0.

104. Fioramonti M, Santini D, Iuliani M, Ribelli G, Manca P, Papapietro N, Spiezia F, Vincenzi B, Denaro V, Russo A, Tonini G, Pantano F. Cabozantinib targets bone microenvironment modulating human osteoclast and osteoblast functions. *Oncotarget*. 8:20113-20121, 2017. doi: 10.18632/oncotarget.15390.

105. Fizazi K, Carducci M, Smith M, Damião R, Brown J, Karsh L, Milecki P, Shore N, Rader M, Wang H, Jiang Q, Tadros S, Dansey R, Goessl C. Denosumab versus zoledronic acid for treatment of bone metastases in men with castration-resistant prostate cancer: a randomised, double-blind study. *Lancet*. 377:813-822, 2011. doi: 10.1016/S0140-6736(10)62344-6.

106. Florio M, Gunasekaran K, Stolina M, Li X, Liu L, Tipton B, Salimi-Moosavi H, Asuncion FJ, Li C, Sun B, Tan HL, Zhang L, Han CY, Case R, Duguay AN, Grisanti M, Stevens J, Pretorius JK, Pacheco E, Jones H, Chen Q, Soriano BD, Wen J, Heron B, Jacobsen FW, Brisan E, Richards WG, Ke HZ, Ominsky MS. A bispecific antibody targeting sclerostin and DKK-1 promotes bone mass accrual and fracture repair. *Nat Commun*. 7:11505, 2016. doi: 10.1038/ncomms11505.

107. Fournier PG, Juárez P, Jiang G, Clines GA, Niewolna M, Kim HS, Walton HW, Peng XH, Liu Y, Mohammad KS, Wells CD, Chirgwin JM, Guise TA. The TGF-β signaling regulator PMEPA1 suppresses prostate cancer metastases to bone. *Cancer Cell* 27:809-821, 2015. doi: 10.1016/j.ccell.2015.04.009.

108. Fradet A, Bouchet M, Delliaux C, Gervais M, Kan C, Benetollo C, Pantano F, Vargas G, Bouazza L, Croset M, Bala Y, Leroy X, Rosol TJ, Rieusset J, Bellahcène A, Castronovo V, Aubin JE, Clézardin P, Duterque-Coquillaud M, Bonnelye E. Estrogen related receptor alpha in castration-resistant prostate cancer cells promotes tumor progression in bone. *Oncotarget* 7: 77071-77086, 2016. doi: 10.18632/oncotarget.12787.

109. Fradet A, Sorel H, Bouazza L, Goehrig D, Dépalle B, Bellahcène A, Castronovo V, Follet H, Descotes F, Aubin JE, Clézardin P, Bonnelye E. Dual function of ERRα in breast cancer and bone metastasis formation: implication of VEGF and osteoprotegerin. *Cancer Res* 71: 5728-5738, 2011. doi: 10.1158/0008-5472.CAN-11-1431.

110. Frees S, Breuksch I, Haber T, Bauer HK, Chavez-Munoz C, Raven P, Moskalev I, D Costa N, Tan Z, Daugaard M, Thüroff JW, Haferkamp A, Prawitt D, So A, Brenner W. Calcium-sensing

receptor (CaSR) promotes development of bone metastasis in renal cell carcinoma. *Oncotarget*. 9:15766-15779, 2018. doi: 10.18632/oncotarget.24607.

111. Fujisaki J, Wu J, Carlson AL, Silberstein L, Putheti P, Larocca R, Gao W, Saito TI, Lo Celso C, Tsuyuzaki H, Sato T, Côté D, Sykes M, Strom TB, Scadden DT, Lin CP. In vivo imaging of Treg cells providing immune privilege to the haematopoietic stem-cell niche. *Nature* 474: 216-219, 2011. doi: 10.1038/nature10160.

112. Fukuda T, Takeda S, Xu R, Ochi H, Sunamura S, Sato T, Shibata S, Yoshida Y, Gu Z, Kimura A, Ma C, Xu C, Bando W, Fujita K, Shinomiya K, Hirai T, Asou Y, Enomoto M, Okano H, Okawa A, Itoh H. Sema3A regulates bone-mass accrual through sensory innervations. *Nature* 497: 490-493, 2013. doi: 10.1038/nature12115.

113. Furlan F, Galbiati C, Jorgensen NR, Jensen JE, Mrak E, Rubinacci A, Talotta F, Verde P, Blasi F. Urokinase plasminogen activator receptor affects bone homeostasis by regulating osteoblast and osteoclast function. *J Bone Miner Res* 22:1387-96, 2007. doi: 10.1359/jbmr.070516.

114. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol.* 9: 162-174, 2009. doi: 10.1038/nri2506.

115. Galasko CS. Monitoring of bone metastases. *Schweiz Med Wochenschr.* 111:1873-1875, 1981.

116. Gartrell BA, Saad F. Managing bone metastases and reducing skeletal related events in prostate cancer. *Nat Rev Clin Oncol*. 11:335-345, 2014. doi: 10.1038/nrclinonc.2014.70.

117. Gawrzak S, Rinaldi L, Gregorio S, Arenas EJ, Salvador F, Urosevic J, Figueras-Puig C, Rojo F, Del Barco Barrantes I, Cejalvo JM, Palafox M, Guiu M, Berenguer-Llergo A, Symeonidi A, Bellmunt A, Kalafatovic D, Arnal-Estapé A, Fernández E, Müllauer B, Groeneveld R, Slobodnyuk K, Stephan-Otto Attolini C, Saura C, Arribas J, Cortes J, Rovira A, Muñoz M, Lluch A, Serra V, Albanell J, Prat A, Nebreda AR, Benitah SA, Gomis RR. MSK1 regulates luminal

cell differentiation and metastatic dormancy in ER+ breast cancer. *Nat Cell Biol.* 20: 211-221, 2018. doi: 10.1038/s41556-017-0021-z.

118. Geissmann F, Manz MG, Jung S, Sieweke MH, Merad M, Ley K. Development of monocytes, macrophages, and dendritic cells. *Science.* 327: 656-61, 2010. doi: 10.1126/science.

119. Ghajar CM, Peinado H, Mori H, Matei IR, Evason KJ, Brazier H, Almeida D, Koller A, Hajjar KA, Stainier DY, Chen EI, Lyden D, Bissell MJ. The perivascular niche regulates breast tumour dormancy. *Nat Cell Biol* 15: 807-817, 2013. doi: 10.1038/ncb2767.

120. Gnant M, Baselga J, Rugo HS, Noguchi S, Burris HA, Piccart M, Hortobagyi GN, Eakle J, Mukai H, Iwata H, Geberth M, Hart LL, Hadji P, El-Hashimy M, Rao S, Taran T, Sahmoud T, Lebwohl D, Campone M, Pritchard KI. Effect of everolimus on bone marker levels and progressive disease in bone in BOLERO-2. *J Natl Cancer Inst* 105(9):654-663, 2013. doi: 10.1093/jnci/djt026.

121. Gnant M, Pfeiler G, Dubsky PC, Hubalek M, Greil R, Jakesz R, Wette V, Balic M, Haslbauer F, Melbinger E, Bjelic-Radisic V, Artner-Matuschek S, Fitzal F, Marth C, Sevelda P, Mlineritsch B, Steger GG, Manfreda D, Exner R, Egle D, Bergh J, Kainberger F, Talbot S, Warner D, Fesl C, Singer CF; Austrian Breast and Colorectal Cancer Study Group. Adjuvant denosumab in breast cancer (ABCSG-18): a multicentre, randomised, double-blind, placebo-controlled trial. *Lancet*. 386:433-443, 2015. doi: 10.1016/S0140-6736(15)60995-3.

122. Gnant M, Pfeiler G, Steger GG, Egle D, Greil R, Fitzal F, Wette V, Balic M, Haslbauer F, Melbinger-Zeinitzer E, Bjelic-Radisic V, Jakesz R, Marth C, Sevelda P, Mlineritsch B, Exner R, Fesl C, Frantal S, Singer CF, on behalf of Austrian Breast and Colorectal Cancer Study Group. Adjuvant denosumab in postmenopausal patients with hormone receptor-positive breast cancer (ABCSG-18): disease-free survival results from a randomized, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol*. 20: 339-351, 2019. doi: 10.1016/S1470-2045(18)30862-3.

123. Gnant MF, Mlineritsch B, Luschin-Ebengreuth G, Grampp S, Kaessmann H, Schmid M, Menzel C, Piswanger-Soelkner JC, Galid A, Mittlboeck M, Hausmaninger H, Jakesz R; Austrian Breast and Colorectal Cancer Study Group. Zoledronic acid prevents cancer treatment-induced bone loss in premenopausal women receiving adjuvant endocrine therapy for hormone-responsive breast cancer: a report from the Austrian Breast and Colorectal Cancer Study Group. J Clin Oncol. 25:820-828, 2007. doi: 10.1200/JCO.2005.02.7102.

124. Goto T, Nakao K, Gunjigake KK, Kido MA, Kobayashi S, Tanaka T. Substance P stimulates late-stage rat osteoblastic bone formation through neurokinin-1 receptors. *Neuropeptides* 41: 25-31, 2007. doi: 10.1016/j.npep.2006.11.002.

125. Grimaud E, Soubigou L, Couillaud S, Coipeau P, Moreau A,Passuti N, Gouin F, Redini F, Heymann D. Receptor activator of nuclear factor kappaB ligand (RANKL)/osteoprotegerin (OPG) ratio is increased in severe osteolysis. *Am. J Pathol*. 163: 2021-2031, 2003. doi: 10.1016/s0002-9440(10)63560-2.

126. Guise TA, Yin JJ, Taylor SD, Kumagai Y, Dallas M, Boyce BF, Yoneda T, Mundy GR. Evidence for a causal role of parathyroid hormone-related protein in the pathogenesis of human breast cancer-mediated osteolysis. *J Clin Invest*. 98:1544-1549, 1996. DOI: 10.1172/JCI118947.

127. Hadji P, Aapro MS, Body JJ, Gnant M, Brandi ML, Reginster JY, Zillikens MC, Glüer CC, de Villiers T, Baber R, Roodman GD, Cooper C, Langdahl B, Palacios S, Kanis J, Al-Daghri N, Nogues X, Eriksen EF, Kurth A, Rizzoli R, Coleman RE. Management of Aromatase Inhibitor-Associated Bone Loss (AIBL) in postmenopausal women with hormone sensitive breast cancer: Joint position statement of the IOF, CABS, ECTS, IEG, ESCEO IMS, and SIOG. *J Bone Oncol*. 7:1-12, 2017. doi: 10.1016/j.jbo.2017.03.001.

128. Hadji P, Coleman RE, Wilson C, Powles TJ, Clézardin P, Aapro M, Costa L, Body JJ, Markopoulos C, Santini D, Diel I, Di Leo A, Cameron D, Dodwell D, Smith I, Gnant M, Gray R,

Harbeck N, Thurlimann B, Untch M, Cortes J, Martin M, Albert US, Conte PF, Ejlertsen B, Bergh J, Kaufmann M, Holen I. Adjuvant bisphosphonates in early breast cancer: consensus guidance for clinical practice from a European Panel. *Ann Oncol.* 27:379-390, 2016. doi: 10.1093/annonc/mdv617.

129. Hall CL, Bafico A, Dai J, Aaronson SA, Keller ET. Prostate cancer cells promote osteoblastic bone metastases through Wnts. *Cancer Res*. 65:7554-7560, 2005. doi: 10.1158/0008-5472.CAN-05-1317.

130. Hall CL, Daignault SD, Shah RB, Pienta KJ, Keller ET. Dickkopf-1 expression increases early in prostate cancer development and decreases during progression from primary tumor to metastasis. *Prostate*. 68:1396-1404, 2008. doi: 10.1002/pros.20805.

131. Hall CL, Zhang H, Baile S, Ljungman M, Kuhstoss S, Keller ET. p21CIP-1/WAF-1 induction is required to inhibit prostate cancer growth elicited by deficient expression of the Wnt inhibitor Dickkopf-1. Cancer Res. 70:9916-9926, 2010. doi: 10.1158/0008-5472.CAN-10-0440.

132. Hamidi H, Ivaska J. Every step of the way: integrins in cancer progression and metastasis. *Nat Rev Cancer* 2018. doi: 10.1038/s41568-018-0038-z.

133. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 144: 646-674, 2011. doi: 10.1016/j.cell.2011.02.013.

134. Hardaway AL, Herroon MK, Rajagurubandara E, Podgorski I. Bone marrow fat: linking adipocyte-induced inflammation with skeletal metastases. *Cancer Metastasis Rev*. 33:527-453, 2014. doi: 10.1007/s10555-013-9484-y.

135. Hardaway AL, Herroon MK, Rajagurubandara E, Podgorski I. Marrow adipocyte-derived CXCL1 and CXCL2 contribute to osteolysis in metastatic prostate cancer. *Clin Exp Metastasis.* 32:353-368, 2015. doi: 10.1007/s10585-015-9714-5.

136. Harms JF, Welch DR, Samant RS, Shevde LA, Miele ME, Babu GR, Goldberg SF, Gilman VR, Sosnowski DM, Campo DA, Gay CV, Budgeon LR, Mercer R, Jewell J, Mastro AM, Donahue

HJ, Erin N, Debies MT, Meehan WJ, Jones AL, Mbalaviele G, Nickols A, Christensen ND, Melly R, Beck LN, Kent J, Rader RK, Kotyk JJ, Pagel MD, Westlin WF, Griggs DW. A small molecule antagonist of the alpha(v)beta3 integrin suppresses MDA-MB-435 skeletal metastasis. *Clin Exp Metastasis* 21:119-128, 2004.

137. Hashimoto K, Ochi H, Sunamura S, Kosaka N, Mabuchi Y, Fukuda T, Yao K, Kanda H, Ae K, Okawa A, Akazawa C, Ochiya T, Futakuchi M, Takeda S, Sato S. Cancer-secreted hsa-miR-940 induces an osteoblastic phenotype in the bone metastatic microenvironment via targeting ARHGAP1 and FAM134A. *Proc Natl Acad Sci USA* 115: 2204-2209, 2018. doi: 10.1073/pnas.1717363115.

138. Haug JS, He XC, Grindley JC, Wunderlich JP, Gaudenz K, Ross JT, Paulson A, Wagner KP, Xie Y, Zhu R, Yin T, Perry JM, Hembree MJ, Redenbaugh EP, Radice GL, Seidel C, Li L. N-cadherin expression level distinguishes reserved versus primed states of hematopoietic stem cells. Cell Stem Cell 2:367-379, 2008. doi: 10.1016/j.stem.2008.01.017.

139. Henry DH, Costa L, Goldwasser F, Hirsh V, Hungria V, Prausova J, Scagliotti GV, Sleeboom H, Spencer A, Vadhan-Raj S, von Moos R, Willenbacher W, Woll PJ, Wang J, Jiang Q, Jun S, Dansey R, Yeh H. Randomized, double-blind study of denosumab versus zoledronic acid in the treatment of bone metastases in patients with advanced cancer (excluding breast and prostate cancer) or multiple myeloma. *J Clin Oncol*. 29:1125-1132, 2011. doi: 10.1200/JCO.2010.31.3304.

140. Herroon MK, Diedrich JD, Rajagurubandara E, Martin C, Maddipati KR, Kim S, Heath EI, Granneman J, Podgorski I. Prostate Tumor Cell-Derived IL1β Induces an Inflammatory Phenotype in Bone Marrow Adipocytes and Reduces Sensitivity to Docetaxel via Lipolysis-Dependent Mechanisms. *Mol Cancer Res*. 17:2508-2521, 2019. doi: 10.1158/1541-7786.MCR-19-0540.

141. Herroon MK, Rajagurubandara E, Diedrich JD, Heath EI, Podgorski I. Adipocyte-activated oxidative and ER stress pathways promote tumor survival in bone via upregulation of Heme Oxygenase 1 and Survivin. *Sci Rep*. 8:40, 2018. doi: 10.1038/s41598-017-17800-5.

142. Herroon MK, Rajagurubandara E, Hardaway AL, Powell K, Turchick A, Feldmann D, Podgorski I. Bone marrow adipocytes promote tumor growth in bone via FABP4-dependent mechanisms. *Oncotarget*. 4:2108-2123, 2013. doi: 10.18632/oncotarget.1482.

143. Hesse E, Schröder S, Brandt D, Pamperin J, Saito H, Taipaleenmäki H. Sclerostin inhibition alleviates breast cancer-induced bone metastases and muscle weakness. *JCI Insight*. pii: 125543, 2019. doi: 10.1172/jci.insight.125543.

144. Higano CS. Understanding treatments for bone loss and bone metastases in patients with prostate cancer: a practical review and guide for the clinician. *Urol Clin North Am*. 3: 331-352, 2004. 10.1016/j.ucl.2004.01.001.

145. Himelstein AL, Foster JC, Khatcheressian JL, Roberts JD, Seisler DK, Novotny PJ, Qin R, Go RS, Grubbs SS, O'Connor T, Velasco MR Jr, Weckstein D, O'Mara A, Loprinzi CL, Shapiro CL. Effect of Longer-Interval vs Standard Dosing of Zoledronic Acid on Skeletal Events in Patients With Bone Metastases: A Randomized Clinical Trial. *JAMA*. 317:48-58, 2017. doi: 10.1001/jama.2016.19425.

146. Hiraga T, Kizaka-Kondoh S, Hirota K, Hiraoka M, Yoneda T. Hypoxia and hypoxia-inducible factor-1 expression enhance osteolytic bone metastases of breast cancer. *Cancer Res.* 67: 4157-4163, 2007. doi: 10.1158/0008-5472.CAN-06-2355.

147. Hiraga T, Myoui A, Hashimoto N, Sasaki A, Hata K, Morita Y, Yoshikawa H, Rosen CJ, Mundy GR, Yoneda T. Bone-derived IGF mediates crosstalk between bone and breast cancer cells in bony metastases. *Cancer Res*. 72:4238-4249, 2012. doi: 10.1158/0008-5472.CAN-11-3061.

148. Hiraoka K, Zenmyo M, Watari K, Iguchi H, Fotovati A, Kimura YN, Hosoi F, Shoda T, Nagata K, Osada H, Ono M, Kuwano M. Inhibition of bone and muscle metastases of lung cancer cells

by a decrease in the number of monocytes/ macrophages. *Cancer Sci*. 99:1595-602, 2008. doi: 10.1111/j.1349-7006.2008.00880.x.

149. Hiratsuka S, Watanabe A, Aburatani H, Maru Y. Tumour-mediated upregulation of chemoattractants and recruitment of myeloid cells predetermines lung metastasis. *Nat Cell Biol*. 8:1369-1375, 2006. doi: 10.1038/ncb1507.

150. Hofbauer LC, Rachner TD, Coleman RE, Jakob F. Endocrine aspects of bone metastases. *Lancet Diabetes Endocrinol*. 2:500-512, 2014. doi: 10.1016/S2213-8587(13)70203-1.

151. Holen I, Lefley DV, Francis SE, Rennicks S, Bradbury S, Coleman RE, Ottewell P. IL-1 drives breast cancer growth and bone metastasis in vivo. *Oncotarget* 7: 75571-75584, 2016. doi: 10.18632/oncotarget.12289.

152. Hortobagyi GN. Everolimus plus exemestane for the treatment of advanced breast cancer: a review of subanalyses from BOLERO-2. *Neoplasia* 17:279-288, 2015. doi: 10.1016/j.neo.2015.01.005.

153. Hortobagyi GN, Theriault RL, Porter L, Blayney D, Lipton A, Sinoff C, Wheeler H, Simeone JF, Seaman J, Knight RD. Efficacy of pamidronate in reducing skeletal complications in patients with breast cancer and lytic bone metastases. Protocol 19 Aredia Breast Cancer Study Group. *N Engl J Med*. 335:1785-1791, 1996. doi: 10.1056/NEJM199612123352401.

154. Huang CF, Lira C, Chu K, Bilen MA, Lee YC, Ye X, Kim SM, Ortiz A, Wu FL, Logothetis CJ, Yu-Lee LY, Lin SH. Cadherin-11 increases migration and invasion of prostate cancer cells and enhances their interaction with osteoblasts. Cancer Res 70: 4580-4589, 2010. doi: 10.1158/0008-5472.CAN-09-3016.

155. Huang J, Gu T, Ying J. A meta-analysis survey of appropriate bone turnover markers in the detection of bone metastasis in lung cancer. *Int J Clin Oncol.* 22:1015-1025, 2017. doi: 10.1007/s10147-017-1159-1.

156. Iuliani M, Pantano F, Buttigliero C, Fioramonti M, Bertaglia V, Vincenzi B, Zoccoli A, Ribelli G, Tucci M, Vignani F, Berruti A, Scagliotti GV, Tonini G, Santini D. Biological and clinical effects of abiraterone on anti-resorptive and anabolic activity in bone microenvironment. *Oncotarget*. 6:12520-12528, 2015. doi: 10.18632/oncotarget.3724.

157. James ND, Sydes MR, Clarke NW, Mason MD, Dearnaley DP, Spears MR, Ritchie AW, Parker CC, Russell JM, Attard G, de Bono J, Cross W, Jones RJ, Thalmann G, Amos C, Matheson D, Millman R, Alzouebi M, Beesley S, Birtle AJ, Brock S, Cathomas R, Chakraborti P, Chowdhury S, Cook A, Elliott T, Gale J, Gibbs S, Graham JD, Hetherington J, Hughes R, Laing R, McKinna F, McLaren DB, O'Sullivan JM, Parikh O, Peedell C, Protheroe A, Robinson AJ, Srihari N, Srinivasan R, Staffurth J, Sundar S, Tolan S, Tsang D, Wagstaff J, Parmar MK; STAMPEDE investigators. Addition of docetaxel, zoledronic acid, or both to first-line long-term hormone therapy in prostate cancer (STAMPEDE): survival results from an adaptive, multiarm, multistage, platform randomised controlled trial. *Lancet*. 387:1163-1177, 2016. doi: 10.1016/S0140-6736(15)01037-5.

158. Jamieson WL, Shimizu S, D'Ambrosio JA, Meucci O, Fatatis A. CX3CR1 is expressed by prostate epithelial cells and androgens regulate the levels of CX3CL1/fractalkine in the bone marrow: potential role in prostate cancer bone tropism. *Cancer Res*. 68:1715-22, 2008. doi: 10.1158/0008-5472.CAN-07-1315.

159. Jamieson-Gladney WL, Zhang Y, Fong AM, Meucci O, Fatatis A. The chemokine receptor CX_3CR1 is directly involved in the arrest of breast cancer cells to the skeleton. *Breast Cancer Res*. 13:R91, 2011. doi: 10.1186/bcr3016.

160. Jensen AB, Wynne C, Ramirez G, He W, Song Y, Berd Y, Wang H, Mehta A, Lombardi A. The cathepsin K inhibitor odanacatib suppresses bone resorption in women with breast cancer and established bone metastases: results of a 4-week, double-blind, randomized, controlled trial. *Clin Breast Cancer*. 10:452-458, 2010. doi: 10.3816/CBC.2010.n.059.

161. Jiao S, Subudhi SK, Aparicio A, Ge Z, Guan B, Miura Y, Sharma P. Differences in Tumor Microenvironment Dictate T Helper Lineage Polarization and Response to Immune Checkpoint Therapy. *Cell*. 179:1177-1190.e13, 2019. doi: 10.1016/j.cell.2019.10.029.

162. Jimenez-Andrade JM, Ghilardi JR, Castañeda-Corral G, Kuskowski MA, Mantyh PW. Preventive or late administration of anti-NGF therapy attenuates tumor-induced nerve sprouting, neuroma formation, and cancer pain. *Pain*. 152:2564-2574, 2011. doi: 10.1016/j.pain.2011.07.020.

163. Johnson RW, Finger EC, Olcina MM, Vilalta M, Aguilera T, Miao Y, Merkel AR, Johnson JR, Sterling JA, Wu JY, Giaccia AJ. Induction of LIFR confers a dormancy phenotype in breast cancer cells disseminated to the bone marrow. *Nat Cell Biol* 18: 1078-1089, 2016. doi: 10.1038/ncb3408.

164. Jones DH, Nakashima T, Sanchez OH, Kozieradzki I, Komarova SV, Sarosi I, Morony S, Rubin E, Sarao R, Hojilla CV, Komnenovic V, Kong YY, Schreiber M, Dixon SJ, Sims SM, Khokha R, Wada T, Penninger JM. Regulation of cancer cell migration and bone metastasis by RANKL. *Nature* 440: 692-696, 2006. doi: 10.1038/nature04524.

165. Jung K, Lein M. Bone turnover markers in serum and urine as diagnostic, prognostic and monitoring biomarkers of bone metastasis. *Biochim Biophys Acta*. 1846:425-438, 2014. doi: 10.1016/j.bbcan.2014.09.001.

166. Jung Y, Kim JK, Shiozawa Y, Wang J, Mishra A, Joseph J, Berry JE, McGee S, Lee E, Sun H, Wang J, Jin T, Zhang H, Dai J, Krebsbach PH, Keller ET, Pienta KJ, Taichman RS. Recruitment of mesenchymal stem cells into prostate tumours promotes metastasis. *Nat Commun*. 4:1795, 2013. doi: 10.1038/ncomms2766.

167. Jung Y, Wang J, Lee E, McGee S, Berry JE, Yumoto K, Dai J, Keller ET, Shiozawa Y, Taichman RS. Annexin 2-CXCL12 interactions regulate metastatic cell targeting and growth in the bone marrow. *Mol Cancer Res.* 13:197-207, 2015. doi: 10.1158/1541-7786.MCR-14-0118.

168. Kakonen SM, Selander KS, Chirgwin JM, Yin JJ, Burns S, Rankin WA, Grubbs BG, Dallas M, Cui Y, Guise TA. Transforming growth factor-beta stimulates parathyroid hormone-related protein and osteolytic metastases via Smad and mitogen-activated protein kinase signaling pathways. *J Biol Chem* 277:24571-24578, 2002. doi: 10.1074/jbc.M202561200.

169. Kaplan RN, Riba RD, Zacharoulis S, Bramley AH, Vincent L, Costa C, MacDonald DD, Jin DK, Shido K, Kerns SA, Zhu Z, Hicklin D, Wu Y, Port JL, Altorki N, Port ER, Ruggero D, Shmelkov SV, Jensen KK, Rafii S, Lyden D. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. *Nature*. 438:820-7, 2005. doi: 10.1038/nature04186.

170. Katoh M, Katoh M. Molecular genetics and targeted therapy of WNT-related human diseases. *Int J Mol Med* 40 :587-606, 2017. doi: 10.3892/ijmm.2017.3071.

171. Katsuno Y, Hanyu A, Kanda H, Ishikawa Y, Akiyama F, Iwase T, Ogata E, Ehata S, Miyazono K, Imamura T. Bone morphogenetic protein signaling enhances invasion and bone metastasis of breast cancer cells through Smad pathway. *Oncogene*. 27:6322-6333, 2008. doi: 10.1038/onc.2008.232.

172. Keskinov AA, Shurin MR. Myeloid regulatory cells in tumor spreading and metastasis. *Immunobiology* 220: 236-242, 2015. doi: 10.1016/j.imbio.

173. Khoo WH, Ledergor G, Weiner A, Roden DL, Terry RL, McDonald MM, Chai RC, De Veirman K, Owen KL, Opperman KS, Vandyke K, Clark JR, Seckinger A, Kovacic N, Nguyen A, Mohanty ST, Pettitt JA, Xiao Y, Corr AP, Seeliger C, Novotny M, Lasken RS, Nguyen TV, Oyajobi BO, Aftab D, Swarbrick A, Parker B, Hewett DR, Hose D, Vanderkerken K, Zannettino ACW, Amit I, Phan TG, Croucher PI. A niche-dependent myeloid transcriptome signature defines dormant myeloma cells. *Blood* 134:30-43, 2019. doi: 10.1182/blood.2018880930.

174. Kimura T, Kuwata T, Ashimine S, Yamazaki M, Yamauchi C, Nagai K, Ikehara A, Feng Y, Dimitrov DS, Saito S, Ochiai A. Targeting of bone-derived insulin-like growth factor-II by a

human neutralizing antibody suppresses the growth of prostate cancer cells in a human bone environment. *Clin Cancer Res*. 16:121-129, 2010. doi: 10.1158/1078-0432.CCR-09-0982.

175. Kirschenbaum A, Izadmehr S, Yao S, O'Connor-Chapman KL, Huang A, Gregoriades EM, Yakar S, Levine AC. Prostatic Acid Phosphatase Alters the RANKL/OPG System and Induces Osteoblastic Prostate Cancer Bone Metastases. *Endocrinology* 157:4526-4533, 2016. doi: 10.1210/en.2016-1606.

176. Kitagawa Y, Dai J, Zhang J, Keller JM, Nor J, Yao Z, Keller ET. Vascular endothelial growth factor contributes to prostate cancer-mediated osteoblastic activity. *Cancer Res* 65:10921-10929, 2005. doi: 10.1158/0008-5472.CAN-05-1809.

177. Kobayashi A, Okuda H, Xing F, Pandey PR, Watabe M, Hirota S, Pai SK, Liu W, Fukuda K, Chambers C, Wilber A, Watabe K. Bone morphogenetic protein 7 in dormancy and metastasis of prostate cancer stem-like cells in bone. *J Exp Med* 208: 2641-2655, 2011. doi: 10.1084/jem.20110840.

178. Koeneman KS, Yeung F, Chung LW. Osteomimetic properties of prostate cancer cells: a hypothesis supporting the predilection of prostate cancer metastasis and growth in the bone environment. Prostate 39:246-261, 1999. doi: 10.1002/(sici)1097-0045(19990601)39:4<246::aid-pros5>3.0.co;2-u.

179. Kohno N, Aogi K, Minami H, Nakamura S, Asaga T, Iino Y, Watanabe T, Goessl C, Ohashi Y, Takashima S. Zoledronic acid significantly reduces skeletal complications compared with placebo in Japanese women with bone metastases from breast cancer: a randomized, placebo-controlled trial. *J Clin Oncol*. 23:3314-3321, 2005. doi: 10.1200/JCO.2005.05.116.

180. Koopmans N, de Jong IJ, Breeuwsma AJ, van der Veer E. Serum bone turnover markers (PINP and ICTP) for the early detection of bone metastases in patients with prostate cancer: a longitudinal approach. *J Urol*. 178: 849-853, 2007. doi: 10.1016/j.juro.2007.05.029.

181. Krzeszinski JY, Wei W, Huynh H, Jin Z, Wang X, Chang TC, Xie XJ, He L, Mangala LS, Lopez-Berestein G, Sood AK, Mendell JT, Wan Y. miR-34a blocks osteoporosis and bone metastasis by inhibiting osteoclastogenesis and Tgif2. *Nature*. 512:431-435, 2014. doi: 10.1038/nature13375.

182. Kusumbe AP, Ramasamy SK, Adams RH. Coupling of angiogenesis and osteogenesis by a specific vessel subtype in bone. *Nature* 507:323-328, 2014. doi: 10.1038/nature13145.

183. Lacey DL, Boyle WJ, Simonet WS, Kostenuik PJ, Dougall WC, Sullivan JK, San Martin J, Dansey R. Bench to bedside: elucidation of the OPG-RANK-RANKL pathway and the development of denosumab. *Nat Rev Drug Discov* 11: 401-419, 2012. doi: 10.1038/nrd3705.

184. Lamiche C, Clarhaut J, Strale PO, Crespin S, Pedretti N, Bernard FX, Naus CC, Chen VC, Foster LJ, Defamie N, Mesnil M, Debiais F, Cronier L. The gap junction protein Cx43 is involved in the bone-targeted metastatic behaviour of human prostate cancer cells. *Clin Exp Metastasis*. 29:111-122, 2012. doi: 10.1007/s10585-011-9434-4.

185. Lamoureux F, Baud'huin M, Rodriguez Calleja L, Jacques C, Berreur M, Rédini F, Lecanda F, Bradner JE, Heymann D, Ory B. Selective inhibition of BET bromodomain epigenetic signalling interferes with the bone-associated tumour vicious cycle. *Nat Commun*. 5:3511, 2014. doi: 10.1038/ncomms4511.

186. Langdahl B, Binkley N, Bone H, Gilchrist N, Resch H, Rodriguez Portales J, Denker A, Lombardi A, Le Bailly De Tilleghem C, Dasilva C, Rosenberg E, Leung A. Odanacatib in the treatment of postmenopausal women with low bone mineral density: five years of continued therapy in a phase 2 study. *J Bone Miner Res.* 27:2251-2258, 2012. doi: 10.1002/jbmr.1695.

187. Lara PN Jr, Ely B, Quinn DI, Mack PC, Tangen C, Gertz E, Twardowski PW, Goldkorn A, Hussain M, Vogelzang NJ, Thompson IM, Van Loan MD. Serum biomarkers of bone metabolism in castration-resistant prostate cancer patients with skeletal metastases: results from SWOG 0421. *J Natl Cancer Inst.* 106:dju013, 2014. doi: 10.1093/jnci/dju013.

188. Larson SR, Chin J, Zhang X, Brown LG, Coleman IM, Lakely B, Tenniswood M, Corey E, Nelson PS, Vessella RL, Morrissey C. Prostate cancer derived prostatic acid phosphatase promotes an osteoblastic response in the bone microenvironment. *Clin Exp Metastasis* 31: 247-256, 2014. doi: 10.1007/s10585-013-9625-2.

189. Lawson MA, McDonald MM, Kovacic N, Hua Khoo W, Terry RL, Down J, Kaplan W, Paton-Hough J, Fellows C, Pettitt JA, Neil Dear T, Van Valckenborgh E, Baldock PA, Rogers MJ, Eaton CL, Vanderkerken K, Pettit AR, Quinn JM, Zannettino AC, Phan TG, Croucher PI. Osteoclasts control reactivation of dormant myeloma cells by remodelling the endosteal niche. *Nat Commun* 6:8983, 2015. doi: 10.1038/ncomms9983.

190. Leblanc R, Lee SC, David M, Bordet JC, Norman DD, Patil R, Miller D, Sahay D, Ribeiro J, Clézardin P, Tigyi GJ, Peyruchaud O. Interaction of platelet-derived autotaxin with tumor integrin αVβ3 controls metastasis of breast cancer cells to bone. *Blood* 124:3141-3150, 2014. doi: 10.1182/blood-2014-04-56868.

191. Leblanc R, Peyruchaud O. Metastasis: new functional implications of platelets and megakaryocytes. *Blood* 128:24-31, 2016. doi: 10.1182/blood-2016-01-636399.

192. Lee C, Whang YM, Campbell P, Mulcrone PL, Elefteriou F, Cho SW, Park SI. Dual targeting c-met and VEGFR2 in osteoblasts suppresses growth and osteolysis of prostate cancer bone metastasis. *Cancer Lett*. 414:205-213, 2018. doi: 10.1016/j.canlet.2017.11.016.

193. Lee JH, Kim HN, Kim KO, Jin WJ, Lee S, Kim HH, Ha H, Lee ZH. CXCL10 promotes osteolytic bone metastasis by enhancing cancer outgrowth and osteoclastogenesis. *Cancer Res* 72: 3175-3186, 2012. doi: 10.1158/0008-5472.CAN-12-0481.

194. Lee RJ, Smith MR. Targeting MET and vascular endothelial growth factor receptor signaling in castration-resistant prostate cancer. *Cancer J.* 19:90-98, 2013. doi: 10.1097/PPO.0b013e318281e280.

195. Lee YC, Cheng CJ, Bilen MA, Lu JF, Satcher RL, Yu-Lee LY, Gallick GE, Maity SN, Lin SH. BMP4 promotes prostate tumor growth in bone through osteogenesis. *Cancer Res* 71: 5194-5203, 2011. doi: 10.1158/0008-5472.CAN-10-4374.

196. Le Gall C, Bellahcène A, Bonnelye E, Gasser JA, Castronovo V, Green J, Zimmermann J, Clézardin P. A cathepsin K inhibitor reduces breast cancer induced osteolysis and skeletal tumor burden. *Cancer Res*. 67:9894-9902, 2007. doi: 10.1158/0008-5472.CAN-06-3940.

197. Lemma S, Di Pompo G, Porporato PE, Sboarina M, Russell S, Gillies RJ, Baldini N, Sonveaux P, Avnet S. MDA-MB-231 breast cancer cells fuel osteoclast metabolism and activity: A new rationale for the pathogenesis of osteolytic bone metastases. *Biochim Biophys Acta Mol Basis Dis.* 1863:3254-3264, 2017. doi: 10.1016/j.bbadis.2017.08.030.

198. Leto G. Activin A and bone metastasis. *J Cell Physiol*. 225:302-309, 2010. doi: 10.1002/jcp.22272.

199. Lev DC, Kim SJ, Onn A, Stone V, Nam DH, Yazici S, Fidler IJ, Price JE. Inhibition of platelet-derived growth factor receptor signaling restricts the growth of human breast cancer in the bone of nude mice. *Clin Cancer Res*. 11:306-314, 2005.

200. Li XQ, Lu JT, Tan CC, Wang QS, Feng YM. RUNX2 promotes breast cancer bone metastasis by increasing integrin α5-mediated colonization. *Cancer Lett* 380: 78-86, 2016. doi: 10.1016/j.canlet.2016.06.007.

201. Li ZG, Mathew P, Yang J, Starbuck MW, Zurita AJ, Liu J, Sikes C, Multani AS, Efstathiou E, Lopez A, Wang J, Fanning TV, Prieto VG, Kundra V, Vazquez ES, Troncoso P, Raymond AK, Logothetis CJ, Lin SH, Maity S, Navone NM. Androgen receptor-negative human prostate cancer cells induce osteogenesis in mice through FGF9-mediated mechanisms. *J Clin Invest* 118: 2697-2710, 2008. doi: 10.1172/JCI33093.

202. Li ZG, Yang J, Vazquez ES, Rose D, Vakar-Lopez F, Mathew P, Lopez A, Logothetis CJ, Lin SH, Navone NM. Low-density lipoprotein receptor-related protein 5 (LRP5) mediates the

prostate cancer-induced formation of new bone. *Oncogene* 27:596-603, 2008. doi: 10.1038/sj.onc.1210694.

203. Liao J, Li X, Koh AJ, Berry JE, Thudi N, Rosol TJ, Pienta KJ, McCauley LK. Tumor expressed PTHrP facilitates prostate cancer-induced osteoblastic lesions. *Int J Cancer* 123: 2267-78, 2008. doi: 10.1002/ijc.23602.

204. Lin SC, Lee YC, Yu G, Cheng CJ, Zhou X, Chu K, Murshed M, Le NT, Baseler L, Abe JI, Fujiwara K, deCrombrugghe B, Logothetis CJ, Gallick GE, Yu-Lee LY, Maity SN, Lin SH. Endothelial-to-osteoblast conversion generates osteoblastic metastasis of prostate cancer. *Dev Cell* 41: 467-480, 2017. doi: 10.1016/j.devcel.2017.05.005.

205. Lindemann F, Schlimok G, Dirschedl P, Witte J, Riethmüller G. Prognostic significance of micrometastatic tumour cells in bone marrow of colorectal cancer patients. Lancet. 340:685-689, 1992. doi: 10.1016/0140-6736(92)92230-d.

206. Lipton A, Smith MR, Fizazi K, Stopeck AT, Henry D, Brown JE, Shore ND, Saad F, Spencer A, Zhu L, Warner DJ. Changes in Bone Turnover Marker Levels and Clinical Outcomes in Patients with Advanced Cancer and Bone Metastases Treated with Bone Antiresorptive Agents. *Clin Cancer Res*. 22:5713-5721, 2016. doi: 10.1158/1078-0432.CCR-15-3086.

207. Lipton A, Theriault RL, Hortobagyi GN, Simeone J, Knight RD, Mellars K, Reitsma DJ, Heffernan M, Seaman JJ. Pamidronate prevents skeletal complications and is effective palliative treatment in women with breast carcinoma and osteolytic bone metastases: long term follow-up of two randomized, placebo-controlled trials. *Cancer*. 88:1082-1090, 2000. doi: 10.1002/(sici)1097-0142(20000301)88:5<1082::aid-cncr20>3.0.co;2-z.

208. Liu Z, Liu H, He J, Lin P, Tong Q, Yang J. Myeloma cells shift osteoblastogenesis to adipogenesis by inhibiting the ubiquitin ligase MURF1 in mesenchymal stem cells. *Sci Signal*. 13:eaay8203, 2020. doi: 10.1126/scisignal.aay8203.

209. Llombart A, Frassoldati A, Paija O, Sleeboom HP, Jerusalem G, Mebis J, Deleu I, Miller J, Schenk N, Neven P. Immediate Administration of Zoledronic Acid Reduces Aromatase

Inhibitor-Associated Bone Loss in Postmenopausal Women With Early Breast Cancer: 12-month analysis of the E-ZO-FAST trial. *Clin Breast Cancer*. 12:40-48, 2012. doi: 10.1016/j.clbc.2011.08.002.

210. Logothetis CJ, Lin SH. Osteoblasts in prostate cancer metastasis to bone. *Nat Rev Cancer* 5:21-28, 2005. doi: 10.1038/nrc1528.

211. López-Soto A, Gonzalez S, Smyth MJ, Galluzzi L. Control of Metastasis by NK Cells. Cancer Cell. 32:135-154, 2017. doi:10.1016/j.ccell.2017.06.009

212. Lotinun S, Pearsall RS, Davies MV, Marvell TH, Monnell TE, Ucran J, Fajardo RJ, Kumar R, Underwood KW, Seehra J, Bouxsein ML, Baron R. A soluble activin receptor Type IIA fusion protein (ACE-011) increases bone mass via a dual anabolic-antiresorptive effect in Cynomolgus monkeys. *Bone*. 46:1082-1088, 2010. doi: 10.1016/j.bone.2010.01.370.

213. Lu X, Kang Y. Chemokine (C-C motif) ligand 2 engages CCR2+ stromal cells of monocytic origin to promote breast cancer metastasis to lung and bone. *J Biol Chem*. 284:29087-29096, 2009. doi: 10.1074/jbc.M109.035899.

214. Lu X, Mu E, Wei Y, Riethdorf S, Yang Q, Yuan M, Yan J, Hua Y, Tiede BJ, Lu X, Haffty BG, Pantel K, Massagué J, Kang Y. VCAM-1 promotes osteolytic expansion of indolent bone micrometastasis of breast cancer by engaging α4β1-positive osteoclast progenitors. *Cancer Cell* 20: 701-714, 2011. doi: 10.1016/j.ccr.2011.11.002.

215. Luo J, Yang Z, Ma Y, Yue Z, Lin H, Qu G, Huang J, Dai W, Li C, Zheng C, Xu L, Chen H, Wang J, Li D, Siwko S, Penninger JM, Ning G, Xiao J, Liu M. LGR4 is a receptor for RANKL and negatively regulates osteoclast differentiation and bone resorption. *Nat Med.* 22:539-546, 2016. doi: 10.1038/nm.4076.

216. Luo X, Fu Y, Loza AJ, Murali B, Leahy KM, Ruhland MK, Gang M, Su X, Zamani A, Shi Y, Lavine KJ, Ornitz DM, Weilbaecher KN, Long F, Novack DV, Faccio R, Longmore GD, Stewart

SA. Stromal-Initiated Changes in the Bone Promote Metastatic Niche Development. *Cell Rep*. 14:82-92, 2016. doi: 10.1016/j.celrep.2015.12.016.

217. Luzzi KJ, MacDonald IC, Schmidt EE, Kerkvliet N, Morris VL, Chambers AF, Groom AC. Multistep nature of metastatic inefficiency: dormancy of solitary cells after successful extravasation and limited survival of early micrometastases. *Am J Pathol.* 153:865-873, 1998. 10.1016/S0002-9440(10)65628-3.

218. Ma Y, Shurin GV, Peiyuan Z, Shurin MR. Dendritic cells in the cancer microenvironment. *J Cancer*. 4:36-44, 2013. doi: 10.7150/jca.5046.

219. Maeda H, Yonou H, Yano K, Ishii G, Saito S, Ochiai A. Prostate-specific antigen enhances bioavailability of insulin-like growth factor by degrading insulin-like growth factor binding protein 5. *Biochem Biophys Res Commun* 381: 311-316, 2009. doi: 10.1016/j.bbrc.2009.01.096.

220. Major PP, Cook RJ, Chen BL, Zheng M. Survival-adjusted multiple-event analysis for the evaluation of treatment effects of zoledronic Acid in patients with bone metastases from solid tumors. *Support Cancer Ther*. 2:234-240, 2005. doi: 10.3816/SCT.2005.n.017.

221. Malanchi I, Santamaria-Martínez A, Susanto E, Peng H, Lehr HA, Delaloye JF, Huelsken J. Interactions between cancer stem cells and their niche govern metastatic colonization. *Nature* 481: 85-89, 2011. doi: 10.1038/nature10694.

222. Manisterski M, Golan M, Amir S, Weisman Y, Mabjeesh NJ. Hypoxia induces PTHrP gene transcription in human cancer cells through the HIF-2α. *Cell Cycle*. 9:3723-3729, 2010.

223. Maroni P. Megakaryocytes in Bone Metastasis: Protection or Progression? Cells. 8: 2019. pii: E134. doi: 10.3390/cells8020134.

224. Maroni P, Bendinelli P, Matteucci E, Locatelli A, Nakamura T, Scita G, Desiderio MA. Osteolytic bone metastasis is hampered by impinging on the interplay among autophagy, anoikis and ossification. *Cell Death Dis* 5:e1005, 2014. doi: 10.1038/cddis.2013.465.

225. Marshall JC, Collins JW, Nakayama J, Horak CE, Liewehr DJ, Steinberg SM, Albaugh M, Vidal-Vanaclocha F, Palmieri D, Barbier M, Murone M, Steeg PS. Effect of inhibition of the lysophosphatidic acid receptor 1 on metastasis and metastatic dormancy in breast cancer. *J Natl Cancer Inst* 104: 1306-1319, 2012. doi: 10.1093/jnci/djs319.

226. McCaffrey G, Thompson ML, Majuta L, Fealk MN, Chartier S, Longo G, Mantyh PW. NGF blockade at early times during bone cancer development attenuates bone destruction and increases limb use. *Cancer Res*. 74:7014-7023, 2014. doi: 10.1158/0008-5472.CAN-14-1220.

227. McDonald MM, Reagan MR, Youlten SE, Mohanty ST, Seckinger A, Terry RL, Pettitt JA, Simic MK, Cheng TL, Morse A, Le LMT, Abi-Hanna D, Kramer I, Falank C, Fairfield H, Ghobrial IM, Baldock PA, Little DG, Kneissel M, Vanderkerken K, Bassett JHD, Williams GR, Oyajobi BO, Hose D, Phan TG, Croucher PI. Inhibiting the osteocyte-specific protein sclerostin increases bone mass and fracture resistance in multiple myeloma. *Blood*. 129:3452-3464, 2017. doi: 10.1182/blood-2017-03-773341.

228. Melchior SW, Corey E, Ellis WJ, Ross AA, Layton TJ, Oswin MM, Lange PH, Vessella RL. Early tumor cell dissemination in patients with clinically localized carcinoma of the prostate. *Clin Cancer Res*. 3:249-256, 1997.

229. Mercadante S, Arcuri E. Breakthrough pain in cancer patients: pathophysiology and treatment. *Cancer Treat Rev.* 24:425-432, 1998. doi: 10.1016/s0305-7372(98)90005-6.

230. Meunier P, Aaron J, Edouard C, Vignon G. Osteoporosis and the replacement of cell populations of the marrow by adipose tissue. A quantitative study of 84 iliac bone biopsies. *Clin Orthop Relat Res*. 80:147-154, 1971. doi: 10.1097/00003086-197110000-00021.

231. Miyamoto K, Yoshida S, Kawasumi M, Hashimoto K, Kimura T, Sato Y, Kobayashi T, Miyauchi Y, Hoshi H, Iwasaki R, Miyamoto H, Hao W, Morioka H, Chiba K, Kobayashi T, Yasuda H, Penninger JM, Toyama Y, Suda T, Miyamoto T. Osteoclasts are dispensable for hematopoietic

stem cell maintenance and mobilization. *J Exp Med* 208: 2175-2181, 2011. doi: 10.1084/jem.20101890.

232. Mizutani K, Sud S, McGregor NA, Martinovski G, Rice BT, Craig MJ, Varsos ZS, Roca H, Pienta KJ. The chemokine CCL2 increases prostate tumor growth and bone metastasis through macrophage and osteoclast recruitment. *Neoplasia*. 11:1235-1242, 2009. doi: 10.1593/neo.09988.

233. Mowers EE, Sharifi MN, Macleod KF. Autophagy in cancer metastasis. *Oncogene*. 2017 36:1619-1630, 2017. doi: 10.1038/onc.2016.333.

234. Mulcrone PL, Campbell JP, Clément-Demange L, Anbinder AL, Merkel AR, Brekken RA, Sterling JA, Elefteriou F. Skeletal Colonization by Breast Cancer Cells Is Stimulated by an Osteoblast and β2AR-Dependent Neo-Angiogenic Switch. *J Bone Miner Res.* 32:1442-1454, 2017. doi: 10.1002/jbmr.3133.

235. Müller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verástegui E, Zlotnik A. Involvement of chemokine receptors in breast cancer metastasis. *Nature* 410: 50-56, 2001. doi: 10.1038/35065016.

236. Mundy GR. Metastasis to bone: causes, consequences and therapeutic opportunities. *Nat Rev Cancer*. 2:584-593, 2002. DOI: 10.1038/nrc867.

237. Murillo-Garzón V, Kypta R. WNT signalling in prostate cancer. *Nat Rev Urol*. 14:683-696, 2017. doi: 10.1038/nrurol.2017.144.

238. Nakai Y, Okamoto K, Terashima A, Ehata S, Nishida J, Imamura T, Ono T, Takayanagi H. Efficacy of an orally active small-molecule inhibitor of RANKL in bone metastasis. *Bone Res*. 7:1, 2019. doi: 10.1038/s41413-018-0036-5.

239. Nandana S, Tripathi M, Duan P, Chu CY, Mishra R, Liu C, Jin R, Yamashita H, Zayzafoon M, Bhowmick NA, Zhau HE, Matusik RJ, Chung LW. Bone Metastasis of Prostate Cancer Can Be

Therapeutically Targeted at the TBX2-WNT Signaling Axis. *Cancer Res* 77:1331-1344, 2017. doi: 10.1158/0008-5472.CAN-16-0497.

240. Nelson JB, Fizazi K, Miller K, Higano C, Moul JW, Akaza H, Morris T, McIntosh S, Pemberton K, Gleave M. Phase 3, randomized, placebo-controlled study of zibotentan (ZD4054) in patients with castration-resistant prostate cancer metastatic to bone. *Cancer*. 118:5709-18, 2012. doi: 10.1002/cncr.27674.

241. Nguyen DX, Bos PD, Massagué J. Metastasis: from dissemination to organ-specific colonization. *Nat Rev Cancer* 9: 274-284, 2009. doi: 10.1038/nrc2622.

242. Nilsson S, Larsen RH, Fossa SD. First clinical experience with a-emitting radium-223 in the treatment of skeletal metastases. *Clin Cancer Res*. 11:4451-4459, 2005.

243. Nutter F, Holen I, Brown HK, Cross SS, Evans CA, Walker M, Coleman RE, Westbrook JA, Selby PJ, Brown JE, Ottewell PD. Different molecular profiles are associated with breast cancer cell homing compared with colonisation of bone: evidence using a novel bone-seeking cell line. *Endocr Relat Cancer.* 21:327-341, 2014. doi: 10.1530/ERC-13-0158.

244. O'Donnell A, Judson I, Dowsett M, Raynaud F, Dearnaley D, Mason M, Harland S, Robbins A, Halbert G, Nutley B, Jarman M. Hormonal impact of the 17alpha-hydroxylase/C(17,20)-lyase inhibitor abiraterone acetate (CB7630) in patients with prostate cancer. Br J Cancer. 90:2317-2325, 2004. doi: 10.1038/sj.bjc.6601879.

245. Okamoto K, Nakashima T, Shinohara M, Negishi-Koga T, Komatsu N, Terashima A, Sawa S, Nitta T, Takayanagi H. Osteoimmunology: The Conceptual Framework Unifying the Immune and Skeletal Systems. *Physiol Rev* 97: 1295-1349, 2017. doi: 10.1152/physrev.00036.

246. Ono M, Kosaka N, Tominaga N, Yoshioka Y, Takeshita F, Takahashi RU, Yoshida M, Tsuda H, Tamura K, Ochiya T. Exosomes from bone marrow mesenchymal stem cells contain a microRNA that promotes dormancy in metastatic breast cancer cells. *Sci Signal*. 7:ra63, 2014. doi: 10.1126/scisignal.2005231.

247. Oskarsson T, Batlle E, Massagué J. Metastatic stem cells: sources, niches, and vital pathways. *Cell Stem Cell* 14: 306-321, 2014. doi: 10.1016/j.stem.2014.02.002.

248. Ottewell PD. The role of osteoblasts in bone metastasis. *J Bone Oncol* 5: 124-127, 2016. doi: 10.1016/j.jbo.2016.03.007.

249. Ottewell PD, Wang N, Brown HK, Fowles CA, Croucher PI, Eaton CL, Holen I. OPG-Fc inhibits ovariectomy-induced growth of disseminated breast cancer cells in bone. *Int J Cancer* 137:968-977, 2015. doi: 10.1002/ijc.29439.

250. Ottewell PD, Wang N, Brown HK, Reeves KJ, Fowles CA, Croucher PI, Eaton CL, Holen I. Zoledronic acid has differential antitumor activity in the pre- and postmenopausal bone microenvironment in vivo. *Clin Cancer Res* 20: 2922-2932, 2014. doi: 10.1158/1078-0432.CCR-13-1246.

251. Ottewell PD, Wang N, Meek J, Fowles CA, Croucher PI, Eaton CL, Holen I. Castration-induced bone loss triggers growth of disseminated prostate cancer cells in bone. *Endocr Relat Cancer* 21: 769-781, 2014. doi: 10.1530/ERC-14-0199.

252. Ouellet V, Tiedemann K, Mourskaia A, Fong JE, Tran-Thanh D, Amir E, Clemons M, Perbal B, Komarova SV, Siegel PM. CCN3 impairs osteoblast and stimulates osteoclast differentiation to favor breast cancer metastasis to bone. *Am J Pathol* 178: 2377-2388, 2011. doi: 10.1016/j.ajpath.2011.01.033.

253. Owen KL, Parker BS. Beyond the vicious cycle: The role of innate osteoimmunity, automimicry and tumor-inherent changes in dictating bone metastasis. *Mol Immunol*. 110: 57-68, 2019. doi: 10.1016/j.molimm.2017.11.023.

254. Pantano F, Rossi E, Iuliani M, Facchinetti A, Simonetti S, Ribelli G, Zoccoli A, Vincenzi B, Tonini G, Zamarchi R, Santini D. Dynamic changes of Receptor activator of nuclear factor-κB expression in Circulating Tumor Cells during Denosumab predict treatment effectiveness in Metastatic Breast Cancer. *Sci Rep*. 10:1288, 2020. doi: 10.1038/s41598-020-58339-2.

255. Pantano F, Croset M, Driouch K, Bednarz-Knoll N, Iuliani M, Ribelli G, Bonnelye E, Wilkman H, Geraci S, Bonin F, Simonetti S, Vincenzi B, Hong SS, Sousa S, Pantel K, Tonini G, Santini D, Clézardin P. Integrin alpha-5 in human breast cancer is a mediator of bone metastasis and a therapeutic target for the treatment of osteolytic lesions. *Oncogene* (accepted for publication).

256. Parfitt AM, Mundy GR, Roodman GD, Hughes DE, Boyce BF. A new model for the regulation of bone resorption with particular reference to the effects of bisphosphonates. *J Bone Min Res*. 11:150-159, 1996.

257. Park SI, Lee C, Sadler WD, Koh AJ, Jones J, Seo JW, Soki FN, Cho SW, Daignault SD, McCauley LK. Parathyroid hormone-related protein drives a CD11b+Gr1+ cell-mediated positive feedback loop to support prostate cancer growth. Cancer Res. 73:6574-6583, 2013. doi: 10.1158/0008-5472.CAN-12-4692.

258. Parker C, Nilsson S, Heinrich D, Helle SI, O'Sullivan JM, Fosså SD, Chodacki A, Wiechno P, Logue J, Seke M, Widmark A, Johannessen DC, Hoskin P, Bottomley D, James ND, Solberg A, Syndikus I, Kliment J, Wedel S, Boehmer S, Dall'Oglio M, Franzén L, Coleman R, Vogelzang NJ, O'Bryan-Tear CG, Staudacher K, Garcia-Vargas J, Shan M, Bruland ØS, Sartor O; ALSYMPCA Investigators. Alpha emitter radium-223 and survival in metastatic prostate cancer. *N Engl J Med*. 369:213-223, 2013. doi: 10.1056/NEJMoa1213755.

259. Pavlovic M, Arnal-Estapé A, Rojo F, Bellmunt A, Tarragona M, Guiu M, Planet E, Garcia-Albéniz X, Morales M, Urosevic J, Gawrzak S, Rovira A, Prat A, Nonell L, Lluch A, Jean-Mairet J, Coleman R, Albanell J, Gomis RR. Enhanced MAF Oncogene Expression and Breast Cancer Bone Metastasis. J Natl Cancer Inst. 107:djv256, 2015. doi: 10.1093/jnci/djv256.

260. Pécheur I, Peyruchaud O, Serre CM, Guglielmi J, Voland C, Bourre F, Margue C, Cohen-Solal M, Buffet A, Kieffer N, Clézardin P. Integrin alpha(v)beta3 expression confers on tumor cells a greater propensity to metastasize to bone. *FASEB J* 16:1266-1268, 2002. doi: 10.1096/fj.01-0911fje.

261. Peinado H, Zhang H, Matei IR, Costa-Silva B, Hoshino A, Rodrigues G, Psaila B, Kaplan RN, Bromberg JF, Kang Y, Bissell MJ, Cox TR, Giaccia AJ, Erler JT, Hiratsuka S, Ghajar CM, Lyden D. Pre-metastatic niches: organ-specific homes for metastases. *Nat Rev Cancer* 17: 302-317, 2017. doi: 10.1038/nrc.2017.6.

262. Peterson LM, O'Sullivan J, Wu QV, Novakova-Jiresova A, Jenkins I, Lee JH, Shields A, Montgomery S, Linden HM, Gralow JR, Gadi VK, Muzi M, Kinahan PE, Mankoff DA, Specht JM. Prospective study of serial 18F-FDG PET and 18F-fluoride (18F-NaF) PET to predict time to skeletal related events, time-to-progression, and survival in patients with bone-dominant metastatic breast cancer. *J Nucl Med* 59: 1823-1830, 2018.doi: 10.2967/jnumed.118.211102.

263. Pez F, Dayan F, Durivault J, Kaniewski B, Aimond G, Le Provost GS, Deux B, Clézardin P, Sommer P, Pouysségur J, Reynaud C. The HIF-1-inducible lysyl oxidase activates HIF-1 via the Akt pathway in a positive regulation loop and synergizes with HIF-1 in promoting tumor cell growth. *Cancer Res* 71: 1647-1657, 2011. doi: 10.1158/0008-5472.CAN-10-1516.

264. Pham JT, Kim RC, Nguyen A, Bota D, Kong XT, Vadera S, Hsu F, Carrillo JA. Intracranial meningioma with carcinoma tumor-to-tumor metastasis: two case reports. CNS Oncol. 7:CNS09, 2018. doi: 10.2217/cns-2017-0022.

265. Powell GJ, Southby J, Danks JA, Stillwell RG, Hayman JA, Henderson MA, Bennett RC, Martin TJ. Localization of parathyroid hormone-related protein in breast cancer metastases: increased incidence in bone compared with other sites. *Cancer Res* 51:3059-3061, 1991.

266. Pratap J, Lian JB, Stein GS. Metastatic bone disease: role of transcription factors and future targets. *Bone* 48: 30-36, 2011. doi: 10.1016/j.bone.2010.05.035.

267. Price TT, Burness ML, Sivan A, Warner MJ, Cheng R, Lee CH, Olivere L, Comatas K, Magnani J, Kim Lyerly H, Cheng Q, McCall CM, Sipkins DA. Dormant breast cancer micrometastases reside in specific bone marrow niches that regulate their transit to and from bone. *Sci Transl Med* 8: 340ra73, 2016. doi: 10.1126/scitranslmed.aad4059.

268. Puisieux A, Brabletz T, Caramel J. Oncogenic roles of EMT-inducing transcription factors. *Nat Cell Biol* 16:488-494, 2014. doi: 10.1038/ncb2976.

269. Rao S, Cronin SJF, Sigl V, Penninger JM. RANKL and RANK: From Mammalian Physiology to Cancer Treatment. *Trends Cell Biol*. 28:213-223, 2018. doi: 10.1016/j.tcb.2017.11.001.

270. Ravnik J, Ravnik M, Bunc G, Glumbic I, Tobi-Veres E, Velnar T. Metastasis of an occult pulmonary carcinoma into meningioma: a case report. World J Surg Oncol 13:292, 2015. doi: 10.1186/s12957-015-0714-3

271. Reagan MR, Rosen CJ. Navigating the bone marrow niche: translational insights and cancer-driven dysfunction. *Nature Rev Rheum* 12: 154-168, 2016. doi: 10.1038/nrrheum.2015.160.

272. Reyes ME, Fujii T, Branstetter D, Krishnamurthy S, Masuda H, Wang X, Reuben JM, Woodward WA, Edwards BJ, Hortobagyi GN, Tripathy D, Dougall WC, Eckhardt BL, Ueno NT. Poor prognosis of patients with triple-negative breast cancer can be stratified by RANK and RANKL dual expression. *Breast Cancer Res Treat* 164: 57-67, 2017. doi: 10.1007/s10549-017-4233-5.

273. Reymond N, d'Água BB, Ridley AJ. Crossing the endothelial barrier during metastasis. *Nat Rev Cancer* 13: 858-870, 2013. doi: 10.1038/nrc3628.

274. Reynaud C, Ferreras L, Di Mauro P, Kan C, Croset M, Bonnelye E, Pez F, Thomas C, Aimond G, Karnoub AE, Brevet M, Clézardin P. Lysyl Oxidase Is a Strong Determinant of Tumor Cell Colonization in Bone. *Cancer Res* 77: 268-278, 2017. doi: 10.1158/0008-5472.CAN-15-2621.

275. Romero-Moreno R, Curtis KJ, Coughlin TR, Miranda-Vergara MC, Dutta S, Natarajan A, Facchine BA, Jackson KM, Nystrom L, Li J, Kaliney W, Niebur GL, Littlepage LE. The CXCL5/CXCR2 axis is sufficient to promote breast cancer colonization during bone metastasis. *Nat Commun* 10: 4404, 2019. doi: 10.1038/s41467-019-12108-6.

276. Rosanò L, Spinella F, Bagnato A. Endothelin 1 in cancer: biological implications and therapeutic opportunities. *Nat Rev Cancer*. 13:637-651, 2013. doi: 10.1038/nrc3546.

277. Rosen LS, Gordon D, Kaminski M, Howell A, Belch A, Mackey J, Apffelstaedt J, Hussein MA, Coleman RE, Reitsma DJ, Chen BL, Seaman JJ. Long-term efficacy and safety of zoledronic acid compared with pamidronate disodium in the treatment of skeletal complications in patients with advanced multiple myeloma or breast carcinoma: a randomized, double-blind, multicenter, comparative trial. *Cancer*. 98:1735-1744, 2003. doi: 10.1002/cncr.11701.

278. Rosen LS, Gordon D, Kaminski M, Howell A, Belch A, Mackey J, Apffelstaedt J, Hussein M, Coleman RE, Reitsma DJ, Seaman JJ, Chen BL, Ambros Y. Zoledronic acid versus pamidronate in the treatment of skeletal metastases in patients with breast cancer or osteolytic lesions of multiple myeloma: a phase III, double-blind, comparative trial. *Cancer J.* 7:377-387, 2001.

279. Rosen LS, Gordon D, Tchekmedyian NS, Yanagihara R, Hirsh V, Krzakowski M, Pawlicki M, De Souza P, Zheng M, Urbanowitz G, Reitsma D, Seaman J. Long-term efficacy and safety of zoledronic acid in the treatment of skeletal metastases in patients with nonsmall cell lung carcinoma and other solid tumors: a randomized, Phase III, double-blind, placebo-controlled trial. *Cancer*. 100:2613-2621, 2004. doi: 10.1002/cncr.20308.

280. Rosen LS, Gordon D, Tchekmedyian S, Yanagihara R, Hirsh V, Krzakowski M, Pawlicki M, de Souza P, Zheng M, Urbanowitz G, Reitsma D, Seaman JJ. Zoledronic acid versus placebo in the treatment of skeletal metastases in patients with lung cancer and other solid tumors: a phase III, double-blind, randomized trial--the Zoledronic Acid Lung Cancer and Other Solid Tumors Study Group. *J Clin Oncol.* 21:3150-3157, 2003. DOI: 10.1200/JCO.2003.04.105.

281. Roskoski R Jr. Src protein-tyrosine kinase structure, mechanism, and small molecule inhibitors. *Pharmacol Res*. 94:9-25, 2015. doi: 10.1016/j.phrs.2015.01.003.

282. Rossnagl S, Ghura H, Groth C, Altrock E, Jakob F, Schott S, Wimberger P, Link T, Kuhlmann JD, Stenzl A, Hennenlotter J, Todenhöfer T, Rojewski M, Bieback K, Nakchbandi IA. A

Subpopulation of Stromal Cells Controls Cancer Cell Homing to the Bone Marrow. Cancer Res 78: 129-142, 2018. doi: 10.1158/0008-5472.CAN-16-3507.

283. Saad F, Gleason DM, Murray R, Tchekmedyian S, Venner P, Lacombe L, Chin JL, Vinholes JJ, Goas JA, Zheng M; Zoledronic Acid Prostate Cancer Study Group. Long-term efficacy of zoledronic acid for the prevention of skeletal complications in patients with metastatic hormone-refractory prostate cancer. *J Natl Cancer Inst*. 96:879-882, 2004. doi: 10.1093/jnci/djh141.

284. Sahay D, Leblanc R, Grunewald TG, Ambatipudi S, Ribeiro J, Clézardin P, Peyruchaud O. The LPA1/ZEB1/miR-21-activation pathway regulates metastasis in basal breast cancer. Oncotarget 6:20604-20620, 2015. doi: 10.18632/oncotarget.3774.

285. San Martin R, Pathak R, Jain A, Jung SY, Hilsenbeck SG, Piña-Barba MC, Sikora AG, Pienta KJ, Rowley DR. Tenascin-C and Integrin α9 Mediate Interactions of Prostate Cancer with the Bone Microenvironment. *Cancer Res* 77: 5977-5988, 2017. doi: 10.1158/0008-5472.CAN-17-0064.

286. Santini D, Schiavon G, Vincenzi B, Gaeta L, Pantano F, Russo A, Ortega C, Porta C, Galluzzo S, Armento G, La Verde N, Caroti C, Treilleux I, Ruggiero A, Perrone G, Addeo R, Clézardin P, Muda AO, Tonini G. Receptor activator of NF-kB (RANK) expression in primary tumors associates with bone metastasis occurrence in breast cancer patients. *PLoS One* 6:e19234, 2011. doi: 10.1371/journal.pone.0019234.

287. Sato MM, Nakashima A, Nashimoto M, Yawaka Y, Tamura M. Bone morphogenetic protein-2 enhances Wnt/beta-catenin signaling-induced osteoprotegerin expression. *Genes Cells* 14: 141-153, 2009. doi: 10.1111/j.1365-2443.2008.01258.x.

288. Sawant A, Deshane J, Jules J, Lee CM, Harris BA, Feng X, Ponnazhagan S. Myeloid-derived suppressor cells function as novel osteoclast progenitors enhancing bone loss in breast cancer. *Cancer Res* 73: 672-82, 2013. doi: 10.1158/0008-5472.

289. Sawant A, Hensel JA, Chanda D, Harris BA, Siegal GP, Maheshwari A, Ponnazhagan S. Depletion of plasmacytoid dendritic cells inhibits tumor growth and prevents bone metastasis of breast cancer cells. *J Immunol*. 189:4258-4265, 2012. doi: 10.4049/jimmunol.1101855.

290. Scalzi P, Baiocco C, Genovese S, Trevisan A, Sirotova Z, Poti C. Evaluation of bone metastases by 18F-choline PET/CT in a patient with castration-resistant prostate cancer treated with radium-223. *Urologia*. 84:61-64, 2017. doi: 10.5301/uro.5000206.

291. Schild T, Low V, Blenis J, Gomes AP. Unique Metabolic Adaptations Dictate Distal Organ-Specific Metastatic Colonization. *Cancer Cell*. 33:347-354, 2018. doi: 10.1016/j.ccell.2018.02.001.

292. Schoemaker MJ, Jones ME, Wright LB, Griffin J2, McFadden E, Ashworth A, Swerdlow AJ. Psychological stress, adverse life events and breast cancer incidence: a cohort investigation in 106,000 women in the United Kingdom. *Breast Cancer Res*. 18:72, 2016. doi: 10.1186/s13058-016-0733-1.

293. Secondini C, Wetterwald A, Schwaninger R, Thalmann GN, Cecchini MG. The role of the BMP signaling antagonist noggin in the development of prostate cancer osteolytic bone metastasis. *PLoS One*. 6:e16078, 2011. doi: 10.1371/journal.pone.0016078.

294. Seeliger C, Karpinski K, Haug AT, Vester H, Schmitt A, Bauer JS, van Griensven M. Five freely circulating miRNAs and bone tissue miRNAs are associated with osteoporotic fractures. *J Bone Miner Res*. 29:1718-1728, 2014. doi: 10.1002/jbmr.2175.

295. Sethi N, Dai X, Winter CG, Kang Y. Tumor-derived JAGGED1 promotes osteolytic bone metastasis of breast cancer by engaging notch signaling in bone cells. *Cancer Cell.* 19: 192-205, 2011. doi: 10.1016/j.ccr.2010.12.022.

296. Shahinian VB, Kuo YF, Freeman JL, Goodwin JS. Risk of fracture after androgen deprivation for prostate cancer. *N Engl J Med*. 352:154-164, 2005. doi: 10.1056/NEJMoa041943.

297. Shao G, Gu W, Guo M, Zang S, Fu J, Liu S, Wang F, Wang Z. Clinical study of (99m)Tc-3P-RGD2 peptide imaging in osteolytic bone metastasis. *Oncotarget*. 8:75587-75596, 2017. doi: 10.18632/oncotarget.17486.

298. Shiozawa Y, Pedersen EA, Havens AM, Jung Y, Mishra A, Joseph J, Kim JK, Patel LR, Ying C, Ziegler AM, Pienta MJ, Song J, Wang J, Loberg RD, Krebsbach PH, Pienta KJ, Taichman RS. Human prostate cancer metastases target the hematopoietic stem cell niche to establish footholds in mouse bone marrow. *J Clin Invest* 121: 1298-1312, 2011. doi: 10.1172/JCI43414.

299. Siclari VA, Mohammad KS, Tompkins DR, Davis H, McKenna CR, Peng X, Wessner LL, Niewolna M, Guise TA, Suvannasankha A, Chirgwin JM. Tumor-expressed adrenomedullin accelerates breast cancer bone metastasis. *Breast Cancer Res*. 16: 458, 2014. doi: 10.1186/s13058-014-0458-y.

300. Singh S, Singh R, Sharma PK, Singh UP, Rai SN, Chung LW, Cooper CR, Novakovic KR, Grizzle WE, Lillard JW Jr. Serum CXCL13 positively correlates with prostatic disease, prostate-specific antigen and mediates prostate cancer cell invasion, integrin clustering and cell adhesion. *Cancer Lett*. 283:29-35, 2009. doi: 10.1016/j.canlet.2009.03.022.

301. Sipkins DA, Wei X, Wu JW, Runnels JM, Côté D, Means TK, Luster AD, Scadden DT, Lin CP. In vivo imaging of specialized bone marrow endothelial microdomains for tumour engraftment. *Nature* 435: 969-973, 2005. doi: 10.1038/nature03703.

302. Sleeman JP. The lymph node pre-metastatic niche. *J Mol Med (Berl).* 93:1173-84, 2015. doi: 10.1007/s00109-015-1351-6.

303. Sloan EK, Pouliot N, Stanley KL, Chia J, Moseley JM, Hards DK, Anderson RL. Tumor-specific expression of alphavbeta3 integrin promotes spontaneous metastasis of breast cancer to bone. *Breast Cancer Res* 8: R20, 2006. doi: 10.1186/bcr1398.

304. Smith M, De Bono J, Sternberg C, Le Moulec S, Oudard S, De Giorgi U, Krainer M, Bergman A, Hoelzer W, De Wit R, Bögemann M, Saad F, Cruciani G, Thiery-Vuillemin A, Feyerabend S,

Miller K, Houédé N, Hussain S, Lam E, Polikoff J, Stenzl A, Mainwaring P, Ramies D, Hessel C, Weitzman A, Fizazi K. Phase III Study of Cabozantinib in Previously Treated Metastatic Castration-Resistant Prostate Cancer: COMET-1. *J Clin Oncol.* 34:3005-3013, 2016. doi: 10.1200/JCO.2015.65.5597.

305. Smith MR, Egerdie B, Hernández Toriz N, Feldman R, Tammela TL, Saad F, Heracek J, Szwedowski M, Ke C, Kupic A, Leder BZ, Goessl C; Denosumab HALT Prostate Cancer Study Group. Denosumab in men receiving androgen-deprivation therapy for prostate cancer. *N Engl J Med*. 361:745-755, 2009. doi: 10.1056/NEJMoa0809003.

306. Smith MR, Saad F, Coleman R, Shore N, Fizazi K, Tombal B, Miller K, Sieber P, Karsh L, Damião R, Tammela TL, Egerdie B, Van Poppel H, Chin J, Morote J, Gómez-Veiga F, Borkowski T, Ye Z, Kupic A, Dansey R, Goessl C. Denosumab and bone-metastasis-free survival in men with castration-resistant prostate cancer: results of a phase 3, randomised, placebo-controlled trial. *Lancet.* 379:39-46, 2012. doi: 10.1016/S0140-6736(11)61226-9.

307. Solomayer EF, Gebauer G, Hirnle P, Janni W, Lück HJ, Becker S, Huober J, Krämer B, Wackwitz B, Wallwiener D, Fehm T. Influence of zoledronic acid on disseminated tumor cells in primary breast cancer patients. *Ann Oncol.* 23:2271-2277, 2012. doi: 10.1093/annonc/mdr612.

308. Sopata M, Katz N, Carey W, Smith MD, Keller D, Verburg KM, West CR, Wolfram G, Brown MT. Efficacy and safety of tanezumab in the treatment of pain from bone metastases. *Pain*. 156:1703-1713, 2015. doi: 10.1097/j.pain.0000000000000211.

309. Sosa MS, Bragado P, Aguirre-Ghiso JA. Mechanisms of disseminated cancer cell dormancy: an awakening field. *Nat Rev Cancer* 14:611-622, 2014. doi: 10.1038/nrc3793.

310. Sottnik JL, Dai J, Zhang H, Campbell B, Keller ET. Tumor-induced pressure in the bone microenvironment causes osteocytes to promote the growth of prostate cancer bone metastases. *Cancer Res*. 75:2151-2158, 2015. doi: 10.1158/0008-5472.CAN-14-2493.

311. Sottnik JL, Daignault-Newton S, Zhang X, Morrissey C, Hussain MH, Keller ET, Hall CL. Integrin alpha2beta 1 (α2β1) promotes prostate cancer skeletal metastasis. *Clin Exp Metastasis* 30:569-578, 2013. doi: 10.1007/s10585-012-9561-6.

312. Sousa S, Clézardin P. Bone-Targeted Therapies in Cancer-Induced Bone Disease. *Calcif Tissue Int.* 102:227-250, 2018. doi: 10.1007/s00223-017-0353-5.

313. Spencer JA, Ferraro F, Roussakis E, Klein A, Wu J, Runnels JM, Zaher W, Mortensen LJ, Alt C, Turcotte R, Yusuf R, Côté D, Vinogradov SA, Scadden DT, Lin CP. Direct measurement of local oxygen concentration in the bone marrow of live animals. *Nature*. 508:269-273, 2014. doi: 10.1038/nature13034.

314. Sterling JA, Oyajobi BO, Grubbs B, Padalecki SS, Munoz SA, Gupta A, Story B, Zhao M, Mundy GR. The hedgehog signaling molecule Gli2 induces parathyroid hormone-related peptide expression and osteolysis in metastatic human breast cancer cells. *Cancer Res.* 66:7548-7553, 2006. doi: 10.1158/0008-5472.CAN-06-0452.

315. Stopeck AT, Lipton A, Body JJ, Steger GG, Tonkin K, de Boer RH, Lichinitser M, Fujiwara Y, Yardley DA, Viniegra M, Fan M, Jiang Q, Dansey R, Jun S, Braun A. Denosumab compared with zoledronic acid for the treatment of bone metastases in patients with advanced breast cancer: a randomized, double-blind study. *J Clin Oncol*. 28:5132-5139, 2010. doi: 10.1200/JCO.2010.29.7101.

316. Sugiyama T, H. Kohara, M. Noda, T. Nagasawa. Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches *Immunity* 25: 977-988, 2006. doi: 10.1016/j.immuni.2006.10.016.

317. Sun YX, Fang M, Wang J, Cooper CR, Pienta KJ, Taichman RS. Expression and activation of alpha v beta 3 integrins by SDF-1/CXC12 increases the aggressiveness of prostate cancer cells. *Prostate* 67: 61-73, 2007. doi: 10.1002/pros.20500.

318. Sun YX, Schneider A, Jung Y, Wang J, Dai J, Wang J, Cook K, Osman NI, Koh-Paige AJ, Shim H, Pienta KJ, Keller ET, McCauley LK, Taichman RS. Skeletal localization and neutralization of the SDF-1(CXCL12)/CXCR4 axis blocks prostate cancer metastasis and growth in osseous sites in vivo. *J Bone Miner Res* 20: 318-329, 2005. doi: 10.1359/JBMR.041109.

319. Suominen MI, Fagerlund KM, Rissanen JP, Konkol YM, Morko JP, Peng Z, Alhoniemi EJ, Laine SK, Corey E, Mumberg D, Ziegelbauer K, Käkönen SM, Halleen JM, Vessella RL, Scholz A. Radium-223 Inhibits Osseous Prostate Cancer Growth by Dual Targeting of Cancer Cells and Bone Microenvironment in Mouse Models. *Clin Cancer Res*. 23:4335-4346, 2017. doi: 10.1158/1078-0432.CCR-16-2955.

320. Taichman RS, Patel LR, Bedenis R, Wang J, Weidner S, Schumann T, Yumoto K, Berry JE, Shiozawa Y, Pienta KJ. GAS6 receptor status is associated with dormancy and bone metastatic tumor formation. *PLoS One* 8:e61873, 2013. doi: 10.1371/journal.pone.0061873.

321. Taipaleenmäki H, Browne G, Akech J, Zustin J, van Wijnen AJ, Stein JL, Hesse E, Stein GS, Lian JB. Targeting of Runx2 by miR-135 and miR-203 impairs progression of breast cancer and metastatic bone disease. *Cancer Res* 75:1433-1444, 2015. doi: 10.1158/0008-5472.CAN-14-1026.

322. Taipaleenmäki H, Farina NH, van Wijnen AJ, Stein JL, Hesse E, Stein GS, Lian JB. Antagonizing miR-218-5p attenuates Wnt signaling and reduces metastatic bone disease of triple negative breast cancer cells. *Oncotarget* 7: 79032-79046, 2016. doi: 10.18632/oncotarget.12593.

323. Tamura D, Hiraga T, Myoui A, Yoshikawa H, Yoneda T. Cadherin-11-mediated interactions with bone marrow stromal/osteoblastic cells support selective colonization of breast cancer cells in bone. *Int J Oncol* 33: 17-24, 2008.

3355 324. Tandon M, Othman AH, Ashok V, Stein GS, Pratap J. The role of Runx2 in facilitating
3356 autophagy in metastatic breast cancer cells. *J Cell Physiol.* 233:559-571, 2018. doi:
3357 10.1002/jcp.25916.
3358 325. Tang ZN, Zhang F, Tang P, Qi XW, Jiang J. Hypoxia induces RANK and RANKL expression
3359 by activating HIF-1α in breast cancer cells. *Biochem Biophys Res Commun*. 408:411-416,
3360 2011. doi: 10.1016/j.bbrc.2011.04.035.
3361 326. Tarragona M, Pavlovic M, Arnal-Estapé A, Urosevic J, Morales M, Guiu M, Planet E,
3362 González-Suárez E, Gomis RR. Identification of NOG as a specific breast cancer bone
3363 metastasis-supporting gene. *J Biol Chem*. 287(25):21346-21355, 2012. doi:
3364 10.1074/jbc.M112.355834.
3365 327. Taverna S, Pucci M, Giallombardo M, Di Bella MA, Santarpia M, Reclusa P, Gil-Bazo I, Rolfo
3366 C, Alessandro R. Amphiregulin contained in NSCLC-exosomes induces osteoclast
3367 differentiation through the activation of EGFR pathway. *Sci Rep*. 7:3170, 2017. doi:
3368 10.1038/s41598-017-03460-y.
3369 328. Templeton ZS, Lie WR, Wang W, Rosenberg-Hasson Y, Alluri RV, Tamaresis JS, Bachmann
3370 MH, Lee K, Maloney WJ, Contag CH, King BL. Breast Cancer Cell Colonization of the Human
3371 Bone Marrow Adipose Tissue Niche. *Neoplasia*. 17:849-861, 2015. doi:
3372 10.1016/j.neo.2015.11.005.
3373 329. Thomas RJ, Guise TA, Yin JJ, Elliott J, Horwood NJ, Martin TJ, Gillespie MT. Breast cancer
3374 cells interact with osteoblasts to support osteoclast formation. *Endocrinology* 140: 4451-4458,
3375 1999. doi: 10.1210/endo.140.10.7037.
3376 330. Thudi NK, Martin CK, Murahari S, Shu ST, Lanigan LG, Werbeck JL, Keller ET, McCauley LK,
3377 Pinzone JJ, Rosol TJ. Dickkopf-1 (DKK-1) stimulated prostate cancer growth and metastasis
3378 and inhibited bone formation in osteoblastic bone metastases. *Prostate*. 71:615-625, 2011. doi:
3379 10.1002/pros.21277.

331. Thysell E, Surowiec I, Hörnberg E, Crnalic S, Widmark A, Johansson AI, Stattin P, Bergh A, Moritz T, Antti H, Wikström P. Metabolomic characterization of human prostate cancer bone metastases reveals increased levels of cholesterol. *PLoS One.* 5:e14175, 2010. doi: 10.1371/journal.pone.0014175.

332. Tian E, Zhan F, Walker R, Rasmussen E, Ma Y, Barlogie B, Shaughnessy JD Jr. The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma. *N Engl J Med*. 349:2483-2494, 2003. doi: 10.1056/NEJMoa030847.

333. Trotter TN, Yang Y. Matricellular proteins as regulators of cancer metastasis to bone. Matrix Biol 52-54:301-314, 2016. doi: 10.1016/j.matbio.2016.01.006.

334. Tsourdi E, Langdahl B, Cohen-Solal M, Aubry-Rozier B, Eriksen EF, Guañabens N, Obermayer-Pietsch B, Ralston SH, Eastell R, Zillikens MC. Discontinuation of Denosumab therapy for osteoporosis: A systematic review and position statement by ECTS. *Bone* 105:11-17, 2017. doi: 10.1016/j.bone.2017.08.003.

335. Tsukasaki M, Hamada K, Okamoto K, Nagashima K, Terashima A, Komatsu N, Win SJ, Okamura T, Nitta T, Yasuda H, Penninger JM, Takayanagi H. LOX Fails to Substitute for RANKL in Osteoclastogenesis. *J Bone Miner Res* 32:434-439, 2017. doi: 10.1002/jbmr.2990.

336. Tulotta C, Lefley DV, Freeman K, Gregory WM, Hanby AM, Heath PR, Nutter-Howard F, Wilkinson JM, Spicer-Hadlington AR, Liu X, Bradbury S, Hambley L, Cookson V, Allocca G, Kruithof-de-Julio M, Coleman RE, Brown JE, Holen I, Ottewell PD. Endogenous production of IL-1B by breast cancer cells drives metastasis and colonisation of the bone microenvironment. *Clin Cancer* Res. 25:2769-2782, 2019. doi: 10.1158/1078-0432.CCR-18-2202.

337. Turner JJO. Hypercalcaemia - presentation and management. *Clin Med (Lond).* 17:270-273, 2017. doi: 10.7861/clinmedicine.

338. Turpin A, Duterque-Coquillaud M, Vieillard MH. Bone Metastasis: Current State of Play. *Transl Oncol.* 13: 308-320, 2019. doi: 10.1016/j.tranon.2019.10.012.

339. Ubellacker JM, Baryawno N, Severe N, DeCristo MJ, Sceneay J, Hutchinson JN, Haider MT, Rhee CS, Qin Y, Gregory WM, Garrido-Castro AC, Holen I, Brown JE, Coleman RE, Scadden DT, McAllister SS. Modulating bone marrow hematopoietic lineage potential to prevent bone metastasis in breast cancer. *Cancer Res*. 78:5300-5314, 2018. doi: 10.1158/0008-5472.CAN-18-0548.

340. Ubellacker JM, Haider MT, DeCristo MJ, Allocca G, Brown NJ, Silver DP, Holen I, McAllister SS. Zoledronic acid alters hematopoiesis and generates breast tumor-suppressive bone marrow cells. *Breast Cancer Res* 19:23, 2017. doi: 10.1186/s13058-017-0815-8.

341. Valencia K, Luis-Ravelo D, Bovy N, Antón I, Martínez-Canarias S, Zandueta C, Ormazábal C, Struman I, Tabruyn S, Rebmann V, De Las Rivas J, Guruceaga E, Bandrés E, Lecanda F. miRNA cargo within exosome-like vesicle transfer influences metastatic bone colonization. *Mol Oncol.* 8:689-703, 2014. doi: 10.1016/j.molonc.2014.01.012.

342. Valta MP, Tuomela J, Bjartell A, Valve E, Väänänen HK, Härkönen P. FGF-8 is involved in bone metastasis of prostate cancer. *Int J Cancer* 123: 22-31, 2008. doi: 10.1002/ijc.23422.

343. Valta MP, Zhao H, Ingels A, Thong AE, Nolley R, Saar M, Peehl DM. Development of a realistic in vivo bone metastasis model of human renal cell carcinoma. Clin Exp Metastasis. 31:573-584, 2014.

344. van der Horst G, van den Hoogen C, Buijs JT, Cheung H, Bloys H, Pelger RC, Lorenzon G, Heckmann B, Feyen J, Pujuguet P, Blanque R, Clément-Lacroix P, van der Pluijm G. Targeting of α(v)-integrins in stem/progenitor cells and supportive microenvironment impairs bone metastasis in human prostate cancer. *Neoplasia* 13: 516-525, 2011.

345. Van Poznak C, Somerfield MR, Barlow WE, Biermann JS, Bosserman LD, Clemons MJ, Dhesy-Thind SK, Dillmon MS, Eisen A, Frank ES, Jagsi R, Jimenez R, Theriault RL, Vandenberg TA, Yee GC, Moy B. Role of Bone-Modifying Agents in Metastatic Breast Cancer:

An American Society of Clinical Oncology-Cancer Care Ontario Focused Guideline Update. *J Clin Oncol*. 35:3978-3986, 2017. doi: 10.1200/JCO.2017.75.4614.

346. Vargas G, Bouchet M, Bouazza L, Reboul P, Boyault C, Gervais M, Kan C, Benetollo C, Brevet M, Croset M, Mazel M, Cayrefourcq L, Geraci S, Vacher S, Pantano F, Filipits M, Driouch K, Bieche I, Gnant M, Jacot W, Aubin JE, Duterque-Coquillaud M, Alix-Panabières C, Clézardin P, Bonnelye E. ERRα promotes breast cancer cell dissemination to bone by increasing RANK expression in primary breast tumors. *Oncogene*. 38:950-964, 2019. doi: 10.1038/s41388-018-0579-3.

347. Vidula N, Yau C, Li J, Esserman LJ, Rugo HS. Receptor activator of nuclear factor kappa B (RANK) expression in primary breast cancer correlates with recurrence-free survival and development of bone metastases in I-SPY1 (CALGB 150007/150012; ACRIN 6657). *Breast Cancer Res Treat* 165: 129-138, 2017. doi: 10.1007/s10549-017-4318-1.

348. Vija Racaru L, Sinigaglia M, Kanoun S, Ben Bouallègue F, Tal I, Brillouet S, Bauriaud-Mallet M, Zerdoud S, Dierickx L, Vallot D, Caselles O, Gabiache E, Pascal P, Courbon F. Fluorine-18-fluorocholine PET/CT parameters predictive for hematological toxicity to radium-223 therapy in castrate-resistant prostate cancer patients with bone metastases: a pilot study. *Nucl Med Commun*. 39:672-679, 2018. doi: 10.1097/MNM.0000000000000850.

349. Vives V, Cres G, Richard C, Busson M, Ferrandez Y, Planson AG, Zeghouf M, Cherfils J, Malaval L, Blangy A. Pharmacological inhibition of Dock5 prevents osteolysis by affecting osteoclast podosome organization while preserving bone formation. *Nat Commun.* 6:6218, 2015. doi: 10.1038/ncomms7218.

350. Voorzanger-Rousselot N, Garnero P. Biochemical markers in oncology. Part I: molecular basis. Part II: clinical uses. *Cancer Treat Rev*. 33:230-283, 2007. doi: 10.1016/j.ctrv.2007.01.008.

351. Wakabayashi H, Wakisaka S, Hiraga T, Hata K, Nishimura R, Tominaga M, Yoneda T. Decreased sensory nerve excitation and bone pain associated with mouse Lewis lung cancer in TRPV1-deficient mice. *J Bone Miner Metab*.36:274-285, 2018. doi: 10.1007/s00774-017-0842-7.

352. Wallitt KL, Khan SR, Dubash S, Tam HH, Khan S, Barwick TD. Clinical PET Imaging in Prostate Cancer. *Radiographics*. 37:1512-1536, 2017. doi: 10.1148/rg.2017170035.

353. Waltregny D, Bellahcène A, Van Riet I, Fisher LW, Young M, Fernandez P, Dewé W, de Leval J, Castronovo V. Prognostic value of bone sialoprotein expression in clinically localized human prostate cancer. *J Natl Cancer Inst*. 90:1000-1008, 1998. doi: 10.1093/jnci/90.13.1000

354. Wang H, Tian L, Liu J, Goldstein A, Bado I, Zhang W, Arenkiel BR, Li Z, Yang M, Du S, Zhao H, Rowley DR, Wong STC, Gugala Z, Zhang XH. The Osteogenic Niche Is a Calcium Reservoir of Bone Micrometastases and Confers Unexpected Therapeutic Vulnerability. *Cancer Cell.* 34:823-839, 2018.e7. doi: 10.1016/j.ccell.2018.10.002.

355. Wang H, Yu C, Gao X, Welte T, Muscarella AM, Tian L, Zhao H, Zhao Z, Du S, Tao J, Lee B, Westbrook TF, Wong ST, Jin X, Rosen JM, Osborne CK, Zhang XH. The osteogenic niche promotes early-stage bone colonization of disseminated breast cancer cells. *Cancer Cell* 27:193-210, 2015. doi: 10.1016/j.ccell.2014.11.017.

356. Wang J, Chen GL, Cao S, Zhao MC, Liu YQ, Chen XX, Qian C. Adipogenic niches for melanoma cell colonization and growth in bone marrow. *Lab Invest*. 97:737-745, 2017. doi: 10.1038/labinvest.2017.14.

357. Wang N, Docherty FE, Brown HK, Reeves KJ, Fowles AC, Ottewell PD, Dear TN, Holen I, Croucher PI, Eaton CL. Prostate cancer cells preferentially home to osteoblast-rich areas in the early stages of bone metastasis: evidence from in vivo models. *J Bone Miner Res* 29:2688-2696, 2014. doi: 10.1002/jbmr.2300.

358. Wang S, Li GX, Tan CC, He R, Kang LJ, Lu JT, Li XQ, Wang QS, Liu PF, Zhai QL, Feng YM. FOXF2 reprograms breast cancer cells into bone metastasis seeds. *Nat Commun*. 10:2707, 2019. doi: 10.1038/s41467-019-10379-7.

359. Waning DL, Mohammad KS, Reiken S, Xie W, Andersson DC, John S, Chiechi A, Wright LE, Umanskaya A, Niewolna M, Trivedi T, Charkhzarrin S, Khatiwada P, Wronska A, Haynes A, Benassi MS, Witzmann FA, Zhen G, Wang X, Cao X, Roodman GD, Marks AR, Guise TA. Excess TGF-β mediates muscle weakness associated with bone metastases in mice. *Nat Med.* 21:1262-1271, 2015. doi: 10.1038/nm.3961.

360. Watanabe K, Hirata M, Tominari T, Matsumoto C, Fujita H, Yonekura K, Murphy G, Nagase H, Miyaura C, Inada M. The MET/Vascular Endothelial Growth Factor Receptor (VEGFR)-targeted Tyrosine Kinase Inhibitor Also Attenuates FMS-dependent Osteoclast Differentiation and Bone Destruction Induced by Prostate Cancer. *J Biol Chem*. 291:20891-20899, 2016.

361. Weilbaecher KN, Guise TA, McCauley LK. Cancer to bone: a fatal attraction. *Nat Rev Cancer* 11: 411-425, 2011. doi: 10.1038/nrc3055.

362. Welm AL, Sneddon JB, Taylor C, Nuyten DS, van de Vijver MJ, Hasegawa BH, Bishop JM. The macrophage-stimulating protein pathway promotes metastasis in a mouse model for breast cancer and predicts poor prognosis in humans. *Proc Natl Acad Sci U S A*. 104:7570-7575, 2007.

363. Wen J, Huang G, Liu S, Wan J, Wang X, Zhu Y, Kaliney W, Zhang C, Cheng L, Wen X, Lu X. Polymorphonuclear MDSCs are enriched in the stroma and expanded in metastases of prostate cancer. *J Pathol Clin Res*. 6:171-177, 2020. doi: 10.1002/cjp2.160.

364. Werner-Klein M, Grujovic A, Irlbeck C, Obradović M, Hoffmann M, Koerkel-Qu H, Lu X, Treitschke S, Köstler C, Botteron C, Weidele K, Werno C, Polzer B, Kirsch S, Gužvić M, Warfsmann J, Honarnejad K, Czyz Z, Feliciello G, Blochberger I, Grunewald S, Schneider E, Haunschild G, Patwary N, Guetter S, Huber S, Rack B, Harbeck N, Buchholz S, Rümmele P,

Heine N, Rose-John S, Klein CA. Interleukin-6 trans-signaling is a candidate mechanism to drive progression of human DCCs during clinical latency. *Nat Commun* 11:4977, 2020. doi: 10.1038/s41467-020-18701-4.

365. Westbrook JA, Cairns DA, Peng J, Speirs V, Hanby AM, Holen I, Wood SL, Ottewell PD, Marshall H, Banks RE, Selby PJ, Coleman RE, Brown JE. CAPG and GIPC1: Breast Cancer Biomarkers for Bone Metastasis Development and Treatment. *J Natl Cancer Inst.* 108(4), 2016. doi: 10.1093/jnci/djv360.

366. Westbrook JA, Wood SL, Cairns DA, McMahon K, Gahlaut R, Thygesen H, Shires M, Roberts S, Marshall H, Oliva MR, Dunning MJ, Hanby AM, Selby PJ, Speirs V, Mavria G, Coleman RE, Brown JE. Identification and validation of DOCK4 as a potential biomarker for risk of bone metastasis development in patients with early breast cancer. *J Pathol.* 247:381-391, 2019. doi: 10.1002/path.5197.

367. Wong R, Wiffen PJ. Bisphosphonates for the relief of pain secondary to bone metastases. Cochrane *Database Syst Rev*. CD002068, 2002.

368. Wortzel I, Dror S, Kenific CM, Lyden D. Exosome-Mediated Metastasis: Communication from a Distance. *Dev Cell*. 49:347-360, 2019. doi: 10.1016/j.devcel.2019.04.011.

369. Wu AC, He Y, Broomfield A, Paatan NJ, Harrington BS, Tseng HW, Beaven EA, Kiernan DM, Swindle P, Clubb AB, Levesque JP, Winkler IG, Ling MT, Srinivasan B, Hooper JD, Pettit AR. CD169(+) macrophages mediate pathological formation of woven bone in skeletal lesions of prostate cancer. *J Pathol.* 239:218-230, 2016. doi: 10.1002/path.4718.

370. Xiang L, Gilkes DM. The Contribution of the Immune System in Bone Metastasis Pathogenesis. *Int J Mol Sci*. 20(4). pii: E999, 2019. doi: 10.3390/ijms20040999.

371. Xu Z, Liu X, Wang H, Li J, Dai L, Li J, Dong C. Lung adenocarcinoma cell-derived exosomal miR-21 facilitates osteoclastogenesis. *Gene* 666: 116-122, 2018. doi: 10.1016/j.gene.2018.05.008.

372. Yaccoby S, Ling W, Zhan F, Walker R, Barlogie B, Shaughnessy JD Jr. Antibody-based inhibition of DKK1 suppresses tumor-induced bone resorption and multiple myeloma growth in vivo. *Blood*. 109:2106-2111, 2007. doi: 10.1182/blood-2006-09-047712.

373. Yang C, Siebert JR, Burns R, Gerbec ZJ, Bonacci B, Rymaszewski A, Rau M, Riese MJ, Rao S, Carlson KS, Routes JM, Verbsky JW, Thakar MS, Malarkannan S. Heterogeneity of human bone marrow and blood natural killer cells defined by single-cell transcriptome. *Nat Commun*. 10:3931, 2019. doi: 10.1038/s41467-019-11947-7.

374. Yang MH, Wu MZ, Chiou SH, Chen PM, Chang SY, Liu CJ, Teng SC, Wu KJ. Direct regulation of TWIST by HIF-1alpha promotes metastasis. *Nat Cell Biol*. 10:295-305, 2008. doi: 10.1038/ncb1691.

375. Yazici O, Aksoy S, Sendur MA, Babacan T, Ozdemir N, Ozisik Y, Zengin N, Altundag K. The effect of obesity on recurrence pattern in early breast cancer patients. J BUON. 20:954-962, 2015.

376. Ye Y, Li SL, Ma YY, Diao YJ, Yang L, Su MQ, Li Z, Ji Y, Wang J, Lei L, Fan WX, Li LX, Xu Y, Hao XK. Exosomal miR-141-3p regulates osteoblast activity to promote the osteoblastic metastasis of prostate cancer. *Oncotarget* 8: 94834-94849, 2017. doi: 10.18632/oncotarget.22014.

377. Yi B, Williams PJ, Niewolna M, Wang Y, Yoneda T. Tumor-derived platelet-derived growth factor-BB plays a critical role in osteosclerotic bone metastasis in an animal model of human breast cancer. *Cancer Res* 62: 917-923, 2002.

378. Yin JJ, Mohammad KS, Käkönen SM, Harris S, Wu-Wong JR, Wessale JL, Padley RJ, Garrett IR, Chirgwin JM, Guise TA. A causal role for endothelin-1 in the pathogenesis of osteoblastic bone metastases. *Proc Natl Acad Sci USA* 100: 10954-10959, 2003. doi: 10.1073/pnas.1830978100.

379. Yonou H, Aoyagi Y, Kanomata N, Kamijo T, Oda T, Yokose T, Hasebe T, Nagai K, Hatano T, Ogawa Y, Ochiai A. Prostate-specific antigen induces osteoplastic changes by an autonomous mechanism. *Biochem Biophys Res Commun* 289: 1082-1087, 2001. doi: 10.1006/bbrc.2001.6129.

380. Yonou H, Horiguchi Y, Ohno Y, Namiki K, Yoshioka K, Ohori M, Hatano T, Tachibana M. Prostate-specific antigen stimulates osteoprotegerin production and inhibits receptor activator of nuclear factor-kappaB ligand expression by human osteoblasts. *Prostate* 67:840-848, 2007. doi: 10.1002/pros.20574.

381. Yu-Lee LY, Yu G, Lee YC, Lin SC, Pan J, Pan T, Yu KJ, Liu B, Creighton CJ, Rodriguez-Canales J, Villalobos PA, Wistuba II, de Nadal E, Posas F, Gallick GE, Lin SH. Osteoblast-Secreted Factors Mediate Dormancy of Metastatic Prostate Cancer in the Bone via Activation of the TGFβRIII-p38MAPK-pS249/T252RB Pathway. *Cancer Res*. 78: 2911-2924, 2018. doi: 10.1158/0008-5472.CAN-17-1051.

382. Yumoto K, Eber MR, Wang J, Cackowski FC, Decker AM, Lee E, Nobre AR, Aguirre-Ghiso JA, Jung Y, Taichman RS. Axl is required for TGF-β2-induced dormancy of prostate cancer cells in the bone marrow. *Sci Rep*. 6:36520, 2016. doi: 10.1038/srep36520.

383. Zang L, Ma M, Hu J, Qiu H, Huang B, Chu T. The effects of lung and prostate cancer bone metastasis on serum osteoprotegerin levels: a meta-analysis. *Sci Rep* 5:18324, 2015. doi: 10.1038/srep18324.

384. Zhang J, Mao F, Niu G, Peng L, Lang L, Li F, Ying H, Wu H, Pan B, Zhu Z, Chen X. (68)Ga-BBN-RGD PET/CT for GRPR and Integrin α(v)β(3) Imaging in Patients with Breast Cancer. *Theranostics*. 8:1121-1130, 2018. doi: 10.7150/thno.22601.

385. Zhang J, Pang Y, Xie T, Zhu L. CXCR4 antagonism in combination with IDO1 inhibition weakens immune suppression and inhibits tumor growth in mouse breast cancer bone metastases. *Onco Targets Ther*. 12:4985-4992, 2019. doi:10.2147/OTT.S200643

386. Zhang K, Kim S, Cremasco V, Hirbe AC, Collins L, Piwnica-Worms D, Novack DV, Weilbaecher K, Faccio R. CD8+ T cells regulate bone tumor burden independent of osteoclast resorption. *Cancer Res.* 71:4799-4808, 2011. doi: 10.1158/0008-5472.CAN-10-3922.

387. Zhang XH, Wang Q, Gerald W, Hudis CA, Norton L, Smid M, Foekens JA, Massagué J. Latent bone metastasis in breast cancer tied to Src-dependent survival signals. *Cancer Cell*. 16: 67-78, 2009. doi: 10.1016/j.ccr.2009.05.017.

388. Zhao Y, Bachelier R, Treilleux I, Pujuguet P, Peyruchaud O, Baron R, Clément-Lacroix P, Clézardin P. Tumor alphavbeta3 integrin is a therapeutic target for breast cancer bone metastases. *Cancer Res* 67: 5821-5830, 2007. DOI: 10.1158/0008-5472.CAN-06-4499.

389. Zhao E, Wang L, Dai J, Kryczek I, Wei S, Vatan L, Altuwaijri S, Sparwasser T, Wang G, Keller ET, Zou W. Regulatory T cells in the bone marrow microenvironment in patients with prostate cancer. *Oncoimmunology*. 1:152-161, 2012. doi: 10.4161/onci.1.2.18480.

390. Zheng H, Bae Y, Kasimir-Bauer S, Tang R, Chen J, Ren G, Yuan M, Esposito M, Li W, Wei Y, Shen M, Zhang L, Tupitsyn N, Pantel K, King C, Sun J, Moriguchi J, Jun HT, Coxon A, Lee B, Kang Y. Therapeutic Antibody Targeting Tumor- and Osteoblastic Niche-Derived Jagged1 Sensitizes Bone Metastasis to Chemotherapy. *Cancer Cell*. 32:731-747, 2017. doi: 10.1016/j.ccell.2017.11.002.

391. Zheng D, Decker KF, Zhou T, Chen J, Qi Z, Jacobs K, Weilbaecher KN, Corey E, Long F, Jia L. Role of WNT7B-induced noncanonical pathway in advanced prostate cancer. *Mol Cancer Res* 11: 482-493, 2013. doi: 10.1158/1541-7786.MCR-12-0520.

392. Zhou JZ, Riquelme MA, Gao X, Ellies LG, Sun LZ, Jiang JX. Differential impact of adenosine nucleotides released by osteocytes on breast cancer growth and bone metastasis. *Oncogene* 34: 1831-42, 2015. doi: 10.1038/onc.2014.113.

393. Zhou JZ, Riquelme MA, Gu S, Kar R, Gao X, Sun L, Jiang JX. Osteocytic connexin hemichannels suppress breast cancer growth and bone metastasis. *Oncogene*. 35:5597-5607, 2016. doi: 10.1038/onc.2016.101.

394. Zhu L, Narloch JL, Onkar S, Joy M, Broadwater G, Luedke C, Hall A, Kim R, Pogue-Geile K, Sammons S, Nayyar N, Chukwueke U, Brastianos PK, Anders CK, Soloff AC, Vignali DAA, Tseng GC, Emens LA, Lucas PC, Blackwell KL, Oesterreich S, Lee AV. Metastatic breast cancers have reduced immune cell recruitment but harbor increased macrophages relative to their matched primary tumors. J Immunother Cancer. 7:265, 2019. doi: 10.1186/s40425-019-0755-1.

395. Zhu M, Liu C, Li S, Zhang S, Yao Q, Song Q. Sclerostin induced tumor growth, bone metastasis and osteolysis in breast cancer. *Sci Rep*. 7:11399, 2017. doi: 10.1038/s41598-017-11913-7.

396. Zhuang X, Zhang H, Li X, Li X, Cong M, Peng F, Yu J, Zhang X, Yang Q, Hu G. Differential effects on lung and bone metastasis of breast cancer by Wnt signalling inhibitor DKK1. *Nat Cell Biol* 19:1274-1285, 2017. doi: 10.1038/ncb3613.

Figure legends:

Figure 1: Patterns of bone metastases from solid tumors ranging from mostly destructive (osteolytic) to mostly bone-forming (osteoblastic). Representative radiographs and histology of bone metastases with osteolytic (white arrow) or osteoblastic (black arrow) lesions are shown. For bone tumor sections, mineralized bone is stained green, whereas bone marrow and tumor cells (*) are stained red. Of note, in osteoblastic lesions, extensive new woven bone (stained dark red) can be observed, leading to the formation of new trabecular bone that fills the bone marrow cavity (white arrow).

Figure 2: Bone colonization by tumor cells is a stepwise sequence of events that include *(i)* the formation of a pre-metastatic niche to attract circulating tumor cells (CTCs) in bone, *(ii)* the extravasation and homing of CTCs within the pre-metastatic niche where they bind to bone extracellular matrix proteins, and *(iii)* the maintenance of tumor cells in the vascular niche and the osteoblastic niche where tumor cells become quiescent through specific adhesive interactions with host cells. ANXA2: annexin A2; AXL: tyrosine kinase receptor; CAR cells: CXCL-12-abundant reticular cells; CDH: cadherin; Cx43: connexin 43; CXCL: chemokine; CXCR: chemokine receptor; ECM: extracellular matrix; GAS-6: growth arrest-specific 6; IL-1: interleukin-1; IL-6: interleukin-6; LOX: lysyl oxidase; RANK-L: receptor activator of nuclear factor kappa-B (RANK) ligand; SNO: spindle-shaped N-cadherin+ osteoblast; TSP-1: thrombospondin-1.

Figure 3: The fate of bone-resident tumor cells is determined by a balance between activities of multiple activated protein kinases (MAPK) ERK1/2 and p38, where a switch towards ERK1/2 phosphorylation favors proliferation whereas activation of p38 leads to quiescence. This balance between ERK1/2 and p38 activities is governed by several factors that either promote dormancy (boxes in green), helping tumor cells to survive in the vascular and osteoblastic niches, or enhance tumor cell reactivation and proliferation (boxes in red). However, proliferative tumor cells become vulnerable to immune

surveillance, leading to tumor cell killing by $CD8^+$ T cells and NK cells. The bone microenvironment also contains immunosuppressive cells (MDSCs, Treg, pDC) that help tumor cells to escape from adaptive immunity. AXL: tyrosine kinase receptor; BMP-7: bone morphogenetic protein-7; C CAR cells: CXCL-12-abundant reticular cells; CXCL-12: chemokine;: GAS-6: growth arrest-specific 6; IFN-γ: interferon γ; LIF: leukemia inhibitory factor; MDSCs: myeloid-derived suppressor cells; MSK1: mitogen- and stress-activated kinase 1; NK cell: natural killer cell; POSTN: periostin; pDC: plasmacytoid dendritic cell; PTHrP: parathyroid hormone-related peptide; RANK-L: receptor activator of nuclear factor kappa-B (RANK) ligand; SNO: spindle-shaped N-cadherin+ osteoblast; TGFβ: transforming growth factor beta; Treg: regulatory T cells; TSP-1: thrombospondin-1; TYRO3: tyrosine kinase receptor; VCAM-1: vascular cell adhesion protein 1; VEGF: vascular endothelial growth factor.

Figure 4: Mechanisms governing the formation of osteolytic bone metastases. Several factors secreted by tumor cells enhance osteoclast-mediated bone resorption, either directly (*e.g.*, IL-8) or indirectly (*e.g.*, PTHrP, IL-6) *via* stimulation of RANK-L secretion and inhibition of OPG production by osteoblasts. In turn, the binding of RANK-L to RANK on osteoclast precursors leads to the formation of new osteoclasts. LPA by binding to its receptor LPA1 at the tumor cell surface promotes tumor cell proliferation and the production of IL-6 and IL-8, further enhancing osteoclast-mediated bone resorption. In addition, tumor-derived LOX and IL-1beta accelerate RANKL-induced osteoclastogenesis. Consequently, growth factors (TGFβ, IGFs, PDGF) and calcium are released from the resorbed bone matrix. TGFβ acts on tumor cells and stimulates the expression of factors such as PTHrP and Notch ligand Jagged-1. In turn, Jagged/Notch signaling promotes osteoclast differentiation. IGFs and calcium promote tumor cell proliferation. Calcium also stimulates the secretion of PTHrP and epiregulin by tumor cells. Tumor-derived epiregulin decreases *OPG* expression in osteoblasts. Thus, there is a vicious cycle where tumor cells stimulate bone destruction and factors released from resorbed bone stimulate tumor growth. This cycle is enhanced by the secretion of tumor-derived factors (DKK-1, SOST-1, noggin,

activin A) that inhibit osteoblast activity, thereby worsening the imbalance between bone formation and bone resorption, and promoting bone destruction.

DKK-1: dickkopf-1; IGF: insulin-like growth factor; IL-6: interleukin-6; LOX: lysyl oxidase; LPA: lysophosphatidic acid; OPG: osteoprotegerin; PDGF: platelet-derived growth factor; PTHrP: parathyroid hormone-related peptide; RANK-L: receptor activator of nuclear factor kappa-B (RANK) ligand; SOST-1: sclerostin; TGFβ: transforming growth factor beta.

Figure 5: Mechanisms governing the formation of osteoblastic bone metastases. Several factors secreted by tumor cells directly enhance osteoblast differentiation (ET-1, BMP-2, BMP-6, Wnts). BMP-4 mediates conversion of endothelial cells into osteoblasts. The stimulation of osteoblast differentiation is associated with increased OPG production, whereas RANK-L secretion is decreased. Tumor cells also produce OPG. Tumor-derived ET-1 directly acts onto mature osteoclasts to inhibit osteoclast activity. Therefore, there is a strong imbalance between bone formation and bone resorption, leading to aberrant bone formation. In addition, tumor-derived PSA and uPA increase the bioavailability of tumor growth-promoting factors to the bone microenvironment, such as IGF-I and TGF-β.

BMP: bone morphogenetic protein; ET-1: endothelin-1; IGF: insulin-like growth factor; OPG: osteoprotegerin; PSA: prostate specific antigen; TGFβ: transforming growth factor beta; uPA: urokinase.

Figure 6: Contribution of immune cells to bone metastasis formation. The innate and adaptive immune cells in the bone tissue microenvironment harbor both tumor-promoting and tumor-suppressing activities. $CD8^+$ T cells and natural killer (NK) cells eliminate tumor cells through the production of interferon (IFN)-γ or TRAIL/FASL-induced apoptosis. However, these tumor cells may escape to the cytotoxic activity of immune cells (*e.g.*, $CD8^+$ T cells), by inducing the recruitment of myeloid derived suppressor cells (MDSC), plasmacytoid dendritic cells (pDC) and regulatory T cells (Treg) that induce

an immunosuppressive state within the bone tissue microenvironment. Beside tumor-suppressing activities, MDSCs can differentiate into functional osteoclasts. Furthermore, tumor-associated macrophages and a population of specialist osteal tissue macrophages termed osteomacs facilitate bone metastasis formation. RANK-L: receptor activator of nuclear factor kappa-B (RANK) ligand.

Figure 7: Metabolic pathways associated with bone metastasis progression. In order to increase glucose uptake, cancer cells up-regulate glucose transporters, notably glucose transporter 1 (GLUT1). Glucose is then utilized for ATP generation through lactate production (aerobic glycolysis), *via* glucose-6-phosphate (G6P) and the pentose phosphate pathway (PPP) for nucleotide synthesis and through the tricarboxylic acid (TCA) cycle for lipid biosynthesis and protein acetylation. Lactate is released from tumor cells by monocarboxylate transporter 4 (MCT4) and then uptaken by osteoclasts through the transporter MCT1. Lactate stimulates osteoclast-mediated bone resorption, whereas fatty acids, cholesterol and nucleotides stimulate tumor cell proliferation.

Figure 8: (A) Clinical presentation of a solitary asymptomatic bone metastasis in a 54-year-old patient with breast cancer. A hot spot localized on L1 left pedicle (black arrow) was initially detected using technetium-99m (99m Tc)-bisphosphonate (BP) planar whole-body scintigraphy. Further analysis was conducted using fluorodeoxyglucose (FDG) positron emission tomography (PET) imaging. FDG/PET imaging displayed a hypermetabolic focus (white arrow) congruent to 99mTc-BP scintigraphy. (B) Clinical presentation of a solitary asymptomatic bone metastasis in a 67-year-old patient with prostate cancer. A hot spot localized on L3 vertebral body (black arrow) was initially detected using 99mTc-BP planar whole-body scintigraphy. Further analysis was conducted using fluorocholine (FCH)/PET imaging, displaying a hypermetabolic focus (white arrow) on L3, which was congruent to 99mTc-BP scintigraphy.

Figure 9: Current and emerging bone-targeted therapies. Summary of cellular and molecular targets and corresponding bone-targeted agents that are approved by the FDA and EMA for use in oncology or evaluated in phase trials. NCT: ClinicalTrials.gov identifier (ID) number.

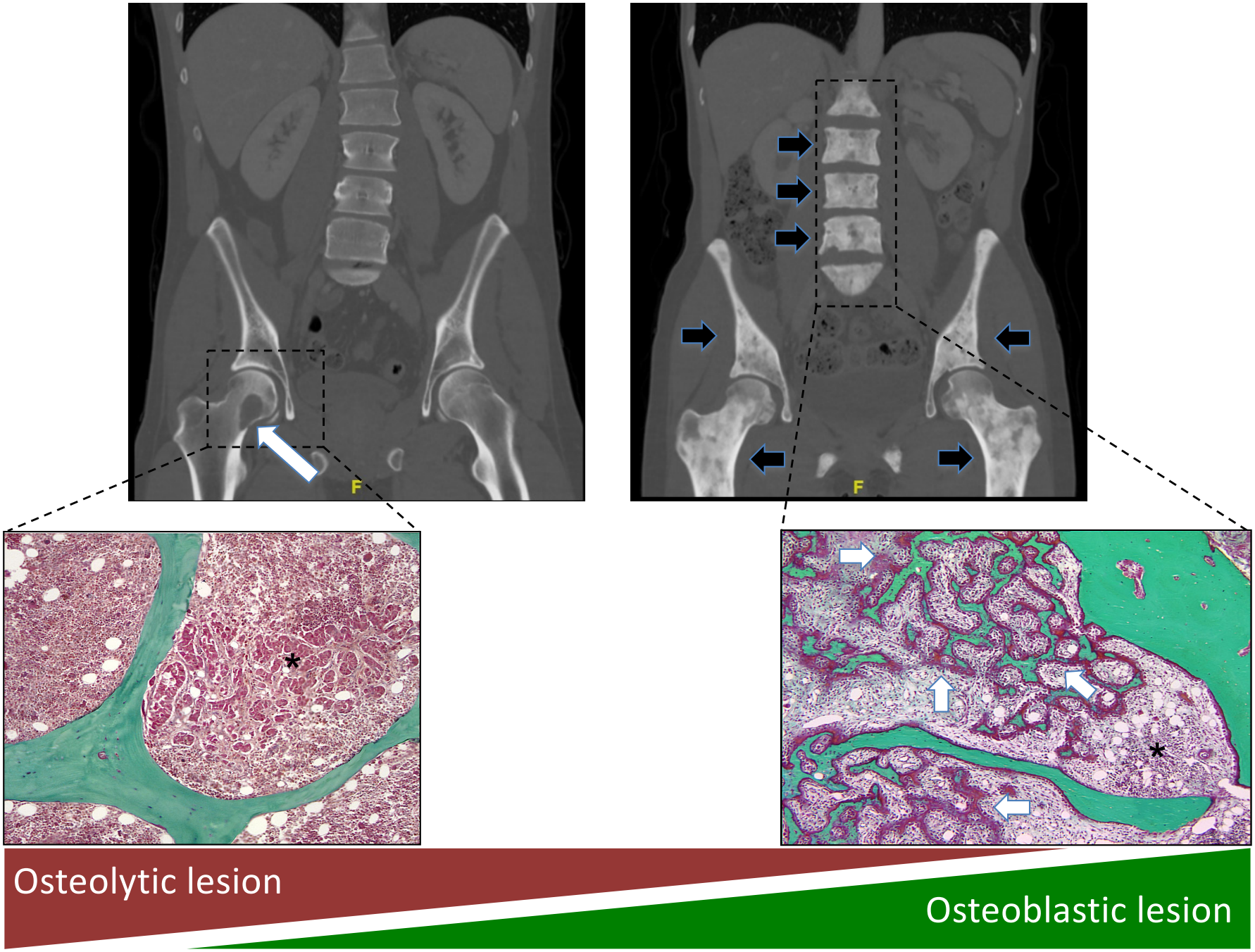


Figure 1

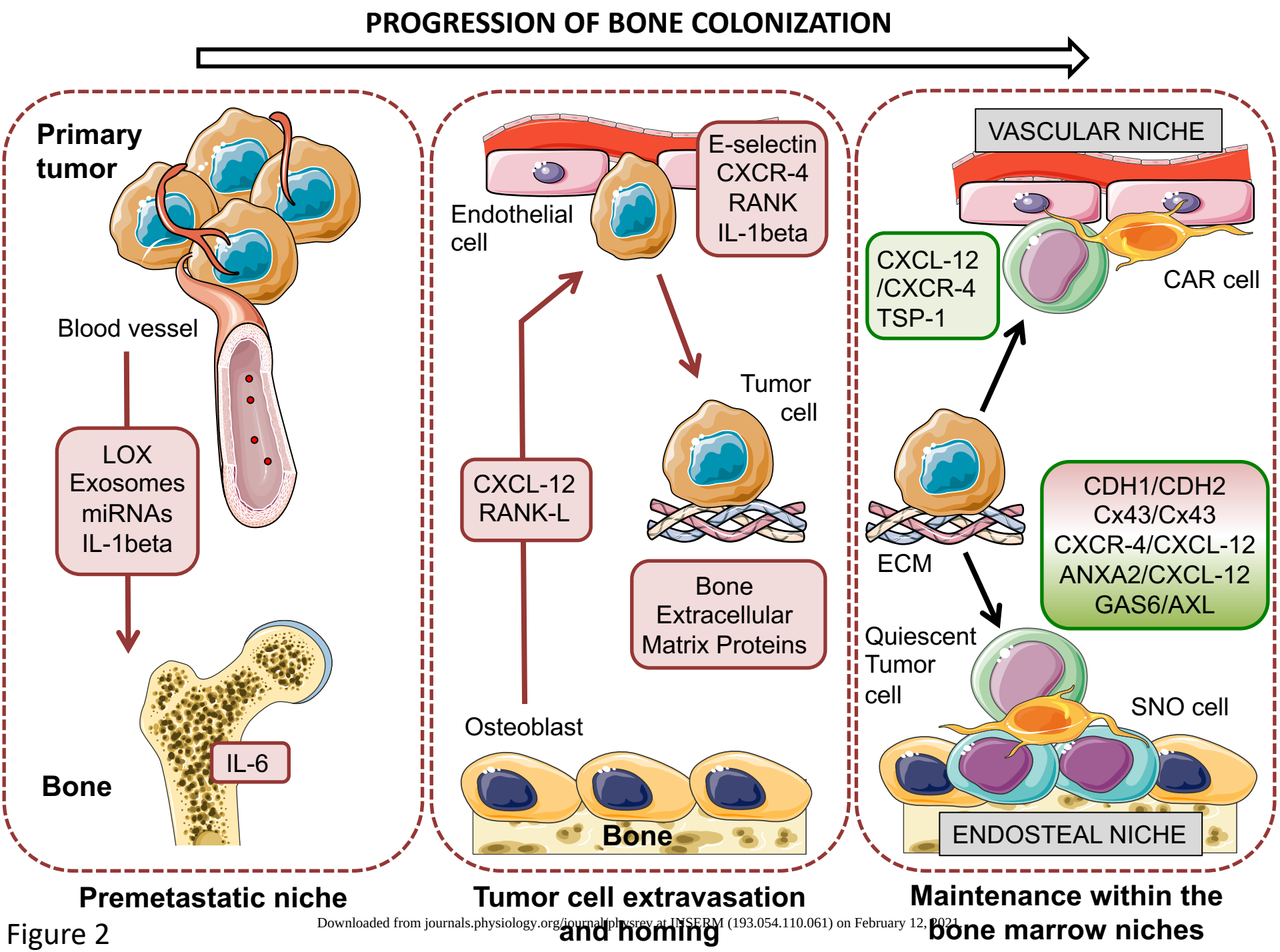


Figure 2

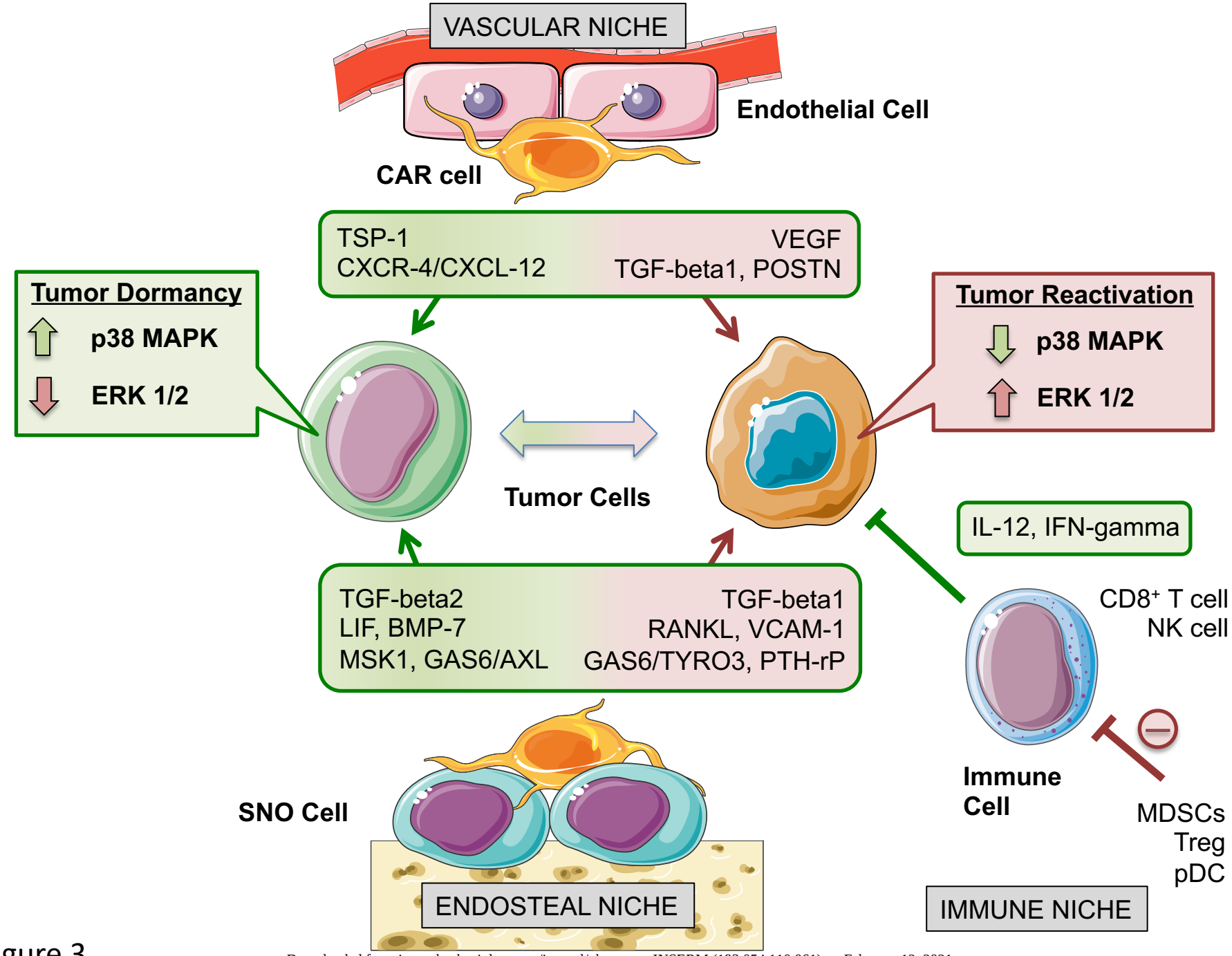


Figure 3

Figure 4

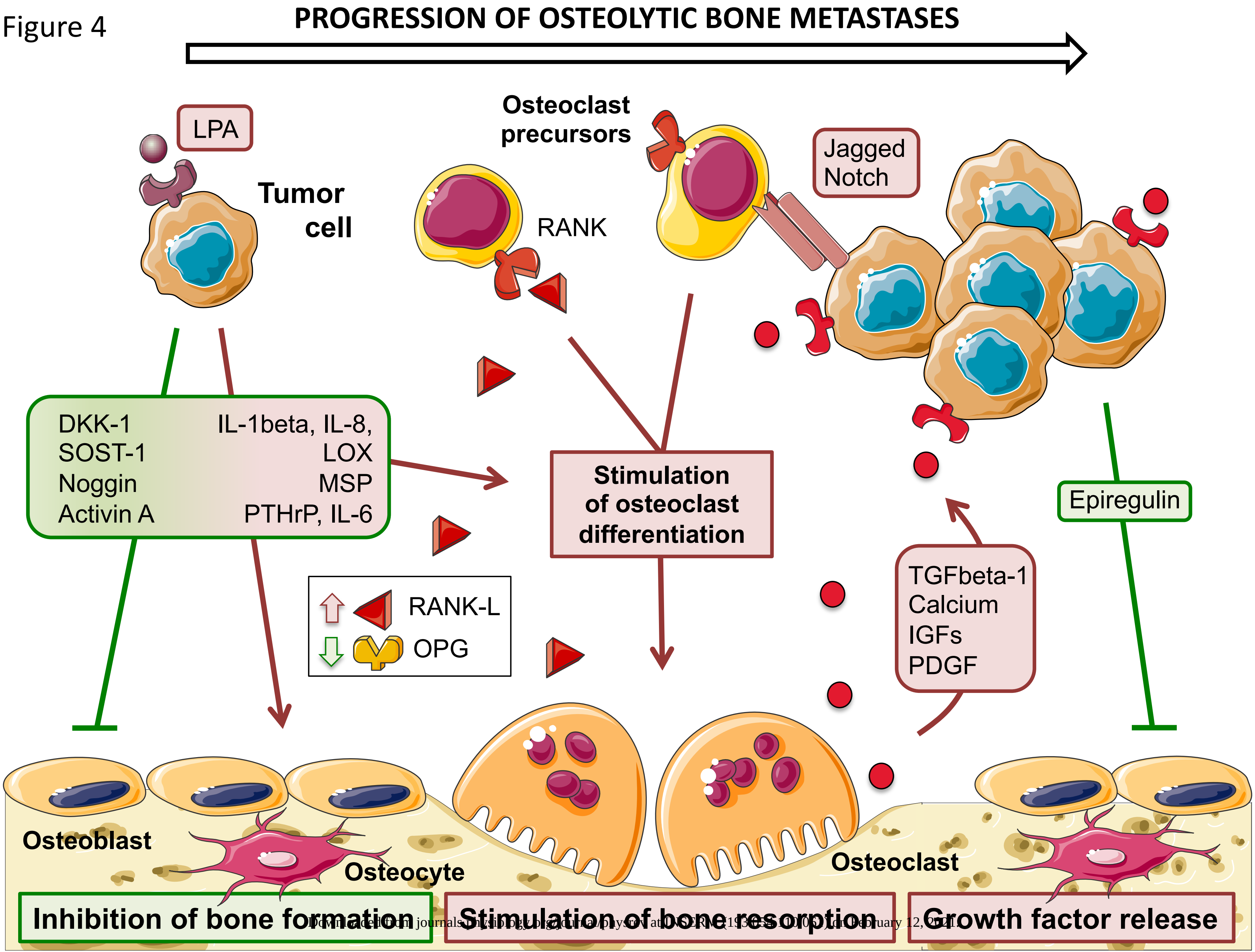
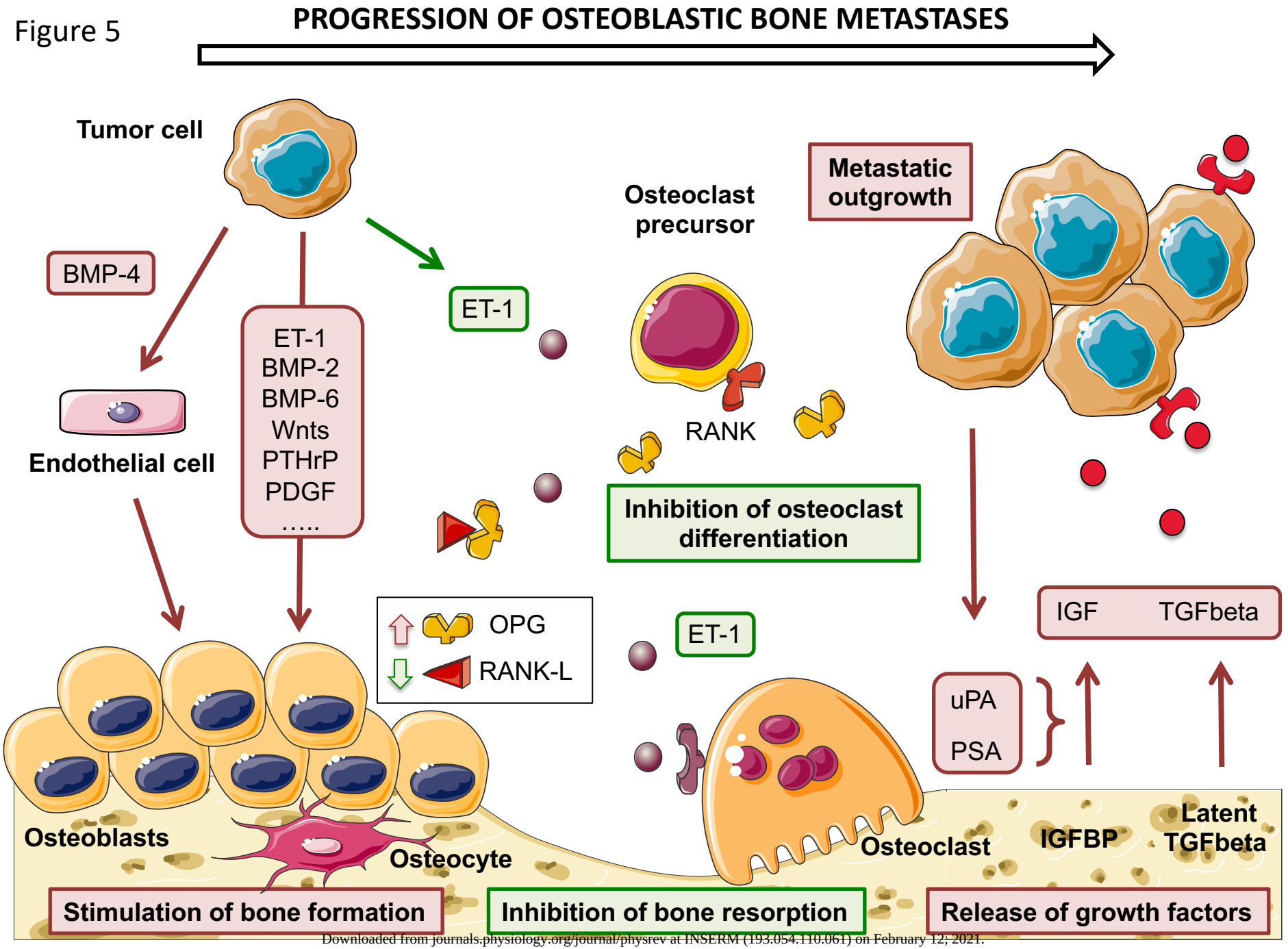
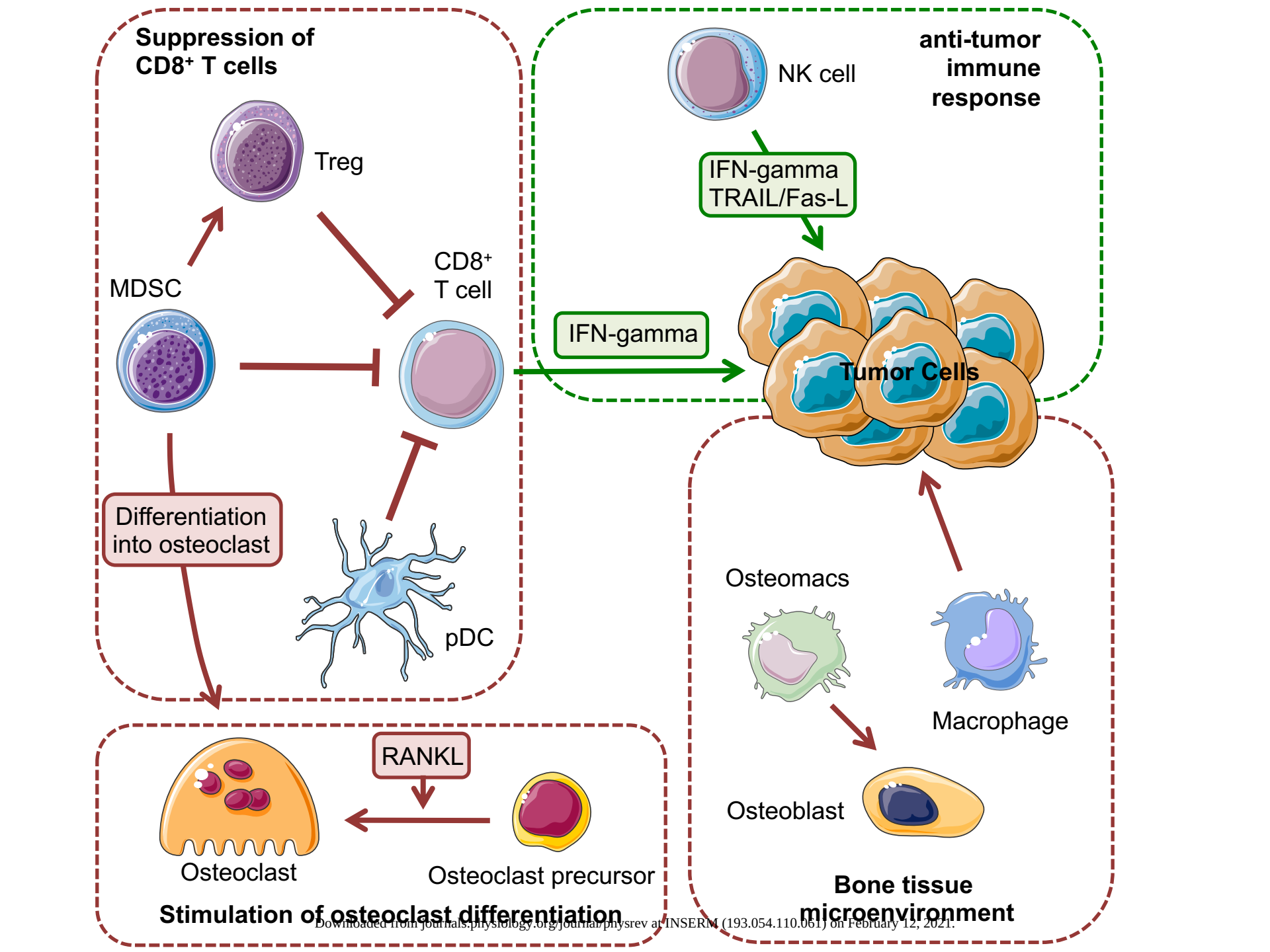


Figure 5





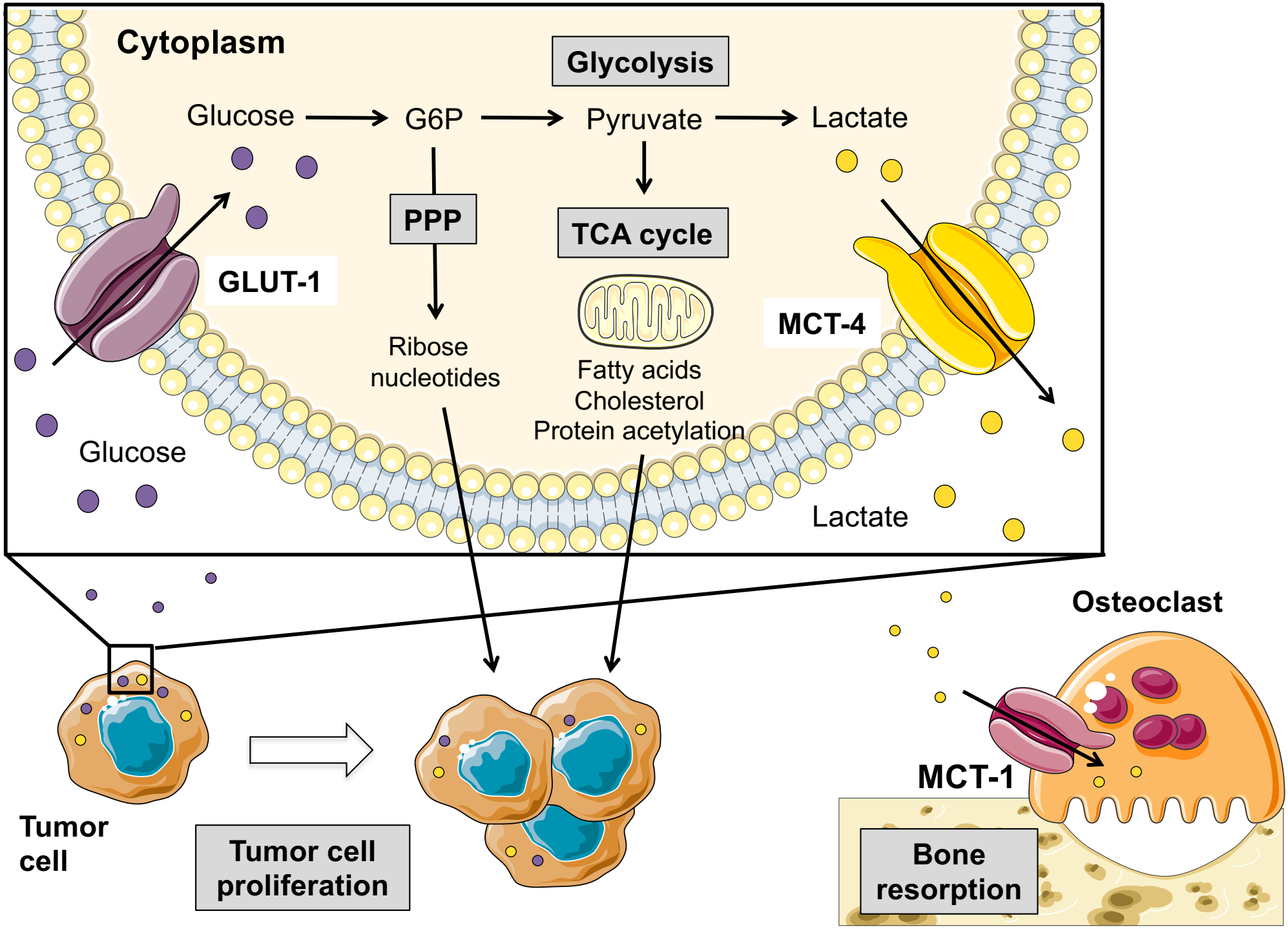
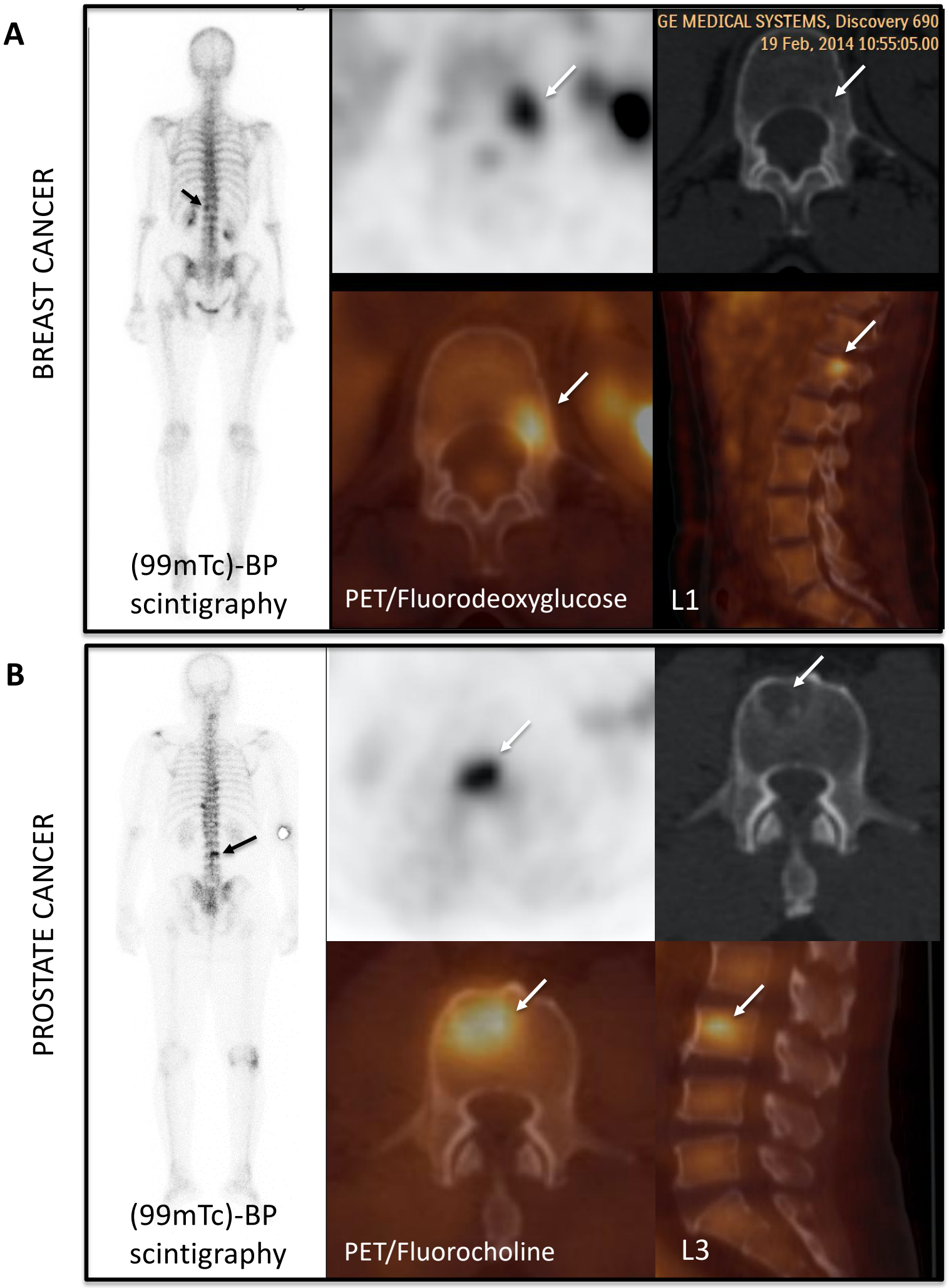


Figure 7



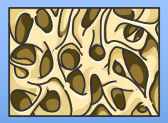
Matrix, Cell	Molecular target	Drug	Phase / Trial ID (NCT)
 Bone matrix	Calcium	Bisphosphonates, Radium-223, Samarium-223, Strontium-89	Approved
	TGFβ	Galinusertib	II / NCT02452008
 Osteoclast	FPPS	Bisphosphonates	Approved
	RANKL	Denosumab	Approved
	mTOR	Everolimus	Approved
	VEGFR, c-MET	Cabozantinib	Approved
	RON	BMS777607/ASLAN002	I / NCT01721148, NCT00605618
 Osteoblast	Androgen receptor	Enzalutamide	Approved
	CYP17A	Abiraterone	Approved
	Activin A	Sotatercept	II / NCT01562405, NCT00747123
	DKK-1	BHQ880	II / NCT01302886, NCT01337752, NCT00741377
 Nerve cell	NGF	Tanezumab	III / NCT00545129, NCT02609828

Table 1. *Chemokines and their receptors involved in the formation of bone metastasis.*

Chemokine receptor	Chemokine Ligand	Tumor type	Function in bone metastasis	Reference
CXCR-2	CXCL-1, CXCL-2	Prostate	Marrow adipocyte-derived CXCL-1 and CXCL-2 contribute to osteolysis in metastatic prostate cancer	135
CXCR-2	CXCL-5	Breast	The CXCR-2/CXCL-5 axis promotes tumor cell colonization in the bone marrow	275
CXCR-2	CXCL-8 (IL8)	Breast	Tumor-derived IL8 contributes to osteoclastic bone destruction in bone metastasis	18, 26, 27, 361
CXCR-3	CXCL-10	Breast, melanoma	CXCL-10 facilitates trafficking of CXCR-3-expressing cancer cells to bone	193
CXCR-4	CXCL-12	Breast, prostate	The CXCR-4/CXCL-12 axis regulates tumor cell entry in the bone marrow	235, 267, 273
CXCR-5	CXCL-13	Prostate	CXCL-13 mediates attachment of CXCR-5-expressing tumor cells to bone marrow endothelial cells *in vitro*	300
CX3CR-1	CX3CL-1	Breast, prostate	CX3CR-1 and CX3CL-1 interactions promote tumor cell extravasation in the bone marrow	158,159
CCR-2	CCL-2	Breast, prostate	CCL2-expressing tumor cells engage CCR-2-expressing macrophages and pre-osteoclasts to facilitate colonization in bone	213, 232

Table 2. *Potential clinical utility of bone turnover biomarkers.*

Biomarker	Abbreviation	Clinical application	Reference
Bone formation marker			
Bone alkaline phosphatase	BALP	Diagnosis of bone metastasis in solid tumors. Prognosis of bone metastasis in solid tumors. Risk of skeletal related events. Prognosis during anti-resorptive therapy. Prediction of response to treatment.	61, 67, 90, 91, 155, 187, 206
procollagen I carboxyl-terminal propeptide	PICP	Diagnosis of bone metastasis in prostate cancer. Prediction of response to atrasentan in prostate cancer.	187
Procollagen I amino-terminal propeptide	PINP	Diagnosis of bone metastasis in breast and prostate cancer.	35, 90, 180
Bone resorption marker			
C-telopeptide	CTX	Diagnosis of bone metastasis in prostate cancer. Prognosis of bone metastasis in breast cancer.	33, 35
N-telopeptide	NTX	Diagnosis of bone metastasis in prostate and lung cancer. Prognosis of bone metastasis in solid tumors. Risk of skeletal related events. Prognosis during anti-resorptive therapy. Prediction	35, 61, 67,90, 155, 187, 206

		of response to treatment.	
carboxyterminal telopeptide of type I collagen	ICTP	Diagnosis of bone metastasis in lung cancer. Prognosis of bone metastasis in breast cancer.	33, 155
Tartrate resistant acid phosphatase 5b	TRACP	Diagnosis of bone metastasis in breast cancer.	90, 155
pyridinoline	PYD	Prediction of response to atrasentan in prostate cancer.	186
Receptor activator of nuclear factor κB-ligand/osteoprotegerin	RANKL/OPG	Diagnosis of bone metastasis in solid tumors	125, 165, 183
microRNAs	miRNAs	Diagnosis of bone metastasis in breast cancer.	96, 294