Bone metastasis: the importance of the neighbourhood

Peter I. Croucher[1,2,3], Michelle M. McDonald[1,2] and T. John Martin[4,5]

Abstract | During the past decade preclinical studies have defined many of the mechanisms used by tumours to hijack the skeleton and promote bone metastasis. This has led to the development and widespread clinical use of bone-targeted drugs to prevent skeletal-related events. This understanding has also identified a critical dependency between colonizing tumour cells and the cells of bone. This is particularly important when tumour cells first arrive in bone, adapt to their new microenvironment and enter a long-lived dormant state. In this Review, we discuss the role of different bone cell types in supporting disseminated tumour cell dormancy and reactivation, and highlight the new opportunities this provides for targeting the bone microenvironment to control dormancy and bone metastasis.

The dissemination of cancer cells from sites of primary tumour growth is necessary for metastasis; however, few disseminated tumour cells (DTCs) survive to grow in distant organs. In addition to the invasive properties that these primary cancer cells require, they need the ability to interact with the microenvironment of the organ of metastasis in ways that favour colonization, survival and growth[1].

In patients with certain common cancers, including those of the breast and prostate, the skeleton is the most frequent site for metastasis[2]. Understanding of this owes much to Stephen Paget who, on the basis of autopsy studies of women with breast cancer, made the seminal observation that "in cancer of the breast the bones suffer in a special way"[3]. These insights led Paget to propose the "seed and soil" hypothesis in which the 'soil' of bone supports the survival and growth of the breast cancer cell 'seed'[2,3]. This concept has been expanded to the modern view that cancer cells need characteristics that enable them to grow in distant individual organs, and the specific organ microenvironment is critical to the development of metastases. Each metastatic microenvironment, whether it is brain, lung, liver or bone, exerts specific, and probably unique, functions that either support or oppose colonization by metastatic cells[4].

In the skeleton, the multi-step process of metastasis development (FIG. 1) begins with colonization, when circulating cells enter the bone marrow compartment and engage in specialized microenvironments or 'niches'. The second steps involve survival and dormancy, such that colonizing DTCs adapt to their new microenvironment, evade the immune system and reside in a dormant state for long periods, possibly decades. The third step, reactivation and development, requires an ability to escape from the dormant state to proliferate actively and form a micrometastasis. The final step, growth, occurs when cells grow uncontrollably, become independent of the microenvironment and ultimately modify bone as the metastasis flourishes.

Although progress has been made in understanding how tumour cells bring about changes to bone and how the bone microenvironment can control tumour growth in what is termed the 'vicious cycle'[5], we have limited knowledge of the crucial early events of colonization, survival and dormancy, and reactivation. Yet arguably, this is when tumour cells are most vulnerable to therapeutic targeting and are likely to provide the best opportunity to eradicate disease. Fortunately, the development of new technologies has facilitated the study of rare cells in bone, including dormant cells, and stimulated renewed interest in understanding these early steps in metastasis development. As the term 'dormancy' can be used to denote different phenomena, we use this term to define individual tumour cells that are resident in the skeleton in a quiescent state, or state of low cell cycling, for sustained periods of time but with the capability to give rise to overt tumours.

In this Review, we consider first, the properties of bone that enable it to host tumour growth and, secondly, the early critical events in metastasis to the bone by DTCs from solid cancers and also by cells of haematological malignancies, notably multiple myeloma, which are commonly located in bone. Although it is clear that these different tumours originate from different tissues and indeed different cellular lineages, they share features in common, particularly an ability to localize to similar locations in bone. It is therefore likely that they share common cellular and molecular features that control

[1]*Division of Bone Biology, Garvan Institute of Medical Research, 384 Victoria Street, Sydney, New South Wales 2010, Australia.*
[2]*St Vincent's Clinical School, University of New South Wales Medicine, Sydney, New South Wales 2052, Australia.*
[3]*School of Biotechnology and Biomolecular Sciences, University of New South Wales Australia, Sydney, New South Wales 2052, Australia.*
[4]*St Vincent's Institute of Medical Research, 9 Princes Street, Fitzroy, Melbourne, Victoria 3065, Australia.*
[5]*Department of Medicine, University of Melbourne, St Vincent's Hospital, Melbourne, Victoria 3065, Australia.*
Correspondence to P.I.C. or T.J.M.
p.croucher@garvan.org.au; jmartin@svi.edu.au

doi:10.1038/nrc.2016.44
Published online 25 May 2016

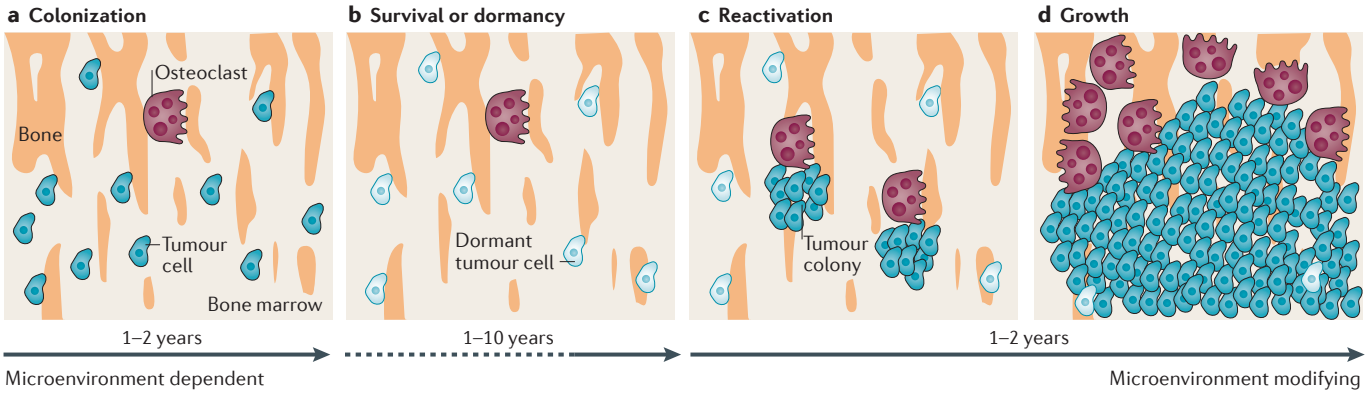

Figure 1 | **The multi-step process of bone metastasis development.** Bone metastasis development begins with tumour cells colonizing (panel **a**) the bone marrow microenvironment, a process that can occur early in the disease. Tumour cells survive and adapt to the local environment and, depending on their location, either grow immediately or enter dormancy (panel **b**), a phase that may last years or even decades. Of those cells that survive, a limited number will be reactivated (panel **c**) to form micrometastases, which ultimately enter a growth phase (panel **d**) and form microenvironment-modifying bone metastases. Tumour cells become progressively less dependent on the bone microenvironment for survival signals.

this behaviour. Therefore, this Review will consider these cancer types together and confine its comments to early events associated with colonization, survival and dormancy, and reactivation, as much has already been written about the molecular pathways that control active tumour growth in bone[5,6].

The skeleton as a site for metastasis

Common though metastasis is, tumour growth at metastatic sites is relatively less efficient than at primary sites, owing to the challenges faced by DTCs upon arrival at distant organs[7–9]. Although the skeleton is a permissive environment, the physical properties make it a harsh and unwelcoming site for colonizing DTCs. In experimental models of breast and prostate cancer dissemination, and multiple myeloma, substantial numbers of DTCs are present in the skeleton, but there are limited numbers of bone metastases or myeloma colonies[10–13]. Similarly, clinical studies in women with breast cancer show DTCs in the bone marrow but not all patients develop metastatic bone disease[14–16]. Furthermore, only 24% of patients with circulating tumour cells are reported to have detectable DTCs in the skeleton[14], suggesting that there are considerable barriers to cells colonizing and growing in the bone microenvironment. The bone microenvironment encountered by colonizing tumour cells is certainly remarkably heterogeneous and constantly changing, features that must be navigated by cancer cells if they are to survive and grow.

Bone cells and bone remodelling. The skeleton is maintained by continuous removal and replacement of bone throughout life. This is controlled by the coordinated activity of specific bone cells, which also influence tumour cell behaviour. Osteoclasts are highly specialized multinucleated cells, derived from haematopoietic precursors in the myeloid lineage, that are the only cells capable of bone resorption[17] (FIG. 2). Osteoclast formation is controlled by the receptor activator of nuclear factor-κB ligand (RANKL; also known as TNFSF11) and macrophage colony-stimulating factor (M-CSF; also known as CSF1), which are provided by cells of the osteoblast lineage[18]. Osteoclast-mediated bone resorption occurs in a sealed microenvironment in the area opposing the bone matrix. The resorption space is acidified by H^+ ions, resulting in dissolution of the bone mineral, exposing the organic matrix to proteolytic enzymes, including cathepsin K, that degrade the bone matrix[19]. Bone resorption also releases growth factors deposited in bone, which can act locally on osteoblasts and tumour cells in the microenvironment. Following resorption, sites of bone remodelling are found associated with a poorly defined cell type known as the reversal cell[20]. These are believed to have the capacity to produce proteinases and prepare the resorbed bone surface for bone formation by osteoblasts[21] and would also be well placed to act on tumour cells.

Osteoblastic bone formation results from the proliferation of primitive skeletal stem cells (SSCs), their differentiation into osteoblast precursors (osteoprogenitors and preosteoblasts), maturation to become osteoblasts, formation of bone matrix and, finally, mineralization[22] (BOX 1). Precursors of osteoblasts are derived from SSCs in the bone marrow, from blood and from pericytes and may also be found on the endosteal surface as components of the bone lining cell population or even the newly identified 'canopy' (discussed further below)[21,23]. Their fate is to become bone lining cells or to become embedded in bone as osteocytes (FIG. 2). Although the term 'osteoblast' is used to describe the cells responsible for synthesizing bone matrix, the osteoblast family also includes osteoblast precursors, bone lining cells and osteocytes, which change in phenotype as they transition through differentiation and each may have distinct roles when interacting with tumour cells (BOX 1). Gene expression patterns define the different stages[24] and their location in bone, as well as the influence of local and humoral factors. The latter include signals between cells in the osteoblast lineage as well as signals from immune cells

Bone resorption
The process by which bone-resorbing cells break down bone.

Osteoblast lineage
The lineage of cells responsible for making new bone.

Osteoprogenitors
Cells that are derived from skeletal stem cells with the ability to transition through the osteogenic lineage.

Preosteoblasts
Cells that are committed to forming functional bone-forming osteoblasts.

Mineralization
The process by which mineral, including hydroxyapatite, is precipitated and deposited on a collagen matrix.

Pericytes
Cells located adjacent to endothelial cells of capillaries.

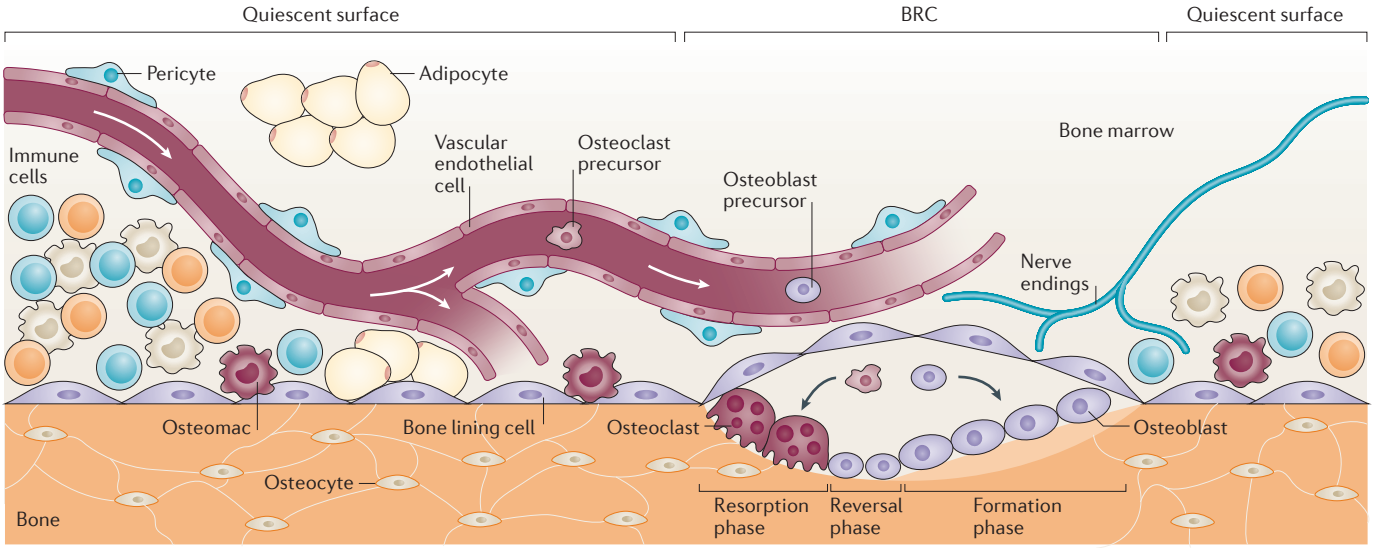

Figure 2 | **The endosteal bone surface.** The majority (approximately 80%) of the endosteal bone surface is quiescent and covered by bone lining cells, adipocytes, osteomacs, cells of the immune system and neurons. Blood vessels, lined by vascular endothelial cells and pericytes, supply the quiescent bone surface. A minority of the endosteal surface (approximately 20%) undergoes active bone remodelling at any one time. This occurs in the bone remodelling compartment (BRC), which is separated from the bone marrow compartment by a canopy of bone lining cells or cells of the myeloid lineage. Adjacent to the BRC is a network of capillary blood vessels, which can provide nutrients and oxygenation. They contain osteoclast precursors and early cells of the osteogenic lineage that can differentiate into functional bone-resorbing osteoclasts and bone matrix-synthesizing osteoblasts, respectively (see text for details). The events leading to activation of bone remodelling and the formation of a BRC are complex and probably under the control of osteocytes embedded in the bone matrix.

acting on cells of the osteoblast lineage[25,26,27]. For that reason, the cells of this lineage need to be identified *in situ*[28,29].

The activity of osteoclasts and osteoblasts is coordinated in time and space through the process of bone remodelling[30]. Bone remodelling occurs asynchronously at anatomically distinct sites called basic multicellular units (BMUs), and is crucial for adaptation of the skeleton to load and for repair of damaged bone (FIG. 2). The idea that bone cells might communicate with each other developed when it was proposed that cells of the osteoblast lineage produced factors that regulate the formation and activity of osteoclasts[31], and led to the discovery that RANKL, its signalling pathway and the decoy receptor osteoprotegerin (OPG; also known as TNFRSF11B), are essential regulators of osteoclast formation and activity[17]. This supported the concept of the supremacy of the osteoblast lineage over other lineages in control mechanisms within bone[31]. Furthermore, just as the osteoblast lineage regulates the osteoclast, communication also takes place in the reverse direction, with products of osteoclasts or resorption controlling bone formation in a process known as 'coupling'[32,33]. Accordingly, osteoblast differentiation and bone formation within BMUs are programmed by activities that arise both from osteoclasts themselves and from the bone matrix[25,34,35]. The dynamic nature of the BMU and its role in controlling cell behaviour may therefore be crucial in controlling tumour development in the skeleton (discussed further below) and speaks to the vicious cycle theory of tumour growth in bone[36,37].

The bone remodelling compartment. New evidence suggests that additional structures may control the behaviour of the BMU. A canopy of CD56 (also known as NCAM1)$^+$ bone lining cells and capillaries covering the BMU has been described[38–40], although a less well characterized layer of cells called the bone marrow envelope has recently been reported to cover the bone lining cells[41]. This structure is now referred to as the bone remodelling compartment (BRC)[39] (FIG. 2). In a study of patients with hyperparathyroidism, chosen for the large number of sites of bone remodelling, canopies were identified over virtually all remodelling sites[40]. An abundant network of capillaries is present adjacent to the canopy surface (FIG. 2), and is even proposed to penetrate the canopy[40,42] to provide entry, via the circulation, for osteoclast and osteoblast precursors. The opening of capillaries into the BRC has not been directly demonstrated, but preosteoclasts have been identified in adjacent capillaries as well as within the BRC, and accessibility to the circulation has been demonstrated by the fact that 5 minutes after ferritin injection in rabbits, ferritin was present within canopies[38]. Bone marrow stromal progenitor cells and pericytes that adhere to nearby capillaries may also be important sources of osteoblast precursors[22]. It is conceivable that tumour cells may be delivered directly to the BRC, or the immediate microenvironment, by the capillaries associated with this structure, which could have implications for the development of tumours in bone. In support of this, myeloma cell–osteoclast hybrids have been reported[43].

Osteomacs
Macrophages associated with the bone surface.

Osteogenic lineage
The lineage that gives rise to cells that can make new bone; these include bone lining cells, osteoblasts and osteocytes.

Basic multicellular units
The cells responsible for the coordinated removal and replacement of a quantum of bone in bone remodelling.

Hyperparathyroidism
A disorder of the parathyroid gland resulting in increased concentrations of circulating parathyroid hormone.

Bone marrow stromal progenitor cells
A population of stromal cells found in the bone marrow that can differentiate into a range of cell types including cells of the osteogenic lineage.

Reversal phase
The phase of bone remodelling between bone formation and bone resorption.

Endochondral ossification
One of the essential processes by which bone is formed via a cartilage cell precursor.

Canaliculi
The microscopic canals that link the lacunae occupied by osteocytes.

The endosteal bone surface. Although the BRC is an important feature of the bone microenvironment, it represents only a small proportion of the bone surface. Indeed, only 1–2% of the bone surface is undergoing bone resorption and approximately 20% is undergoing bone formation in humans at any one time[38,39,44]. Even if we consider the reversal phase, this would argue that less than 25% of the endosteal surface is actively involved in bone remodelling. The remaining endosteal surface is covered largely by bone lining cells and is relatively quiescent (FIG. 2). Capillaries are found associated with both quiescent and actively resorbing endosteal surfaces, although their frequency is higher at resorbing surfaces. The quiescent surface is also innervated and populated by cells other than bone lining cells, including F4/80 (also known as ADGRE1)$^+$ macrophages, which are also referred to as osteomacs)[45].

The fact that colonizing tumour cells may be delivered to different bone microenvironments could be key to their fate. Those delivered to the BRC will be exposed to a rich microenvironment containing factors that promote growth and survival and thus the cells may grow immediately, whereas those arriving at quiescent surfaces will find a microenvironment that promotes tumour cell dormancy. Given that the quiescent endosteal surface predominates, it is conceivable that colonizing tumour cells may be more likely to become dormant than to proliferate when they arrive in bone. In this regard, it is already clear that cells present on the bone surface can provide a localized supportive microenvironment, or niche, in which specialized cells reside[46]. Although this has been best studied in relation to haematopoietic stem cells (HSCs), this may have direct implications for understanding how cancer cells survive in bone.

Bone cells and stem cell niches in bone. Early studies showed that HSCs home to spindle-shaped, N-cadherin (also known as cadherin 2)$^+$ CD45 (also known as PTPRC)$^-$ osteoblasts in endosteal regions of bone[47–49] and consequently, the HSC niche was referred to as the "osteoblast" or "endosteal" niche[48,49]. However, these studies could not specify which cells in the osteoblast lineage are present in these niches[50,51]. Studies in mice in which the parathyroid hormone/parathyroid hormone-related protein receptor (PTH/PTHrP receptor; which is encoded by *Pth1r*) was overexpressed in the osteoblast lineage, or in which the bone morphogenetic protein (BMP) receptor type 1A (*Bmpr1a*) gene was deleted, demonstrated the importance of the osteoblast lineage, or preosteoblasts to the osteoblast or endosteal niche[48,49]. The importance of early osteoblast precursors in niche formation is supported by the discovery of pericyte involvement in the HSC niche[52]. Pericytes are mesenchymal stem cells (MSCs) that adhere to blood vessels in the bone marrow and are SSC precursors of osteoblasts[22,28,29,53,54] (FIG. 2). *Ex vivo* real-time and intravital imaging in animals indicates a close association between the endosteal osteoblastic HSC niche and vasculature, and that endochondral ossification is required for HSC niche formation[55–57]. Furthermore, activating mutations in β-catenin that drive osteoblast differentiation have been shown to promote development of acute myeloid leukaemia, which also develops in the bone marrow[58]. However, recent evidence points to cells early in the osteoblastic lineage playing a part in the HSC niche and there was no evidence in these studies that mature, bone-synthesizing osteoblasts have a role[29,59,60]. Although the precise nature of the cells that comprise the HSC niche has not been fully defined, it is clear that cells present on the endosteal bone surface have the capacity to support HSCs and may support colonizing tumour cells.

Tumour cell colonization of bone

The success of colonizing cancer cells in establishing themselves in the bone microenvironment is likely to be determined by intrinsic properties of the DTCs, which are governed by the primary tumour microenvironment, as well as by acquired characteristics determined by the distant bone microenvironment. There are certainly many aspects of the biology of bone that are unique in providing influences that are unlikely to be available in other metastatic sites.

Box 1 | **Cells of the osteoblast lineage and putative roles in tumour cell dormancy**

Tumour cells interact with cells of the osteoblast lineage and influence their bone-forming ability. In the case of osteolytic disease this is usually associated with osteoblast suppression, whereas in osteosclerotic disease this results in increased osteoblast formation and function, and leads to inappropriate bone deposition. Osteoblast lineage cells also have a role in the early tumour cell colonization of bone and may prove to be pivotal in controlling tumour cell dormancy in the skeleton. Cells of this lineage have different roles at different stages and they should not be considered as a single entity:

- Skeletal stem cells have the capacity to give rise to osteoprogenitors and cells of the osteogenic lineage.
- Osteoprogenitors are derived from skeletal stem cells and undergo transition through the osteogenic lineage.
- Osteoblasts are bone-forming cells; their short-lived nature makes them the least likely among the lineage to have a role in supporting the long-term dormancy of tumour cells in bone.
- Bone lining cells are derived from osteoblasts that have completed their functional bone-forming activity. They cover the endosteal bone surface and are abundant, long-lived and relatively quiescent. They can be reawakened to form bone and are well placed to support the long-term dormancy of tumour cells that have successfully colonized bone.
- Osteocytes are derived from osteoblasts that have become embedded in mineralizing bone. They respond to mechanical strain and are pivotal regulators of bone remodelling, communicating with the bone surface via canaliculi. Osteocytes are likely to respond to the presence of tumour cells that either are on the endosteal surface or have modified the bone microenvironment.

Primary tumour determinants of bone metastasis. Gene expression studies have identified signatures in primary cancers in humans that are associated with poor outcome and metastasis[61–63]. In human breast cancer cells, bone metastatic signatures, which include expression of interleukin-11 (*IL11*), connective tissue growth factor (*CTGF*) and *SRC* signatures have been defined[64,65]. Expression of transforming growth factor-β (TGFβ) in the primary tumour also induces angiopoietin-like protein 4 (ANGPTL4) and promotes retention of metastatic cancer cells in the lungs, but not bone[66]. Furthermore, cancer associated fibroblasts (CAFs) in the primary tumour can impose selective pressure for tumour cell clones with a propensity to grow in bone, which seems to be mediated by C-X-C motif chemokine ligand 12 (CXCL12; also known as SDF1) and insulin-like growth factor 1 (IGF1) that are secreted by the CAFs[67,68]. By contrast, expression of interferon regulatory factor 7 (IRF7)-regulated genes in primary breast cancers is associated with increased bone metastasis-free survival, and interferon treatment or overexpression of IRF7 in 4T1 tumour cells prevents bone metastasis in mouse models[69]. This supports the notion that the control of the innate immune system by primary breast cancer cells restricts tumour immune surveillance, facilitating bone metastasis. Moreover, in prostate cancer, expression of CXCL16 by tumour cells mediates recruitment of MSCs into the primary tumour, controls their differentiation into CAFs, induces epithelial–mesenchymal transition (EMT) and aids metastasis to bone[68].

Although studies have found that matched primary cancers and metastases were more similar than those from different patients[63], this has not been widely replicated and indeed different gene expression profiles have been reported in primary and metastatic sites of breast and colon cancers[70–72]. This is not surprising, as signatures from a primary cancer will be driven by exposure to a different microenvironment from that experienced in the metastatic site. Furthermore, independent progression of metastases arising from early DTCs is also likely to result in increased intraclonal variation between the primary tumour and individual bone metastases, a concept supported by the intraclonal heterogeneity described in prostate cancer bone metastases[73,74,75]. Thus, it may not be a surprise if gene signatures in the primary tumour differ from those of the bone metastasis.

The regulation of tumour cell colonization of bone. In bone, tumour cells initiate colonization through a process controlled by the physical microenvironment, specific chemokines and adhesion molecules. Tumour cells entering bone encounter a physical environment different from that of other organs. The bone extracellular matrix is more than 10^5 times more rigid than the extracellular matrix found in soft tissue, and matrix rigidity controls cell behaviour, transformation and morphogenesis[76,77]. Synthetic substrates, with elastic modulus values ranging from those of soft tissue to those of bone[78], show that increasing matrix rigidity increases GLI2 and TGFβ signalling, and parathyroid hormone-like protein (*Pthlh*; the gene encoding PTHrP) promoter activity in breast cancer cells[79,80], factors crucial in the development of bone metastasis. This argues that the physical environment, although poorly understood, may be an important determinant in establishing tumours in bone.

Tumour cells express specific repertoires of molecules that facilitate colonization. For example, expression of C-X-C motif chemokine receptor 4 (CXCR4) facilitates chemotaxis and invasion of a range of tumour cells into lymph nodes, lung and bone[81–83]. In bone, CXCR4 binds to CXCL12, which is expressed by mesenchymal cells adjacent to bone surfaces or by pericytes[84,85], and MDA-MB-231 breast cancer cells selected for their propensity to establish themselves in bone express CXCR4 (REF. 64). Human multiple myeloma cells also express CXCR4, which controls integrin-mediated migration and binding to vascular cell adhesion molecule 1 (VCAM1) on local bone cells[86,87] and upregulates macrophage inflammatory protein 1α (MIP1α; also known as CCL3)[88], a pro-osteoclastic factor produced by multiple myeloma cells[88–90]. Importantly, inhibition of CXCR4 releases multiple myeloma cells from the bone and reduces tumour burden by increasing tumour cell sensitivity to the proteasome inhibitor bortezomib[91]. Similarly, release of prostate and breast cancer cells from the control of the bone microenvironment by CXCR4 antagonism increases their sensitivity to chemotherapeutic agents[92,93].

Tumour cells also express integrins, including $\alpha_v\beta_3$ and $\alpha_v\beta_5$ (REF. 94), which can bind to osteopontin (OPN), bone sialoprotein (BSP; also known as IBSP) and vitronectin expressed by cells of the osteoblast lineage[95,96], and E-cadherin, which can bind to N-cadherin on osteoblasts[97]. Tumour cells also express OPN themselves and in women with breast cancer this is associated with bone metastasis[98,99]. BSP is also expressed in breast cancer cells and controls adhesion, migration and proliferation by binding to $\alpha_v\beta_3$ and $\alpha_v\beta_5$; cells lacking BSP expression have reduced bone metastatic potential[100–102]. In contrast, multiple myeloma cells express integrin $\alpha_4\beta_1$ (REF. 103), which mediates interactions with bone cells via VCAM1, and treatment of mice bearing 5TGM1 mouse multiple myeloma cells with anti-integrin β_1 antibody reduces tumour growth[104], arguing for a functional role of integrin β_1 in colonization.

Tumour cells also express cytokine receptors, which may contribute to colonization. For example, breast and prostate cancer cells and B16 melanoma cells express RANK, the receptor for RANKL, and RANKL promotes motility of these cells *in vitro*, whereas inhibition of RANKL signalling with OPG prevented establishment of bone metastasis by intracardiac injection of B16 melanoma cells[105]. In metastatic bone disease and myeloma, tumour cells promote RANKL production by osteoblast lineage cells[106–109], and in experimental models RANKL-targeted agents prevent bone disease and reduce tumour burden[110–113]. Stimulation of the sympathetic nervous system also enhances RANKL production by osteoblastic cells and is associated with a pro-migratory effect on cancer cells *in vitro* and increases in breast cancer bone metastasis *in vivo*[114]. Whether the antitumour effect is mediated by blockade of the vicious cycle, blocking

Innate immune system
The cells and mechanisms present in readiness to fight microorganisms.

Elastic modulus
A measure of the elasticity or stiffness of a material.

Metaphyseal region
The section of a long bone between the diaphysis (shaft) and epiphysis (end).

Diaphyseal region
The midsection or shaft of a long bone.

colonization and engagement in bone or whether it prevents activation of dormant cancer cells (discussed below) is unclear.

Bone cell control of dormancy

Having colonized the skeleton, tumour cells locate to specialized microenvironments, or niches, which support survival and dormancy. Whether colonizing tumour cells hijack HSC and/or progenitor niches[115] or occupy distinct niches is unclear.

The osteoblast lineage, tumour cell survival and dormancy. Like HSCs, there is evidence that colonizing tumour cells localize to niches containing cells of the osteoblast lineage, which support cell survival and control long-term dormancy[13]. However, the pathways that regulate colonization may be distinct from those that control dormancy (FIG. 3).

Tracing of ^{51}Cr-labelled multiple myeloma cells in the 5T mouse experimental model showed that they home selectively to the bone marrow, liver and spleen, but survive only in bone and spleen[116]. The estimate that thousands of multiple myeloma cells arrive in individual bones is supported by direct intravital imaging[13]. These cells localize to bone surfaces, interact directly with type I collagen-expressing cells of the osteoblast lineage and are retained in a dormant state[13].

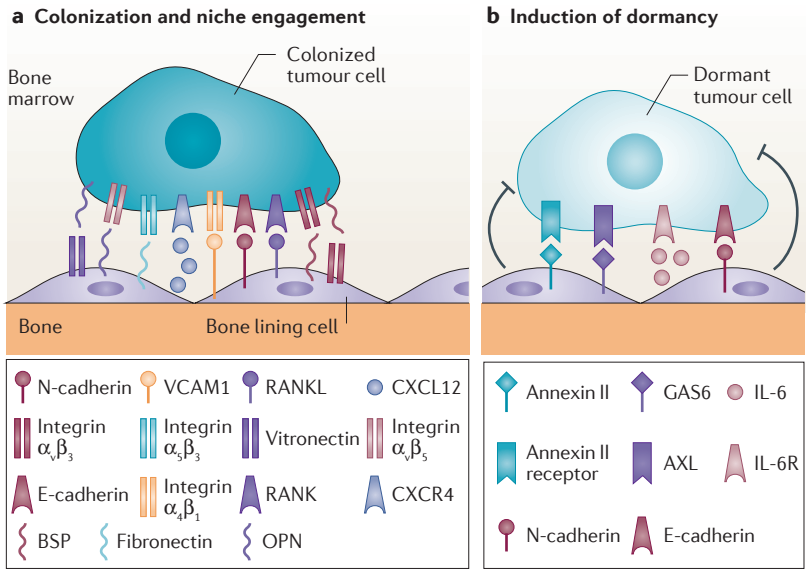

Figure 3 | **Niche engagement and induction of dormancy.** Tumour cells use a repertoire of molecules that are important in colonizing bone and engaging in a niche in bone (panel **a**). These include C-X-C motif chemokine receptor 4 (CXCR4), integrin $\alpha_4\beta_1$, receptor activator of nuclear factor-κB (RANK), integrins $\alpha_v\beta_5$ and $\alpha_5\beta_3$, osteopontin (OPN), bone sialoprotein (BSP) and E-cadherin, which bind to their cognate ligands on cells in the niche: C-X-C motif chemokine ligand 12 (CXCL12), vascular cell adhesion molecule 1 (VCAM1), RANK ligand (RANKL), OPN, BSP, fibronectin, vitronectin, integrin $\alpha_v\beta_3$ and N-cadherin, respectively. The engagement of cells in the niche leads to the expression of genes that can induce dormancy (panel **b**). These include the annexin II receptor and AXL on tumour cells and their ligands annexin II and growth arrest-specific protein 6 (GAS6) on bone lining cells. Local cytokines and growth factors, for example interleukin-6 (IL-6), may also control dormancy by binding IL-6 receptor (IL-6R) on tumour cells.

Indeed, cells of the osteoblast lineage maintain multiple myeloma cells in a dormant state and slow the transition to active growth[13]. Multiple myeloma cells isolated from patients also localize to an osteoblast niche in non-obese diabetic-severe combined immunodeficient (NOD-SCID) mice[117]. Dormant multiple myeloma cells resist chemotherapies, are available to repopulate tumours and may contribute to disease relapse in experimental mouse models[13,117]. Although the crucial signals that support dormancy remain to be defined, cells of the osteoblast lineage express factors that could contribute, including IL-6 (REF. 118), BMPs[119–122], WNT proteins and WNT antagonists[123], decorin[124] and membrane adhesion molecules and receptors such as the annexin II receptor and growth arrest specific protein 6 (GAS6), which bind to annexin II and the AXL receptor tyrosine kinase (AXL, also known as UFO), respectively, on multiple myeloma cells[13,125]. Osteoblast lineage cells also produce OPG, which promotes tumour cell survival by binding to tumour necrosis factor-related apoptosis-inducing ligand (TRAIL; also known as TNFSF10)[126–130].

In patients with solid tumours, DTCs can be detected at early stages of disease[131–135] and in experimental models slow-cycling or dormant tumour cells can be found in the skeleton[10–12,135]. Shiozawa *et al.*[115] demonstrated in mice that prostate cancer cells compete with HSCs for the HSC niche. Although the molecular mechanisms are poorly defined, both prostate cancer cells and multiple myeloma cells express the annexin II receptor, which, by binding to annexin II on bone cells, controls tumour growth[136,125]. In prostate cancer cells this interaction regulates expression of the receptor tyrosine kinases *AXL*, *TYRO3* (also known as *SKY*) and *MER* (also known as *MERTK*)[136], which encode the receptors for GAS6. The balance between the expression of GAS6 and expression of these receptors, particularly AXL, may be important in controlling dormancy, with relatively high levels of AXL being associated with dormancy in human xenograft models of prostate cancer[136–138]. Interestingly, AXL is stabilized by hypoxia[139] and the metaphyseal region of the long bone, a site in which tumours typically develop, is normoxic, whereas the diaphyseal region is more hypoxic[140] and less prone to metastasis development. Furthermore, endosteal regions are less hypoxic than deeper perivascular regions[141], which may also have implications for maintaining cells in a dormant state. The recent demonstration that DTC–niche interactions in breast cancer are mediated by heterotypic adherens junctions, with DTCs expressing E-cadherin, and N-cadherin being expressed by osteogenic cells, is also consistent with osteoblasts having an active role in controlling dormancy[97].

The perivascular microenvironment and tumour cell dormancy. Although cells of the osteoblast lineage may control survival and dormancy, perivascular cells have also been implicated[142]. Disseminated breast cancer cells can be found associated with the vasculature in metastatic target tissues, including bone in mice[143], and thrombospondin 1 (TSP1; also known as THBS1), produced by endothelial cells, maintains associated cells

Metastasis initiating cells
Cancer cells with the ability to give rise to overt metastasis in distant organs.

Cancer stem cells
Cancer cells with self-renewal and tumour-initiating abilities.

in a dormant state[143]. Physical changes to the microenvironment brought about by sprouting new blood vessels remove this suppressive signal, releasing cells from dormancy and increasing breast cancer cell growth[143]. TGFβ and periostin, which are also expressed by osteoblasts[144,145] and implicated in breast cancer cell growth and metastasis[64,146–150], are released from the endothelial tip cells of the neovasculature to promote tumour growth in mice[143].

The location of the bone metastasis niche in the skeleton. Although evidence suggests that the endosteal surface and cells of the osteoblast lineage are important in tumour colonization of bone and may control dormancy, the specific identity of the cells that comprise the niche remains to be determined. Whether these cells are functional bone-synthesizing osteoblasts, osteoprogenitors, bone lining cells or even SSCs has still to be established. Equally, other cells, including osteomacs[45,151], endothelial cells, perivascular cells or neurons, also found associated with the endosteal surface, may have a role.

However, the long latency associated with bone metastasis development suggests that niches are stable microenvironments that do not undergo short-term change. This provides clues to their locations and the identity of the cells that comprise the niche. This is certainly supported by the demonstration that stable microvasculature provides a niche for breast cancer cells, whereas the sprouting microvasculature activates dormant cells[143]. This would argue against functional bone-synthesizing osteoblasts within the BRC being components of the niche, as human bone formation occurs over months rather than years[152], and these cells will change during this period as their functional capacity declines and they become osteocytes or bone lining cells. These changes would result in changes to the niche, removal of suppressive signals and would lead to reactivation of dormant cells. It is therefore likely that cells that exist in a long-term quiescent state such as bone lining cells are better placed to support long-term tumour cell dormancy (FIG. 2).

Control of dormant cell reactivation

The niche is crucial in capturing and retaining tumour cells in a long-term dormant state in bone. This may be a multi-step process. The first step of engagement occurs when tumour cells use a profile of receptors and adhesion molecules to locate and adhere to cells within the niche (FIG. 3). The second step occurs when cells in the niche regulate the phenotype of the colonizing tumour cells to stabilize the cell, ensure that they adapt to this environment and induce dormancy (FIG. 3). This is likely to be an active process requiring induction of new gene expression, and to be niche dependent. However, a crucial step will be the release of cells from the niche to form an overt metastasis. Currently, our understanding of how dormant cells escape control of the niche is limited. The selective release of some but not all dormant cells suggests a complex level of control[13]; however, we have only limited insights into what is arguably one of the most important questions in the field.

VCAM1 may be one tumour cell product that has a role in activation of indolent micrometastasis[153–155]. This is believed to be through recruitment of integrin $\alpha_4\beta_1^+$ osteoclast progenitors, which have a role in establishing a vicious cycle[153]. Although this remains an intriguing hypothesis it does not explain why only selected dormant cells get activated. Indeed, release from dormancy (reactivation)[156] may be under tumour cell-autonomous (intrinsic) control. While dormant, metastasis-initiating cells have many of the characteristics of putative cancer stem cells[156]; only a small proportion are reactivated, suggesting that there is heterogeneity among this population. Whether these features are present in the colonizing tumour cells or acquired once they have entered the bone metastasis niche is unclear (FIG. 3); however, the fact that dormant tumour cells cycle rarely argues that acquisition of new genetic or epigenetic events that facilitate reactivation is unlikely.

An alternative explanation is that release from dormancy is under non-tumour cell (extrinsic) control (FIG. 4). This would require changes to the local microenvironment that result in removal of crucial signals that maintain cells in a dormant state. Alternatively, both models may operate with limited numbers of cells retaining the capacity to be reactivated once changes to the microenvironment remove inhibitory signals. Of course, the possibility that these two models operate independently cannot be excluded. Indeed, it has been proposed that in solid tumours there may be two different metastasis-initiating cells[156] and it is conceivable that these different populations respond differently to changes in the niche. There is certainly increasing evidence that bone cells regulate the behaviour of tumour cells and in doing so have the capacity to release dormant cells from niches in bone, arguing for extrinsic control of reactivation.

Bone turnover and osteoclasts regulate tumour growth. Manipulation of the bone microenvironment in experimental models of solid tumour growth in bone is well known to alter tumour development. Examples include overexpression of PTHrP, vitamin D deficiency, calcium restriction and ovariectomy, which all increase bone turnover and all accelerate tumour development in the skeleton[157–161]. Interestingly, dietary calcium restriction increases osteoclastic resorption and tumour growth but has no effect on osteoblast numbers, suggesting that the effect is mediated by osteoclasts rather than osteoblasts[158]. Furthermore, resorption inhibition, with either OPG treatment or bisphosphonates, reduces tumour burden, arguing for a role for osteoclasts in reactivating tumour growth in bone[162–164]. Ottewell *et al.*[10] recently demonstrated that breast cancer cells develop lesions in the skeleton of young but not mature (in which bone turnover is reduced) mice. This is despite the demonstration that DTCs can be detected in the bones of mature animals. Ovariectomy, which accelerates bone turnover in mature mice, increased the number of bone lesions, but not non-osseous lesions[10]. This is inhibited by blocking osteoclastic resorption with the bisphosphonate zoledronic acid or recombinant OPG[10,165].

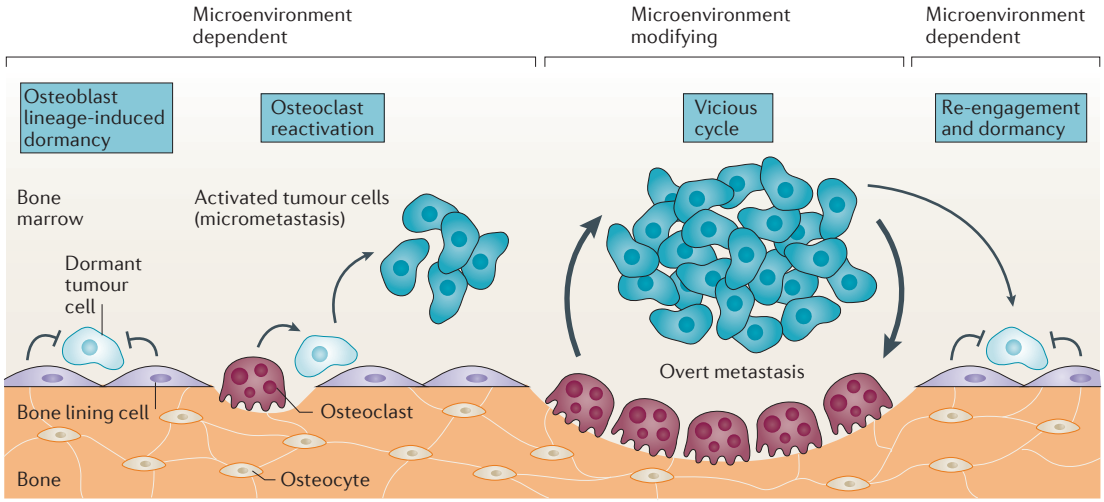

Figure 4 | **Osteoclast remodelling of the endosteal niche reactivates dormant tumour cells.** Tumour cells engage with cells of the osteogenic lineage on the endosteal surface, which induces long-term tumour cell dormancy. Osteoclast-mediated remodelling of the endosteal niche removes bone lining cells and releases dormant tumour cells from niche-dependent control, enabling them to proliferate and form micrometastases. The micrometastases then proliferate to establish the vicious cycle, promote osteoclast formation and become microenvironment modifying. It is possible that as the tumour expands, tumour cells will become environment independent and develop the capacity to leave the metastatic site and colonize other sites. Proliferating cells may retain the potential to re-engage in an endosteal niche and become dormant.

Castration of male mice bearing prostate cancer cells, which also increases bone turnover, also promotes tumour development[11].

Similar data have been reported in models of multiple myeloma. For example, ovariectomy increases multiple myeloma burden in the skeleton[166], whereas inhibitors of bone resorption, including zoledronic acid and inhibitors of RANKL, reduced multiple myeloma burden and increased survival[109,111,167–170].

Osteoclasts reactivate dormant cancer cells in the skeleton. Although promotion of bone turnover and osteoclastic resorption increases tumour growth, the mechanism responsible is unclear. It has been argued that tumour cells produce factors, including PTHrP, that stimulate bone resorption by upregulating RANKL and releasing bone-bound molecules, including TGFβ, that stimulate tumour growth[5,157]. However, the vicious cycle model does not consider the temporal development of the tumour, the part that dormant cells play or the events that initiate the interdependence between osteoclasts and tumour cells. A refinement to this hypothesis is that osteoclasts initiate the process by first remodelling the bone niche to reactivate dormant tumour cells before establishing a microenvironment that modifies the tumour, which can then establish a vicious cycle (FIG. 4). Increasingly, evidence supports this hypothesis. Studies of the HSC niche demonstrated that treatment of mice with granulocyte colony-stimulating factor (G-CSF; also known as CSF3) or soluble RANKL (sRANKL) both stimulates osteoclast resorption and mobilizes HSCs from the niche in bone[171]. Prostate cancer cells compete with HSCs for the endosteal niche and G-CSF treatment releases these cells from this microenvironment[115]. Cathepsin K, one of the major enzymes produced by osteoclasts, can cleave CXCL12 to prevent it interacting with its receptor, which is important in tumour cell engagement in this niche[171]. Recently, intravital imaging has been used to follow the fate of tumour cells colonizing the osteoblast niche and has demonstrated that sRANKL treatment increases osteoclast numbers and decreases the numbers of dormant tumour cells engaged in the endosteal niche[13]. Together these data suggest that osteoclasts, by remodelling the endosteal niche, can release dormant cells from niche control and reactivate them to form overt tumours (FIG. 4).

Implications of osteoclast control of reactivation on the interdependency between tumour cells and bone. If osteoclasts reactivate dormant tumour cells in bone this argues that bone cells have distinct roles at different stages in the evolution of bone metastasis. Tumour cells may engage in a niche containing cells of the osteoblast lineage, which is crucial in retaining them in a dormant state. However, to release and reactivate these cells, alterations in the niche are required to downregulate expression of molecules responsible for maintaining tumour cells in a dormant state. As not all dormant cells are reactivated this suggests that these events are not synchronized, but are associated with events unique to individual niches. Bone resorption events represent the most likely mechanism for remodelling the niche and will be independent of the presence of individual dormant cells. As a consequence, osteoclast-mediated reactivation of dormant tumour cells could be considered a stochastic event, with the frequency of metastases being determined by both the numbers of dormant cells that successfully colonize the endosteal niche and the rate of bone resorption: the greater the rate of resorption the greater the likelihood of osteoclastic changes reactivating dormant cells.

Monoclonal gammopathy of unknown significance (MGUS). A disorder characterized by the presence in the blood of an abnormal protein that is produced by plasma cells that in some individuals can transition to multiple myeloma.

The fact that stimulators of osteoclastic bone resorption accelerate bone metastasis and inhibitors of bone resorption all slow tumour growth in bone (as discussed above), supports this hypothesis. Although this has been interpreted as promoting the vicious cycle model of bone metastasis, it may now be appropriate to consider that osteoclastic resorption could have more than one role in the development of bone metastasis: the first in reactivating dormant tumour cells to promote tumour outgrowth in bone, and the second as part of the vicious cycle when the tumour is established and begins to modify the microenvironment (FIG. 4).

Implications for control of dormancy

With evidence that osteoblasts and osteoclasts control dormancy come important clinical implications (FIG. 5). These could include the use of biochemical markers as early predictors of disease progression. This is certainly supported by histomorphometric studies showing that increases in osteoclastic resorption in patients with monoclonal gammopathy of unknown significance (MGUS) are associated with progression to overt multiple myeloma[172] and that increases in biochemical markers of bone resorption precede disease progression[173]. However, the most important implications may come in the context of treatment. This is likely to include implications for current therapies but also may provide new treatment strategies, including promoting long-term dormancy retention or dormant cell reactivation and targeting to eradicate minimal residual disease[142].

Clinical implications of current therapeutic approaches. Currently used hormonal therapies have a direct impact on the skeleton. For example, androgen deprivation therapy (ADT), which is widely used in men with prostate cancer, is associated with osteoclast-mediated bone loss[174]. This can be prevented by inhibitors of bone resorption, including bisphosphonates[175–178] and the anti-RANKL antibody denosumab[179,180]. Although this is important[181], by increasing resorption ADT may have additional consequences, including the reactivation of dormant tumour cells[11]. This is also the case for aromatase inhibitors (AIs) used to treat women with breast cancer, which increase osteoclast-mediated bone loss[182]. Bisphosphonate treatment also prevents AI-induced bone loss[183] and may limit inadvertent activation of dormant breast cancer cells in bone.

Tumour-targeted agents also affect bone, yet with few exceptions this has rarely been recognized[184]. For example, in mice and humans, proteasome inhibitors stimulate osteoblastic bone formation and suppress osteoclastic bone resorption[185–188]. In contrast, immunomodulatory drugs, such as thalidomide and lenalidomide, inhibit osteoblast differentiation and bone formation *in vitro* and in animal models[189,190], and may inhibit osteoclastic bone resorption *in vitro*[191–193]. Similarly, doxorubicin causes bone loss by increasing bone resorption and suppressing bone formation[194–196] and can accelerate the development of bone lesions in preclinical models[196], whereas the heat shock protein 90 (HSP90) inhibitor 17-*N*-allyamino-17-demethoxy geldanamycin (17-AAG) and methotrexate increase bone loss by promoting osteoclast formation and bone resorption *in vitro* and in mouse models[195,197–199].

These agents that stimulate osteoclasts and bone resorption have the capacity to inadvertently reactivate dormant cells. Conversely, agents that promote osteoblastic bone formation may increase the number of cells that form the endosteal niche, retain dormant cells in the niche and slow progression. However, we cannot exclude the possibility that by creating more bone niches that can be populated by tumour cells this could have long-term deleterious effects. Only direct examination of the fate of dormant cells in the bone metastasis niche following therapeutic intervention will enable us to answer these questions.

New therapeutic opportunities. The discovery that bone cells control tumour cell dormancy opens up new therapeutic opportunities. This includes exploiting bone-active agents to retain cells in a dormant state indefinitely, or reactivating dormant cells and killing them with existing tumour-targeting agents to effect a 'cure'.

Drugs that target osteoclastic resorption, including the bisphosphonates (such as zoledronic acid) and denosumab, are used to treat tumour-induced bone disease. These agents reduce skeletal-related events[200–204]. However, there is increasing evidence to suggest that they also improve survival. In patients with multiple myeloma, bisphosphonates are associated with increases in overall survival, and in the UK Medical Research Council (MRC) Myeloma IX trial this is independent of effects on skeletal disease[204,205]. In women with breast cancer, zoledronic acid treatment is also associated with improvements in recurrence of bone metastasis and overall survival[200,206], although, among women with breast cancer, this is confined to postmenopausal women in whom bone resorption is increased[200,207]. Furthermore, in patients with castration-resistant prostate cancer, inhibiting bone resorption with denosumab increased bone-metastasis-free survival and delayed time to first bone metastasis[208]. Furthermore, recent clinical studies have shown that zoledronic acid treatment, commencing at the time of ADT initiation, improved the time to prostate-specific antigen failure[209,210]. This was not observed when treatment started after ADT initiation [211], which supports the notion that immediate intervention prevents osteoclast reactivation of DTCs. Thus, early intervention with bone resorption inhibitors, particularly when osteoclastic resorption is elevated, may prevent reactivation of dormant cells and metastasis development (FIG. 5).

Interestingly, a new agent in clinical development for osteoporosis, the cathepsin K inhibitor odanacatib, inhibits bone resorption while maintaining non-resorbing osteoclasts[212]. In preclinical studies, osteoclast-specific cathepsin K ablation inhibits bone resorption while maintaining the number of osteoclasts that are proposed to generate sufficient activity to maintain bone formation[213]. This raises the possibility that the various inhibitors of bone resorption may have differential effects on tumour development in bone depending on their mechanism of action. Odanacatib has been shown to reduce

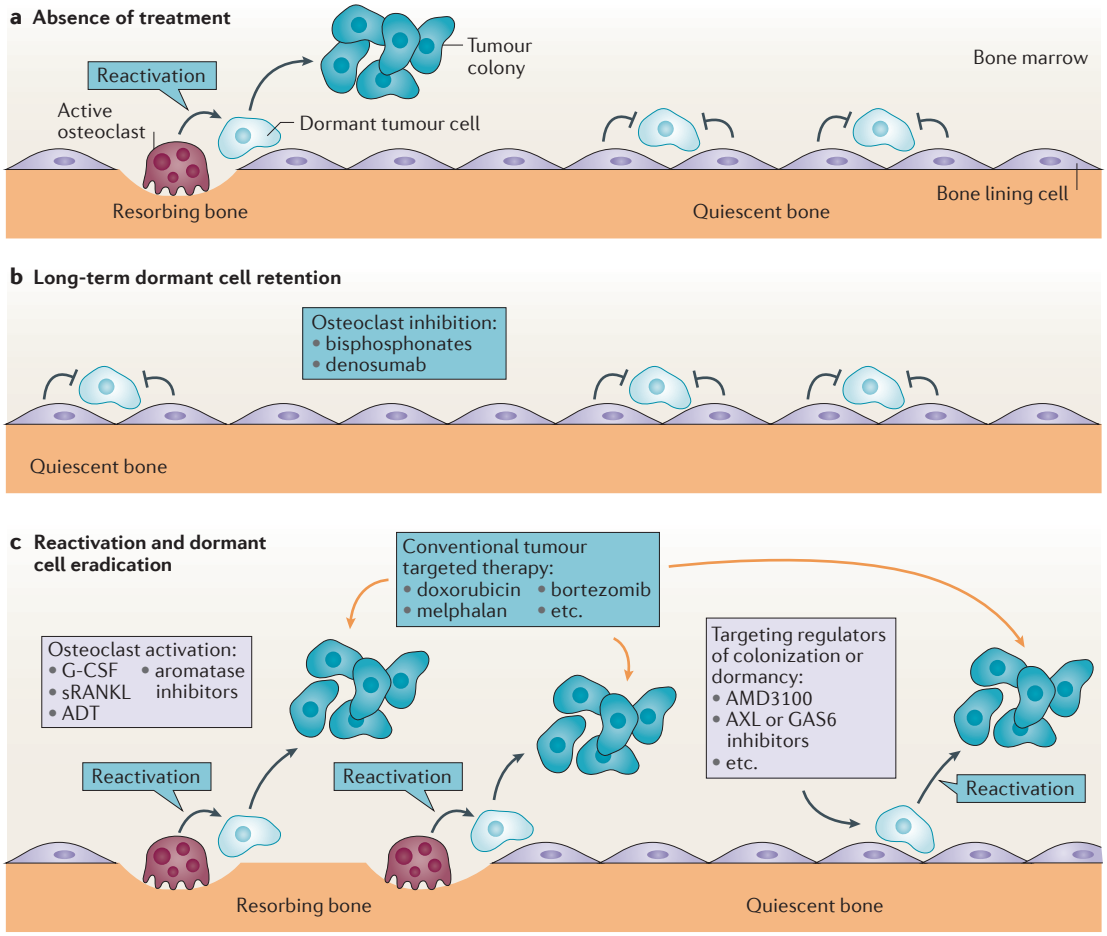

Figure 5 | **Niche-targeted therapies to prevent bone metastasis. a** | In the absence of treatment, dormant cells are retained in an endosteal niche. Stochastic reactivation by osteoclasts releases cells from dormancy. **b** | Treatment with anti-resorptive agents, including bisphosphonates and anti-receptor activator of nuclear factor-κB ligand (RANKL) strategies (such as denosumab), offers the potential for long-term dormant cell retention. **c** | In contrast, stimulation of bone resorption with agents that promote osteoclast formation, including granulocyte colony-stimulating factor (G-CSF), soluble RANKL (sRANKL), or even androgen and oestrogen deprivation (through treatment with androgen deprivation therapy (ADT), or aromatase inhibitors or ovariectomy, respectively), will reactivate dormant cells, making them susceptible to conventional tumour-targeted agents. Equally, promoting reactivation by targeting regulators of colonization and/or dormancy, for example, with AMD3100 or inhibitors of the receptor tyrosine kinase AXL or growth arrest-specific protein 6 (GAS6), will also reactivate cells for conventional targeting. This may facilitate eradication of dormant cells (orange arrows) and effect a 'cure'.

tumour burden in bone in an intratibial nude rat model of breast cancer[214]; however, the effect of this agent on dormant cell reactivation in the bone is unclear.

An alternative approach is to promote release of dormant cells from the protective environment of the niche, and target cells with currently available tumour-targeting agents (FIG. 5). This could be achieved in several ways. First, agents that stimulate osteoclast formation, such as G-CSF or even sRANKL, could be used to promote osteoclast-mediated remodelling of the endosteal niche and reactivation of dormant cells. Once active, these cells could be targeted by conventional antitumour agents (FIG. 5). Secondly, the discovery of specific molecules, for example AXL, expressed by dormant tumour cells engaged in the endosteal niche raises the possibility that these could be targeted to facilitate mobilization of tumour cells and targeting with conventional agents. Finally, it may be possible to use our new understanding of key regulators of dormancy to target dormant cells within the endosteal niche to effect tumour cell killing directly, as has been proposed for cells in the perivascular niche[142].

Perivascular niche
A specialized microenvironment located adjacent to blood vessels that supports the long-term survival of specific cell types, including stem or progenitor cells.

Challenges and future requirements

Despite progress in understanding the early events in bone metastasis development, much greater understanding of colonization, survival and dormancy and the selective reactivation of dormant cells is required if we are to exploit this knowledge for therapeutic benefit.

Improved models of the early events in bone metastasis are required. Most current models are based on dissemination of human cell lines into the skeleton of immunocompromised mice following intracardiac injection. As these cells are presented simultaneously

in large numbers to bone, any property they have of promoting osteoclast formation predominates, favouring their establishment and survival. These models also lack crucial components of the immune system, which is important in metastasis development[69]. Although syngeneic models have attempted to address this[13,111,215], we have some way to go. The heterogeneity found clinically is also not captured in most current models, so systems developed directly from patient bone metastases or primary material will be important, an approach that is now feasible[216,217]. However, where real progress is required is in the development of genetic models. The application of next-generation sequencing to bone metastases isolated from individuals with disease will surely identify genetic drivers of metastasis[74,75], which will be key in the construction of models in the future.

With the development of models comes the need to better visualize the dynamic nature of tumour cell–niche interactions. It will be particularly important to be able to visualize and follow the fate of individual tumour cells as they engage in the niche, acquire a dormant state and are then reactivated to form overt bone metastases. Although intravital microscopy has been used to examine niches in the calvaria[56,218,219], this has not typically been applied to the study of tumour development in other skeletal sites. However, the application of new intravital imaging methodologies now provides opportunities to study these events in different bone compartments in real time[13]. Furthermore, coupling this technology with recent advances in fluorescent probe development, including two-photon photoconversion for tracking the fate of single cells in live animals, provides the real prospect of studying the evolution of bone metastasis[220,221]. In addition, the application of next-generation technologies to understand the intraclonal heterogeneity of bone metastasis, as well as transcriptional profiling of colonizing and dormant tumour cells, will be key to defining the crucial molecular events that define the early steps in metastasis development and identifying tractable therapeutic targets.

Calvaria
The bones of the skull (cranium) that protect the brain.

Two-photon photoconversion
The use of two-photon excitation light to convert the emission spectra of a photoconvertible fluorescent probe from one colour (for example, green) to another (for example, red).

Summary and conclusions

Progress in visualizing and studying the early events of tumour colonization, dormancy and reactivation has provided new insights into the role of bone cells in controlling bone metastasis. Cells of the osteogenic lineage, probably SSCs and bone lining cells, present on quiescent endosteal surfaces, but not functional bone synthesizing osteoblasts, are crucial in providing a niche for the long-term retention of dormant tumour cells that have colonized the skeleton. Conversely, osteoclasts, by remodelling the endosteal bone surface, release dormant tumour cells from the active control of the bone niche, facilitating reactivation and tumour growth, which probably explains why only limited numbers of dormant cells get reactivated. This stochastic reactivation of dormant cells and metastasis initiation by osteoclasts argues that we need to revisit the vicious cycle model of bone metastasis development. Instead of osteoclasts being simply bystanders that respond to tumour-derived products, bone cells may be crucial orchestrators that initiate bone metastasis development. Crucially, recognition of the importance of bone cell control of bone metastasis development provides new opportunities for therapeutic intervention, by facilitating long-term retention of dormant cells, by awakening dormant cells and targeting them directly to eradicate residual disease or by developing new dormancy-targeted therapies. Irrespective of the choice, this may mean we can use currently available agents in better ways.

1. Fidler, I. J. The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. *Nat. Rev. Cancer* **3**, 453–458 (2003).
2. Mundy, G. R. & Martin, T. J. in *Physiology and Pharmacology of Bone* (ed. Mundy, G. R. & Martin, T. J.) 641–671 (Springer-Verlag, 1993).
3. Paget, S. The distribution of secondary growths in cancer of the breast. *Lancet* **1**, 571–573 (1889).
The classic description of the predilection of breast cancer cells to metastasize to the skeleton.
4. Fidler, I. J. & Kripke, M. L. The challenge of targeting metastasis. *Cancer Metastasis Rev.* **34**, 635–641 (2015).
5. Weilbaecher, K. N., Guise, T. A. & McCauley, L. K. Cancer to bone: a fatal attraction. *Nat. Rev. Cancer* **11**, 411–425 (2011).
6. Sterling, J. A., Edwards, J. R., Martin, T. J. & Mundy, G. R. Advances in the biology of bone metastasis: how the skeleton affects tumor behavior. *Bone* **48**, 6–15 (2011).
7. Chambers, A. F., Groom, A. C. & MacDonald, I. C. Dissemination and growth of cancer cells in metastatic sites. *Nat. Rev. Cancer* **2**, 563–572 (2002).
8. Cameron, M. D. *et al.* Temporal progression of metastasis in lung: cell survival, dormancy, and location dependence of metastatic inefficiency. *Cancer Res.* **60**, 2541–2546 (2000).
9. Luzzi, K. J. *et al.* 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. Ottewell, P. D. *et al.* Zoledronic acid has differential antitumor activity in the pre- and postmenopausal bone microenvironment *in vivo*. *Clin. Cancer Res.* **20**, 2922–2932 (2014).
One of the first studies to show that the bone microenvironment can control metastasis development.
11. Ottewell, P. D. *et al.* Castration-induced bone loss triggers growth of disseminated prostate cancer cells in bone. *Endocr. Relat. Cancer* **21**, 769–781 (2014).
12. Wang, N. *et al.* 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).
13. Lawson, M. A. *et al.* Osteoclasts control reactivation of dormant myeloma cells by remodeling the endosteal niche. *Nat. Commun.* **6**, 8983 (2015).
A demonstration of dormant cancer cells in the skeleton and the importance of extrinsic microenvironmental changes in promoting dormant cell reactivation.
14. Fortunato, L. *et al.* Positive bone marrow biopsy is associated with a decreased disease-free survival in patients with operable breast cancer. *Ann. Surg. Oncol.* **16**, 3010–3019 (2009).
15. Tjensvoll, K. *et al.* Persistent tumor cells in bone marrow of non-metastatic breast cancer patients after primary surgery are associated with inferior outcome. *BMC Cancer* **12**, 190 (2012).
16. Domschke, C. *et al.* Prognostic value of disseminated tumor cells in the bone marrow of patients with operable primary breast cancer: a long-term follow-up study. *Ann. Surg. Oncol.* **20**, 1865–1871 (2013).
17. Boyle, W. J., Simonet, W. S. & Lacey, D. L. Osteoclast differentiation and activation. *Nature* **423**, 337–342 (2003).
A review of the discovery of osteoclast formation and activity by the tumour necrosis factor (TNF) receptor and TNF ligand family of molecules.
18. Suda, T. *et al.* Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. *Endocr. Rev.* **20**, 345–357 (1999).
19. Baron, R. Molecular mechanisms of bone resorption by the osteoclast. *Anat. Rec.* **224**, 317–324 (1989).
20. Andersen, T. L. *et al.* Understanding coupling between bone resorption and formation: are reversal cells the missing link? *Am. J. Pathol.* **183**, 235–246 (2013).
21. Delaisse, J. M. The reversal phase of the bone-remodeling cycle: cellular prerequisites for coupling resorption and formation. *Bonekey Rep.* **3**, 561 (2014).
22. Kassem, M. & Bianco, P. Skeletal stem cells in space and time. *Cell* **160**, 17–19 (2015).
A succinct and authoritative summary of the differentiation of osteoblasts from stem cell precursors.
23. Kim, S. W. *et al.* Intermittent parathyroid hormone administration converts quiescent lining cells to active osteoblasts. *J. Bone Miner. Res.* **27**, 2075–2084 (2012).
24. Mizoguchi, T. *et al.* Osterix marks distinct waves of primitive and definitive stromal progenitors during bone marrow development. *Dev. Cell* **29**, 340–349 (2014).
25. Martin, T. J. & Sims, N. A. Coupling the activities of bone formation and resorption: a multitude of signals within the basic multicellular unit. *BoneKey Rep.* **3**, 215 (2014).
26. Sims, N. A. & Martin, T. J. Coupling signals between the osteoclast and osteoblast: how are messages transmitted between these temporary visitors to the bone surface? *Front. Endocrinol. (Lausanne)* **6**, 41 (2015).
A critical review of current ideas behind the mechanisms coupling bone formation and bone resorption.

27. Martin, T. J., Ng, K. W. & Sims, N. A. in *Translational Endocrinology of Bone* (ed. Karsenty, G.) 5–26 (Elsevier Inc, 2013).
28. Kalajzic, Z. *et al.* Use of an alpha-smooth muscle actin GFP reporter to identify an osteoprogenitor population. *Bone* **43**, 501–510 (2008).
29. Acar, M. *et al.* Deep imaging of bone marrow shows non-dividing stem cells are mainly perisinusoidal. *Nature* **526**, 126–130 (2015).
30. Hattner, R., Epker, B. N. & Frost, H. M. Suggested sequential mode of control of changes in cell behaviour in adult bone remodelling. *Nature* **206**, 489–490 (1965).
31. Rodan, G. A. & Martin, T. J. Role of osteoblasts in hormonal control of bone resorption—a hypothesis. *Calcif. Tissue Int.* **33**, 349–351 (1981).
32. Parfitt, A. M. The coupling of bone formation to bone resorption: a critical analysis of the concept and of its relevance to the pathogenesis of osteoporosis. *Metab. Bone Dis. Relat. Res.* **4**, 1–6 (1982).
33. Martin, T. J. & Sims, N. A. Osteoclast-derived activity in the coupling of bone formation to resorption. *Trends Mol. Med.* **11**, 76–81 (2005).
34. Xian, L. *et al.* Matrix IGF-1 maintains bone mass by activation of mTOR in mesenchymal stem cells. *Nat. Med.* **18**, 1095–1101 (2012).
35. Weivoda, M. M. *et al.* Osteoclast TGF-β receptor signaling induces Wnt1 secretion and couples bone resorption to bone formation. *J. Bone Miner. Res.* **31**, 76–85 (2015).
36. Mundy, G. R. Mechanisms of bone metastasis. *Cancer* **80**, 1546–1556 (1997).
37. Kozlow, W. & Guise, T. A. Breast cancer metastasis to bone: mechanisms of osteolysis and implications for therapy. *J. Mammary Gland Biol. Neoplasia* **10**, 169–180 (2005).
38. Andersen, T. L. *et al.* A physical mechanism for coupling bone resorption and formation in adult human bone. *Am. J. Pathol.* **174**, 239–247 (2009).
39. Kristensen, H. B., Andersen, T. L., Marcussen, N., Rolighed, L. & Delaisse, J. M. Increased presence of capillaries next to remodeling sites in adult human cancellous bone. *J. Bone Miner. Res.* **28**, 574–585 (2013).
A detailed description of BRCs in human bone and how blood vessels participate.
40. Hauge, E. M., Qvesel, D., Eriksen, E. F., Mosekilde, L. & Melsen, F. Cancellous bone remodeling occurs in specialized compartments lined by cells expressing osteoblastic markers. *J. Bone Miner. Res.* **16**, 1575–1582 (2001).
41. Kristensen, H. B., Andersen, T. L., Marcussen, N., Rolighed, L. & Delaisse, J. M. Osteoblast recruitment routes in human cancellous bone remodeling. *Am. J. Pathol.* **184**, 778–789 (2014).
42. Eriksen, E. F., Eghbali-Fatourechi, G. Z. & Khosla, S. Remodeling and vascular spaces in bone. *J. Bone Miner. Res.* **22**, 1–6 (2007).
43. Andersen, T. L., Soe, K., Sondergaard, T. E., Plesner, T. & Delaisse, J. M. Myeloma cell-induced disruption of bone remodelling compartments leads to osteolytic lesions and generation of osteoclast-myeloma hybrid cells. *Br. J. Haematol.* **148**, 551–561 (2010).
44. Yamaguchi, K., Croucher, P. I. & Compston, J. E. Comparison between the lengths of individual osteoid seams and resorption cavities in human iliac crest cancellous bone. *Bone Miner.* **23**, 27–33 (1993).
45. Chang, M. K. *et al.* 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).
46. Schofield, R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. *Blood Cells* **4**, 7–25 (1978).
47. Nilsson, S. K., Johnston, H. M. & Coverdale, J. A. Spatial localization of transplanted hemopoietic stem cells: inferences for the localization of stem cell niches. *Blood* **97**, 2293–2299 (2001).
48. Calvi, L. M. *et al.* Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature* **425**, 841–846 (2003).
49. Zhang, J. *et al.* Identification of the haematopoietic stem cell niche and control of the niche size. *Nature* **425**, 836–841 (2003).
50. Bianco, P. Minireview: The stem cell next door: skeletal and hematopoietic stem cell "niches" in bone. *Endocrinology* **152**, 2957–2962 (2011).
51. Askmyr, M., Sims, N. A., Martin, T. J. & Purton, L. E. What is the true nature of the osteoblastic hematopoietic stem cell niche? *Trends Endocrinol. Metab.* **20**, 303–309 (2009).
52. Bianco, P. Bone and the hematopoietic niche: a tale of two stem cells. *Blood* **117**, 5281–5288 (2011).
53. Doherty, M. J. *et al.* Vascular pericytes express osteogenic potential *in vitro* and *in vivo*. *J. Bone Miner. Res.* **13**, 828–838 (1998).
54. Sacchetti, B. *et al.* Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. *Cell* **131**, 324–336 (2007).
55. Xie, Y. *et al.* Detection of functional haematopoietic stem cell niche using real-time imaging. *Nature* **457**, 97–101 (2009).
56. Lo Celso, C. *et al.* Live-animal tracking of individual haematopoietic stem/progenitor cells in their niche. *Nature* **457**, 92–96 (2009).
57. Chan, C. K. *et al.* Endochondral ossification is required for haematopoietic stem-cell niche formation. *Nature* **457**, 490–494 (2009).
58. Kode, A. *et al.* Leukaemogenesis induced by an activating β-catenin mutation in osteoblasts. *Nature* **506**, 240–244 (2014).
59. Ding, L. & Morrison, S. J. Haematopoietic stem cells and early lymphoid progenitors occupy distinct bone marrow niches. *Nature* **495**, 231–235 (2013).
60. Greenbaum, A. *et al.* CXCL12 in early mesenchymal progenitors is required for haematopoietic stem-cell maintenance. *Nature* **495**, 227–230 (2013).
61. Van 't Veer, L. J. *et al.* Gene expression profiling predicts clinical outcome of breast cancer. *Nature* **415**, 530–536 (2002).
62. Van de Vijver, M. J. *et al.* A gene-expression signature as a predictor of survival in breast cancer. *N. Engl. J. Med.* **347**, 1999–2009 (2002).
63. Weigelt, B. *et al.* Gene expression profiles of primary breast tumors maintained in distant metastases. *Proc. Natl Acad. Sci. USA* **100**, 15901–15905 (2003).
64. Kang, Y. *et al.* A multigenic program mediating breast cancer metastasis to bone. *Cancer Cell* **3**, 537–549 (2003).
65. Zhang, X. H. *et al.* Latent bone metastasis in breast cancer tied to Src-dependent survival signals. *Cancer Cell* **16**, 67–78 (2009).
66. Padua, D. *et al.* TGFβ primes breast tumors for lung metastasis seeding through angiopoietin-like 4. *Cell* **133**, 66–77 (2008).
67. Zhang, X. H. *et al.* Selection of bone metastasis seeds by mesenchymal signals in the primary tumor stroma. *Cell* **154**, 1060–1073 (2013).
68. Jung, Y. *et al.* Recruitment of mesenchymal stem cells into prostate tumours promotes metastasis. *Nat. Commun.* **4**, 1795 (2013).
69. Bidwell, B. N. *et al.* Silencing of Irf7 pathways in breast cancer cells promotes bone metastasis through immune escape. *Nat. Med.* **18**, 1224–1231 (2012).
70. Vecchi, M. *et al.* Breast cancer metastases are molecularly distinct from their primary tumors. *Oncogene* **27**, 2148–2158 (2008).
71. Ki, D. H. *et al.* Whole genome analysis for liver metastasis gene signatures in colorectal cancer. *Int. J. Cancer* **121**, 2005–2012 (2007).
72. Stoecklein, N. H. & Klein, C. A. Genetic disparity between primary tumours, disseminated tumour cells, and manifest metastasis. *Int. J. Cancer* **126**, 589–598 (2010).
73. Klein, C. A. Parallel progression of primary tumours and metastases. *Nat. Rev. Cancer* **9**, 302–312 (2009).
74. Hong, M. K. *et al.* Tracking the origins and drivers of subclonal metastatic expansion in prostate cancer. *Nat. Commun.* **6**, 6605 (2015).
A study using next-generation sequencing to define the origins of individual bone metastases in men with prostate cancer.
75. Gundem, G. *et al.* The evolutionary history of lethal metastatic prostate cancer. *Nature* **520**, 353–357 (2015).
76. Butcher, D. T., Alliston, T. & Weaver, V. M. A tense situation: forcing tumour progression. *Nat. Rev. Cancer* **9**, 108–122 (2009).
77. Paszek, M. J. & Weaver, V. M. The tension mounts: mechanics meets morphogenesis and malignancy. *J. Mammary Gland Biol. Neoplasia* **9**, 325–342 (2004).
78. Guelcher, S. A., Dumas, J. Srinivasan, A., Didier, J. E. & Hollinger, J. O. Synthesis, mechanical properties, biocompatibility, and biodegradation of polyurethane networks from lysine polyisocyanates. *Biomaterials* **29**, 1762–1775 (2008).
79. Johnson, R. W. *et al.* TGF-β promotion of Gli2-induced expression of parathyroid hormone-related protein, an important osteolytic factor in bone metastasis, is independent of canonical Hedgehog signaling. *Cancer Res.* **71**, 822–831 (2011).
80. Ruppender, N. S. *et al.* Matrix rigidity induces osteolytic gene expression of metastatic breast cancer cells. *PLoS ONE* **5**, e15451 (2010).
81. Muller, A. *et al.* Involvement of chemokine receptors in breast cancer metastasis. *Nature* **410**, 50–56 (2001).
82. Taichman, R. S. *et al.* Use of the stromal cell-derived factor-1/CXCR4 pathway in prostate cancer metastasis to bone. *Cancer Res.* **62**, 1832–1837 (2002).
83. Corcoran, K. E. *et al.* Mesenchymal stem cells in early entry of breast cancer into bone marrow. *PLoS ONE* **3**, e2563 (2008).
84. Correa, D., Somoza, R. A., Lin, P., Schiemann, W. P. & Caplan, A. I. Mesenchymal stem cells regulate melanoma cancer cells extravasation to bone and liver at their perivascular niche. *Int. J. Cancer* **138**, 417–427 (2015).
85. Loibl, M. *et al.* Direct cell–cell contact between mesenchymal stem cells and endothelial progenitor cells induces a pericyte-like phenotype *in vitro*. *Biomed. Res. Int.* **2014**, 395781 (2014).
86. Parmo-Cabanas, M. *et al.* Integrin $\alpha_4\beta_1$ involvement in stromal cell-derived factor-1α-promoted myeloma cell transendothelial migration and adhesion: role of cAMP and the actin cytoskeleton in adhesion. *Exp. Cell Res.* **294**, 571–580 (2004).
87. Sanz-Rodriguez, F., Hidalgo, A. & Teixido, J. Chemokine stromal cell-derived factor-1α modulates VLA-4 integrin-mediated multiple myeloma cell adhesion to CS-1/fibronectin and VCAM-1. *Blood* **97**, 346–351 (2001).
88. Abe, M., Hiura, K., Ozaki, S., Kido, S. & Matsumoto, T. Vicious cycle between myeloma cell binding to bone marrow stromal cells via VLA-4–VCAM-1 adhesion and macrophage inflammatory protein-1α and MIP-1β production. *J. Bone Miner. Metab.* **27**, 16–23 (2009).
89. Choi, S. J. *et al.* Macrophage inflammatory protein 1-α is a potential osteoclast stimulatory factor in multiple myeloma. *Blood* **96**, 671–675 (2000).
90. Oyajobi, B. O. *et al.* Dual effects of macrophage inflammatory protein-1α on osteolysis and tumor burden in the murine 5TGM1 model of myeloma bone disease. *Blood* **102**, 311–319 (2003).
91. Azab, A. K. *et al.* CXCR4 inhibitor AMD3100 disrupts the interaction of multiple myeloma cells with the bone marrow microenvironment and enhances their sensitivity to therapy. *Blood* **113**, 4341–4351 (2009).
92. Domanska, U. M. *et al.* CXCR4 inhibition with AMD3100 sensitizes prostate cancer to docetaxel chemotherapy. *Neoplasia* **14**, 709–718 (2012).
93. Xiang, J. *et al.* CXCR4 protein epitope mimetic antagonist POL5551 disrupts metastasis and enhances chemotherapy effect in triple-negative breast cancer. *Mol. Cancer Ther.* **14**, 2473–2485 (2015).
94. Liapis, H., Flath, A. & Kitazawa, S. Integrin $\alpha_v\beta_3$ expression by bone-residing breast cancer metastases. *Diagn. Mol. Pathol.* **5**, 127–135 (1996).
95. Carlson, C. S. *et al.* Immunolocalization of noncollagenous bone matrix proteins in lumbar vertebrae from intact and surgically menopausal cynomolgus monkeys. *J. Bone Miner. Res.* **8**, 71–81 (1993).
96. Grzesik, W. J. & Robey, P. G. Bone matrix RGD glycoproteins: immunolocalization and interaction with human primary osteoblastic bone cells *in vitro*. *J. Bone Miner. Res.* **9**, 487–496 (1994).
97. Wang, H. *et al.* The osteogenic niche promotes early-stage bone colonization of disseminated breast cancer cells. *Cancer Cell* **27**, 193–210 (2015).
98. Beausoleil, M. S., Schulze, E. B., Goodale, D., Postenka, C. O. & Allan, A. L. Deletion of the thrombin cleavage domain of osteopontin mediates breast cancer cell adhesion, proteolytic activity, tumorgenicity, and metastasis. *BMC Cancer* **11**, 25 (2011).
99. Klevesath, M. B. *et al.* Patterns of metastatic spread in early breast cancer. *Breast* **22**, 449–454 (2013).
100. Sung, V., Stubbs, J. T. 3rd, Fisher, L., Aaron, A. D. & Thompson, E. W. Bone sialoprotein supports breast cancer cell adhesion proliferation and migration through differential usage of the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins. *J. Cell. Physiol.* **176**, 482–494 (1998).
101. Adwan, H. *et al.* Decreased levels of osteopontin and bone sialoprotein II are correlated with reduced proliferation, colony formation, and migration of GFP-MDA-MB-231 cells. *Int. J. Oncol.* **24**, 1235–1244 (2004).
102. Wang, J. *et al.* BSP gene silencing inhibits migration, invasion, and bone metastasis of MDA-MB-231BO human breast cancer cells. *PLoS ONE* **8**, e62936 (2013).

103. Uchiyama, H., Barut, B. A., Chauhan, D., Cannistra, S. A. & Anderson, K. C. Characterization of adhesion molecules on human myeloma cell lines. *Blood* **80**, 2306–2314 (1992).
104. Mori, Y. *et al.* Anti-α^4 integrin antibody suppresses the development of multiple myeloma and associated osteoclastic osteolysis. *Blood* **104**, 2149–2154 (2004).
105. Jones, D. H. *et al.* Regulation of cancer cell migration and bone metastasis by RANKL. *Nature* **440**, 692–696 (2006).
106. Thomas, R. J. *et al.* Breast cancer cells interact with osteoblasts to support osteoclast formation. *Endocrinology* **140**, 4451–4458 (1999).
107. Nannuru, K. C. *et al.* Enhanced expression and shedding of receptor activator of NF-κB ligand during tumor-bone interaction potentiates mammary tumor-induced osteolysis. *Clin. Exp. Metastasis* **26**, 797–808 (2009).
108. Mundy, G. R. Metastasis to bone: causes, consequences and therapeutic opportunities. *Nat. Rev. Cancer* **2**, 584–593 (2002).
109. Pearse, R. N. *et al.* Multiple myeloma disrupts the TRANCE/osteoprotegerin cytokine axis to trigger bone destruction and promote tumor progression. *Proc. Natl Acad. Sci. USA* **98**, 11581–11586 (2001).
110. Zhang, J. *et al.* Osteoprotegerin inhibits prostate cancer-induced osteoclastogenesis and prevents prostate tumor growth in the bone. *J. Clin. Invest.* **107**, 1235–1244 (2001).
111. Croucher, P. I. *et al.* Osteoprotegerin inhibits the development of osteolytic bone disease in multiple myeloma. *Blood* **98**, 3534–3540 (2001).
112. Canon, J. R. *et al.* Inhibition of RANKL blocks skeletal tumor progression and improves survival in a mouse model of breast cancer bone metastasis. *Clin. Exp. Metastasis* **25**, 119–129 (2008).
113. Armstrong, A. P. *et al.* RANKL acts directly on RANK-expressing prostate tumor cells and mediates migration and expression of tumor metastasis genes. *Prostate* **68**, 92–104 (2008).
114. Campbell, J. P. *et al.* Stimulation of host bone marrow stromal cells by sympathetic nerves promotes breast cancer bone metastasis in mice. *PLoS Biol.* **10**, e1001363 (2012).
115. Shiozawa, Y. *et al.* Human prostate cancer metastases target the hematopoietic stem cell niche to establish footholds in mouse bone marrow. *J. Clin. Invest.* **121**, 1298–1312 (2011).
A key study highlighting the potential of colonizing tumour cells to hijack the HSC niche in bone.
116. Vanderkerken, K. *et al.* Selective initial *in vivo* homing pattern of 5T2 multiple myeloma cells in the C57BL/KalwRij mouse. *Br. J. Cancer* **82**, 953–959 (2000).
117. Chen, Z., Orlowski, R. Z., Wang, M., Kwak, L. & McCarty, N. Osteoblastic niche supports the growth of quiescent multiple myeloma cells. *Blood* **123**, 2204–2208 (2014).
118. Karadag, A., Oyajobi, B. O., Apperley, J. F., Russell, R. G. G. & Croucher, P. I. Human myeloma cells promote the production of interleukin-6 by primary human osteoblasts. *Br. J. Haematol.* **108**, 383–390 (2000).
119. Ro, T. B. *et al.* Bone morphogenetic protein-5, -6 and -7 inhibit growth and induce apoptosis in human myeloma cells. *Oncogene* **23**, 3024–3032 (2004).
120. Hjertner, O. *et al.* Bone morphogenetic protein-4 inhibits proliferation and induces apoptosis of multiple myeloma cells. *Blood* **97**, 516–522 (2001).
121. Kawamura, C. *et al.* Bone morphogenetic protein-2 induces apoptosis in human myeloma cells with modulation of STAT3. *Blood* **96**, 2005–2011 (2000).
122. Holien, T. & Sundan, A. The role of bone morphogenetic proteins in myeloma cell survival. *Cytokine Growth Factor Rev.* **25**, 343–350 (2014).
123. Dosen, G. *et al.* Wnt expression and canonical Wnt signaling in human bone marrow B lymphopoiesis. *BMC Immunol.* **7**, 13 (2006).
124. Li, X., Pennisi, A. & Yaccoby, S. Role of decorin in the antimyeloma effects of osteoblasts. *Blood* **112**, 159–168 (2008).
125. D'Souza, S. *et al.* Annexin II interactions with the annexin II receptor enhance multiple myeloma cell adhesion and growth in the bone marrow microenvironment. *Blood* **119**, 1888–1896 (2012).
126. Holen, I., Croucher, P. I., Hamdy, F. C. & Eaton, C. L. Osteoprotegerin (OPG) is a survival factor for human prostate cancer cells. *Cancer Res.* **62**, 1619–1623 (2002).
127. Shipman, C. M. & Croucher, P. I. Osteoprotegerin is a soluble decoy receptor for tumour necrosis factor-related apoptosis-inducing ligand/Apo2 ligand and can function as a paracrine survival factor for human myeloma cells. *Cancer Res.* **63**, 912–916 (2003).
128. Nyambo, R. *et al.* Human bone marrow stromal cells protect prostate cancer cells from TRAIL-induced apoptosis. *J. Bone Miner. Res.* **19**, 1712–1721 (2004).
129. Neville-Webbe, H. L. *et al.* Osteoprotegerin (OPG) produced by bone marrow stromal cells protects breast cancer cells from TRAIL-induced apoptosis. *Breast Cancer Res. Treat.* **86**, 269–279 (2004).
130. Vitovski, S., Phillips, J. S., Sayers, J. & Croucher, P. I. Investigating the interaction between osteoprotegerin and receptor activator of NF-κB or tumor necrosis factor-related apoptosis-inducing ligand: evidence for a pivotal role for osteoprotegerin in regulating two distinct pathways. *J. Biol. Chem.* **282**, 31601–31609 (2007).
131. Schlimok, G. *et al.* Micrometastatic cancer cells in bone marrow: *in vitro* detection with anti-cytokeratin and *in vivo* labeling with anti-17-1A monoclonal antibodies. *Proc. Natl Acad. Sci. USA* **84**, 8672–8676 (1987).
132. Cote, R. J. *et al.* Monoclonal antibodies detect occult breast carcinoma metastases in the bone marrow of patients with early stage disease. *Am. J. Surg. Pathol.* **12**, 333–340 (1988).
133. Pantel, K. *et al.* Frequency and prognostic significance of isolated tumour cells in bone marrow of patients with non-small-cell lung cancer without overt metastases. *Lancet* **347**, 649–653 (1996).
134. Engel, J. *et al.* The process of metastasisation for breast cancer. *Eur. J. Cancer* **39**, 1794–1806 (2003).
135. Husemann, Y. *et al.* Systemic spread is an early step in breast cancer. *Cancer Cell* **13**, 58–68 (2008).
136. Shiozawa, Y. *et al.* GAS6/AXL axis regulates prostate cancer invasion, proliferation, and survival in the bone marrow niche. *Neoplasia* **12**, 116–127 (2010).
Evidence that AXL may be a key molecule in controlling prostate cancer cell survival in the skeleton.
137. Jung, Y. *et al.* Prevalence of prostate cancer metastases after intravenous inoculation provides clues into the molecular basis of dormancy in the bone marrow microenvironment. *Neoplasia* **14**, 429–439 (2012).
138. Taichman, R. S. *et al.* GAS6 receptor status is associated with dormancy and bone metastatic tumor formation. *PLoS ONE* **8**, e61873 (2013).
139. Mishra, A. *et al.* Hypoxia stabilizes GAS6/Axl signaling in metastatic prostate cancer. *Mol. Cancer Res.* **10**, 703–712 (2012).
140. Kusumbe, A. P., Ramasamy, S. K. & Adams, R. H. Coupling of angiogenesis and osteogenesis by a specific vessel subtype in bone. *Nature* **507**, 323–328 (2014).
141. Spencer, J. A. *et al.* Direct measurement of local oxygen concentration in the bone marrow of live animals. *Nature* **508**, 269–273 (2014).
142. Ghajar, C. M. Metastasis prevention by targeting the dormant niche. *Nat. Rev. Cancer* **15**, 238–247 (2015).
143. Ghajar, C. M. *et al.* The perivascular niche regulates breast tumour dormancy. *Nat. Cell Biol.* **15**, 807–817 (2013).
144. Horiuchi, K. *et al.* Identification and characterization of a novel protein, periostin, with restricted expression to periosteum and periodontal ligament and increased expression by transforming growth factor β. *J. Bone Miner. Res.* **14**, 1239–1249 (1999).
145. Merle, B., Bouet, G., Rousseau, J. C., Bertholon, C. & Garnero, P. Periostin and transforming growth factor β-induced protein (TGFβIp) are both expressed by osteoblasts and osteoclasts. *Cell Biol. Int.* **38**, 398–404 (2014).
146. Malanchi, I. *et al.* Interactions between cancer stem cells and their niche govern metastatic colonization. *Nature* **481**, 85–89 (2012).
147. Lee, Y. J. *et al.* Periostin-binding DNA aptamer inhibits breast cancer growth and metastasis. *Mol. Ther.* **21**, 1004–1013 (2013).
148. Yin, J. J. *et al.* TGF-β signaling blockade inhibits PTHrP secretion by breast cancer cells and bone metastases development. *J. Clin. Invest.* **103**, 197–206 (1999).
149. Kakonen, S. M. *et al.* Transforming growth factor-β stimulates parathyroid hormone-related protein and osteolytic metastases via Smad and mitogen-activated protein kinase signaling pathways. *J. Biol. Chem.* **277**, 24571–24578 (2002).
150. Fournier, P. G. *et al.* The TGF-β signaling regulator PMEPA1 suppresses prostate cancer metastases to bone. *Cancer Cell* **27**, 809–821 (2015).
151. Pettit, A. R., Chang, M. K., Hume, D. A. & Raggatt, L. J. Osteal macrophages: a new twist on coupling during bone dynamics. *Bone* **43**, 976–982 (2008).
152. Parfitt, A. M. Osteonal and hemi-osteonal remodeling: the spatial and temporal framework for signal traffic in adult human bone. *J. Cell Biochem.* **55**, 273–286 (1994).
153. Lu, X. *et al.* VCAM-1 promotes osteolytic expansion of indolent bone micrometastasis of breast cancer by engaging α4β1-positive osteoclast progenitors. *Cancer Cell* **20**, 701–714 (2011).
154. Chen, Q., Zhang, X. H. & Massague, J. Macrophage binding to receptor VCAM-1 transmits survival signals in breast cancer cells that invade the lungs. *Cancer Cell* **20**, 538–549 (2011).
155. Hynes, R. O. Metastatic cells will take any help they can get. *Cancer Cell* **20**, 689–690 (2011).
156. Giancotti, F. G. Mechanisms governing metastatic dormancy and reactivation. *Cell* **155**, 750–764 (2013).
157. Guise, T. A. *et al.* 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).
158. Zheng, Y. *et al.* Bone resorption increases tumour growth in a mouse model of osteosclerotic breast cancer metastasis. *Clin. Exp. Metastasis* **25**, 559–567 (2008).
159. Zheng, Y. *et al.* Vitamin D deficiency promotes prostate cancer growth in bone. *Prostate* **71**, 1012–1021 (2011).
160. Ooi, L. L. *et al.* Vitamin D deficiency promotes growth of MCF-7 human breast cancer in a rodent model of osteosclerotic bone metastasis. *Bone* **47**, 795–803 (2010).
161. Ooi, L. L. *et al.* Vitamin D deficiency promotes human breast cancer growth in a murine model of bone metastasis. *Cancer Res.* **70**, 1835–1844 (2010).
162. Corey, E. *et al.* Osteoprotegerin in prostate cancer bone metastasis. *Cancer Res.* **65**, 1710–1718 (2005).
163. Corey, E. *et al.* Zoledronic acid exhibits inhibitory effects on osteoblastic and osteolytic metastases of prostate cancer. *Clin. Cancer Res.* **9**, 295–306 (2003).
164. Kiefer, J. A. *et al.* The effect of osteoprotegerin administration on the intra-tibial growth of the osteoblastic LuCaP 23.1 prostate cancer xenograft. *Clin. Exp. Metastasis* **21**, 381–387 (2004).
165. Ottewell, P. D. *et al.* OPG-Fc inhibits ovariectomy-induced growth of disseminated breast cancer cells in bone. *Int. J. Cancer* **137**, 968–977 (2015).
166. Libouban, H., Moreau, M. F., Basle, M. F., Bataille, R. & Chappard, D. Increased bone remodeling due to ovariectomy dramatically increases tumoral growth in the 5T2 multiple myeloma mouse model. *Bone* **33**, 283–292 (2003).
167. Croucher, P. I. *et al.* Zoledronic acid treatment of 5T2MM-bearing mice inhibits the development of myeloma bone disease: evidence for decreased osteolysis, tumor burden and angiogenesis, and increased survival. *J. Bone Miner. Res.* **18**, 482–492 (2003).
168. Vanderkerken, K. *et al.* Recombinant osteoprotegerin decreases tumor burden and increases survival in a murine model of multiple myeloma. *Cancer Res.* **63**, 287–289 (2003).
169. Yaccoby, S. *et al.* Myeloma interacts with the bone marrow microenvironment to induce osteoclastogenesis and is dependent on osteoclast activity. *Br. J. Haematol.* **116**, 278–290 (2002).
170. Oyajobi, B. O. & Mundy, G. R. Receptor activator of NF-κB ligand, macrophage inflammatory protein-1α, and the proteasome: novel therapeutic targets in myeloma. *Cancer* **97**, 813–817 (2003).
171. Kollet, O. *et al.* Osteoclasts degrade endosteal components and promote mobilization of hematopoietic progenitor cells. *Nat. Med.* **12**, 657–664 (2006).
172. Bataille, R., Chappard, D. & Basle, M. F. Quantifiable excess of bone resorption in monoclonal gammopathy is an early symptom of malignancy: a prospective study of 87 bone biopsies. *Blood* **87**, 4762–4769 (1996).
173. Lund, T., Abildgaard, N., Andersen, T. L., Delaisse, J. M. & Plesner, T. Multiple myeloma: changes in serum C-terminal telopeptide of collagen type I and bone-specific alkaline phosphatase can be used in daily practice to detect imminent osteolysis. *Eur. J. Haematol.* **84**, 412–420 (2010).

174. Michaelson, M. D., Marujo, R. M. & Smith, M. R. Contribution of androgen deprivation therapy to elevated osteoclast activity in men with metastatic prostate cancer. *Clin. Cancer Res.* **10**, 2705–2708 (2004).
175. Smith, M. R. *et al.* Pamidronate to prevent bone loss during androgen-deprivation therapy for prostate cancer. *N. Engl. J. Med.* **345**, 948–955 (2001).
176. Smith, M. R. *et al.* Randomized controlled trial of zoledronic acid to prevent bone loss in men receiving androgen deprivation therapy for nonmetastatic prostate cancer. *J. Urol.* **169**, 2008–2012 (2003).
177. Greenspan, S. L., Nelson, J. B., Trump, D. L. & Resnick, N. M. Effect of once-weekly oral alendronate on bone loss in men receiving androgen deprivation therapy for prostate cancer: a randomized trial. *Ann. Intern. Med.* **146**, 416–424 (2007).
178. Klotz, L. H. *et al.* A phase 3, double-blind, randomised, parallel-group, placebo-controlled study of oral weekly alendronate for the prevention of androgen deprivation bone loss in nonmetastatic prostate cancer: the Cancer and Osteoporosis Research with Alendronate and Leuprolide (CORAL) study. *Eur. Urol.* **63**, 927–935 (2013).
179. Smith, M. R. *et al.* Denosumab in men receiving androgen-deprivation therapy for prostate cancer. *N. Engl. J. Med.* **361**, 745–755 (2009).
180. Smith, M. R. *et al.* Denosumab and changes in bone turnover markers during androgen deprivation therapy for prostate cancer. *J. Bone Miner. Res.* **26**, 2827–2833 (2011).
181. Nguyen, P. L. *et al.* Adverse effects of androgen deprivation therapy and strategies to mitigate them. *Eur. Urol.* **67**, 825–836 (2015).
182. Perez, E. A. & Weilbaecher, K. Aromatase inhibitors and bone loss. *Oncol. (Williston Park)* **20**, 1029–1039; discussion 1039–1048 (2006).
183. Llombart, A. *et al.* 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).
184. Silbermann, R. & Roodman, G. D. Bone effects of cancer therapies: pros and cons. *Curr. Opin. Support. Palliat. Care* **5**, 251–257 (2011).
185. Oyajobi, B. O. *et al.* Stimulation of new bone formation by the proteasome inhibitor, bortezomib: implications for myeloma bone disease. *Br. J. Haematol.* **139**, 434–438 (2007).
186. Giuliani, N. *et al.* The proteasome inhibitor bortezomib affects osteoblast differentiation *in vitro* and *in vivo* in multiple myeloma patients. *Blood* **110**, 334–338 (2007).
187. Pennisi, A. *et al.* The proteasome inhibitor, bortezomib suppresses primary myeloma and stimulates bone formation in myelomatous and nonmyelomatous bones *in vivo*. *Am. J. Hematol.* **84**, 6–14 (2009).
188. Garcia-Gomez, A. *et al.* Preclinical activity of the oral proteasome inhibitor MLN9708 in myeloma bone disease. *Clin. Cancer Res.* **20**, 1542–1554 (2014).
189. Scullen, T. *et al.* Lenalidomide in combination with an activin A-neutralizing antibody: preclinical rationale for a novel anti-myeloma strategy. *Leukemia* **27**, 1715–1721 (2013).
190. Bolomsky, A. *et al.* Immunomodulatory drugs thalidomide and lenalidomide affect osteoblast differentiation of human bone marrow stromal cells *in vitro*. *Exp. Hematol.* **42**, 516–525 (2014).
191. Breitkreutz, I. *et al.* Lenalidomide inhibits osteoclastogenesis, survival factors and bone-remodeling markers in multiple myeloma. *Leukemia* **22**, 1925–1932 (2008).
192. Munemasa, S. *et al.* Osteoprogenitor differentiation is not affected by immunomodulatory thalidomide analogs but is promoted by low bortezomib concentration, while both agents suppress osteoclast differentiation. *Int. J. Oncol.* **33**, 129–136 (2008).
193. Bolzoni, M. *et al.* Immunomodulatory drugs lenalidomide and pomalidomide inhibit multiple myeloma-induced osteoclast formation and the RANKL/OPG ratio in the myeloma microenvironment targeting the expression of adhesion molecules. *Exp. Hematol.* **41**, 387–397 (2013).
194. Friedlaender, G. E., Tross, R. B., Doganis, A. C., Kirkwood, J. M. & Baron, R. Effects of chemotherapeutic agents on bone. I. Short-term methotrexate and doxorubicin (adriamycin) treatment in a rat model. *J. Bone Joint Surg. Am.* **66**, 602–607 (1984).
195. Van Leeuwen, B. L. *et al.* Effect of single chemotherapeutic agents on the growing skeleton of the rat. *Ann. Oncol.* **11**, 1121–1126 (2000).
196. Rana, T., Chakrabarti, A., Freeman, M. & Biswas, S. Doxorubicin-mediated bone loss in breast cancer bone metastases is driven by an interplay between oxidative stress and induction of TGFβ. *PLoS ONE* **8**, e78043 (2013).
197. Van der Kraan, A. G. *et al.* HSP90 inhibitors enhance differentiation and MITF (microphthalmia transcription factor) activity in osteoclast progenitors. *Biochem. J.* **451**, 235–244 (2013).
198. Chai, R. C. *et al.* Molecular stress-inducing compounds increase osteoclast formation in a heat shock factor 1 protein-dependent manner. *J. Biol. Chem.* **289**, 13602–13614 (2014).
199. King, T. J. *et al.* Methotrexate chemotherapy promotes osteoclast formation in the long bone of rats via increased pro-inflammatory cytokines and enhanced NF-κB activation. *Am. J. Pathol.* **181**, 121–129 (2012).
200. Early Breast Cancer Trialists' Collaborative Group. Adjuvant bisphosphonate treatment in early breast cancer: meta-analyses of individual patient data from randomised trials. *Lancet* **386**, 1353–1361 (2015).
A meta-analysis demonstrating the potential survival benefit of early bisphosphonate treatment in individuals with breast cancer.
201. Gnant, M. *et al.* Adjuvant denosumab in breast cancer (ABCSG-18): a multicentre, randomised, double-blind, placebo-controlled trial. *Lancet* **386**, 433–443 (2015).
202. Fizazi, K. *et al.* 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).
203. Saad, F. *et al.* A randomized, placebo-controlled trial of zoledronic acid in patients with hormone-refractory metastatic prostate carcinoma. *J. Natl Cancer Inst.* **94**, 1458–1468 (2002).
204. Morgan, G. J. *et al.* First-line treatment with zoledronic acid as compared with clodronic acid in multiple myeloma (MRC Myeloma IX): a randomised controlled trial. *Lancet* **376**, 1989–1999 (2010).
205. Morgan, G. J. *et al.* Long-term follow-up of MRC Myeloma IX trial: survival outcomes with bisphosphonate and thalidomide treatment. *Clin. Cancer Res.* **19**, 6030–6038 (2013).
206. Coleman, R., Gnant, M., Morgan, G. & Clezardin, P. Effects of bone-targeted agents on cancer progression and mortality. *J. Natl Cancer Inst.* **104**, 1059–1067 (2012).
207. Coleman, R. E. *et al.* Breast-cancer adjuvant therapy with zoledronic acid. *N. Engl. J. Med.* **365**, 1396–1405 (2011).
208. Smith, M. R. *et al.* 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).
209. Nozawa, M. *et al.* Phase II trial of zoledronic acid combined with androgen-deprivation therapy for treatment-naive prostate cancer with bone metastasis. *Int. J. Clin. Oncol.* **19**, 693–701 (2014).
210. Okegawa, T. *et al.* Zoledronic acid improves clinical outcomes in patients with bone metastatic hormone-naive prostate cancer in a multicenter clinical trial. *Anticancer Res.* **34**, 4415–4420 (2014).
211. Smith, M. R. *et al.* Randomized controlled trial of early zoledronic acid in men with castration-sensitive prostate cancer and bone metastases: results of CALGB 90202 (alliance). *J. Clin. Oncol.* **32**, 1143–1150 (2014).
212. Bone, H. G. *et al.* Odanacatib, a cathepsin-K inhibitor for osteoporosis: a two-year study in postmenopausal women with low bone density. *J. Bone Miner. Res.* **25**, 937–947 (2010).
213. Lotinun, S. *et al.* Osteoclast-specific cathepsin K deletion stimulates S1P-dependent bone formation. *J. Clin. Invest.* **123**, 666–681 (2013).
214. Duong, L. T., Wesolowski, G. A., Leung, P., Oballa, R. & Pickarski, M. Efficacy of a cathepsin K inhibitor in a preclinical model for prevention and treatment of breast cancer bone metastasis. *Mol. Cancer Ther.* **13**, 2898–2909 (2014).
215. Power, C. A. *et al.* A novel model of bone-metastatic prostate cancer in immunocompetent mice. *Prostate* **69**, 1613–1623 (2009).
216. Godebu, E. *et al.* PCSD1, a new patient-derived model of bone metastatic prostate cancer, is castrate-resistant in the bone-niche. *J. Transl Med.* **12**, 275 (2014).
217. Valta, M. P. *et al.* Development of a realistic *in vivo* bone metastasis model of human renal cell carcinoma. *Clin. Exp. Metastasis* **31**, 573–584 (2014).
218. Sipkins, D. A. *et al. In vivo* imaging of specialized bone marrow endothelial microdomains for tumour engraftment. *Nature* **435**, 969–973 (2005).
219. Runnels, J. M. *et al.* Optical techniques for tracking multiple myeloma engraftment, growth, and response to therapy. *J. Biomed. Opt.* **16**, 011006 (2011).
220. Conway, J. R., Carragher, N. O. & Timpson, P. Developments in preclinical cancer imaging: innovating the discovery of therapeutics. *Nat. Rev. Cancer* **14**, 314–328 (2014).
221. Chtanova, T. *et al.* Real-time interactive two-photon photoconversion of recirculating lymphocytes for discontinuous cell tracking in live adult mice. *J. Biophoton.* **7**, 425–433 (2014).

Acknowledgements
P.I.C. acknowledges the support of Mrs Janice Gibson and the Ernest Heine Family Foundation, the Cancer Council New South Wales, the Prostate Cancer Foundation of Australia, the Wellcome Trust, Leukaemia and Lymphoma Research and Cancer Research UK. T.J.M. acknowledges research support from the National Health and Medical Research Council (Australia) and the Victorian Government OIS Program.

Competing interests statement
The authors declare no competing interests.