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## Cancer to bone: a fatal attraction

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### Abstract

When cancer metastasizes to bone, considerable pain and deregulated bone remodelling occurs, greatly diminishing the possibility of cure. Metastasizing tumour cells mobilize and sculpt the bone microenvironment to enhance tumour growth and to promote bone invasion. Understanding the crucial components of the bone microenvironment that influence tumour localization, along with the tumour-derived factors that modulate cellular and protein matrix components of bone to favour tumour expansion and invasion, is central to the pathophysiology of bone metastases. Basic findings of tumour–bone interactions have uncovered numerous therapeutic opportunities that focus on the bone microenvironment to prevent and treat bone metastases.

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Tumours are generally incurable once they have metastasized to bone. Devastating consequences of bone metastases include pathological bone fractures, pain, hypercalcaemia, and spinal cord and nerve-compression syndromes<sup>1</sup>. Bone metastases are a common complication of cancer and occur in 65–80% of patients with metastatic breast and prostate cancers<sup>2,3</sup>. The incidence of bone metastases is also increasing in other cancers, probably owing to improved tumour control at other disease sites<sup>4</sup>. Tumour invasion into bone is associated with osteoclast and osteoblast recruitment, resulting in the liberation of growth factors from the bone matrix, which can feed back to enhance tumour growth resulting in the ‘vicious cycle’ of bone metastases<sup>1,3,5,6</sup>. Indeed, the successful suppression of bone turnover with bisphosphonates in patients who had bone metastases that resulted in high levels of bone resorption markers was associated with improved survival<sup>7</sup>. Beyond the effects on osteoclasts and osteoblasts, tumours in the bone microenvironment recruit and modulate the function of platelets, myeloid cells, immune cells and nerve cells, and induce the formation of new blood vessels. The bone marrow also serves as a reservoir for dormant tumour cells that can resist chemotherapeutic attack, and these tumour cells can emerge later as full-blown metastases in bone or other organs<sup>8–10</sup>.

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#### Competing interests statement

The authors declare [competing financial interests](#). See Web version for details.

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Drugs, such as bisphosphonates or receptor activator of NF- $\kappa$ B ligand (RANKL; also known as TNFSF11) antibodies, that target osteoclastogenesis significantly decrease the incidence of skeletal complications and are the current standard of care for patients with bone metastases<sup>1,11–13</sup>. There are emerging data that these anti-resorptive agents can also have direct antitumour effects. However, 30–50% of patients on such therapies still develop new bone metastases, skeletal complications and disease progression<sup>1</sup>, emphasizing the need for new therapies. Important advances in understanding the basic biology of bone remodelling, haematopoiesis, haematopoietic cell egress and homing to bone marrow have uncovered new therapeutic targets for the prevention and treatment of bone metastasis.

## Bone resorption and formation

The bone microenvironment is comprised of a mineralized extracellular matrix and specific cell types that are under the control of local and systemic factors. This special milieu provides a fertile soil for many cancers to thrive (FIG. 1). Certain types of solid tumours metastasize to bone and induce destructive osteolytic and/or bone-forming osteoblastic lesions, with most solid tumours commonly producing both. Tumour cells secrete a vast array of proteins, many of which interact with resident cells in the bone marrow to induce the differentiation, recruitment and activation of osteoclasts and osteoblasts. During the process of bone resorption, stored growth factors and ionized calcium are released from the mineralized bone matrix, and these factors feed back to promote tumour cell growth and further production of osteolytic and osteoblastic factors. This vicious cycle can support tumour growth in bone<sup>3,14</sup> (FIG. 2).

Osteoclasts are polarized, multinucleated myeloid lineage cells that adhere to the bone surface through  $\alpha$ v $\beta$ 3 integrin, form an actin ring, and secrete acid, collagenases and proteases that demineralize the bone matrix and degrade matricellular proteins such as type I collagen. Macrophage colony stimulating factor (M-CSF) and RANKL are important growth factors that support osteoclastogenesis, and they are primarily produced by osteoblasts. M-CSF and interleukin-34 (IL-34) both bind to the FMS receptor (also known as CSF1R) on myeloid cells and promote osteoclastogenesis<sup>209</sup>. RANKL binds to its cognate receptor, RANK, on osteoclast precursors, to induce osteoclastogenesis through the nuclear factor- $\kappa$ B (NF- $\kappa$ B), NFATc1 and JUN N-terminal kinase signalling pathways<sup>15</sup>. Osteoprotegerin (OPG; also known as TNFRSF11B) is an endogenous decoy receptor of RANKL that inhibits osteoclastogenesis. Deletion of RANK or RANKL, or overexpression of OPG, causes severe osteopetrosis, which is consistent with the central role of this pathway in osteoclastogenesis<sup>16,17</sup>.

Mesenchymal stem cells (MSCs) in the bone marrow are directed along the osteoblast lineage through local factors, such as transforming growth factor- $\beta$  (TGF $\beta$ )<sup>18</sup>, bone morphogenetic proteins (BMPs) and WNT proteins (BOX 1). These pathways lead to the expression of three key transcriptional regulators of osteoblast function: RUNX2 (REF. 19), osterix<sup>20</sup> and activating transcription factors (ATFs)<sup>21,22</sup>. The osteoblast-stimulating activity of metastatic tumour cells is thought to be due to the ability of these cells to express many of the factors listed above that can drive osteoblast formation and activation (FIGS 1, 3).

### Box 1

#### WNT signalling in bone metastasis

WNT proteins are secreted, highly post-translationally modified proteins that have a key role in osteogenesis<sup>199</sup>. The WNT- $\beta$ -catenin signalling pathway is controlled by two cell surface receptors, Frizzled and the LRP5 and LRP6 co-receptors. Canonical WNT signalling activates bone formation, and among the many proteins downstream of WNT

is RUNX2, which is required for osteoblast formation. The WNT signalling inhibitor, dickkopf 1 (DKK1) has a crucial role in osteolytic skeletal metastasis in which osteoblast formation is suppressed and osteoclast activity is enhanced<sup>60,200</sup>. DKK1 has been attributed to the support of osteolytic breast cancer metastasis, and serum DKK1 levels are increased in patients with multiple myeloma and in women with breast cancer skeletal metastasis<sup>200,201</sup>. In experimental models of prostate cancer, DKK1 expression is high during early carcinogenesis and decreases with skeletal progression, suggesting a molecular switch between osteolytic and osteoblastic disease<sup>60</sup>. Tumour-derived parathyroid hormone-related protein decreases DKK1 expression and maybe responsible for such a switch<sup>202</sup>. Endothelin 1, a tumour-derived factor, decreases osteoblast DKK1 expression to cause the deregulated formation of new bone, as observed in prostate cancer bone metastases<sup>181,183</sup>. It is likely that WNT signalling is central to the osteoblast-stimulating activity of metastatic tumours. In contrast to solid tumour metastases in bone, myeloma is largely associated with an inhibition of osteoblastic activity. Inhibitory factors secreted by myeloma cells include DKK1, secreted Frizzled-related protein 2, interleukin-7 and hepatocyte growth factor<sup>203–205</sup>.

## The process of bone colonization

### The pre-metastatic niche

The concept of a pre-metastatic niche has emerged as a means through which a primary tumour is able to prepare sites of metastasis. In preclinical melanoma and lung cancer models, for example, vascular endothelial growth factor receptor 1 (VEGFR1)-positive bone marrow-derived haematopoietic cells home to the sites of future metastasis and form cellular clusters that precede tumour cell arrival and increase fibronectin production in tumour target sites<sup>23</sup>. Inflammatory chemoattractants that are produced in the lung in a pulmonary metastasis model further support the concept of the pre-metastatic niche<sup>24</sup>. However, others have described a lack of effect of bone marrow-derived endothelial cell precursors on tumour growth<sup>25</sup>.

In the bone microenvironment, most evidence in support of a pre-metastatic niche is in the context of endocrine-like actions. Primary tumours may condition the bone marrow through the production of circulating factors that target cells in the bone microenvironment and thus render it conducive to tumour localization and colonization. Examples include heparanase that is produced by breast cancer cells increasing bone resorption<sup>26</sup>; osteopontin (OPN) that is secreted by tumour cells and/or senescent fibroblasts promoting bone marrow cell recruitment or tumour formation<sup>27–29</sup>; and matrix metalloproteinase (MMP) production from osteoclasts supporting prostate cancer skeletal metastasis<sup>30</sup>. Parathyroid hormone-related protein (PTHrP; also known as PTHLH) is produced by various tumours and can promote bone resorption<sup>5,14</sup> and can enhance the production of local factors in the bone marrow, such as the chemokine CCL2 (REF. 31).

### Homing to bone

Tumour cells preferentially adhere to the bone marrow endothelium, a potentially initial and key event in their introduction to the bone microenvironment<sup>32</sup>. Tumour cells that metastasize to bone can use the same physiological mechanisms as those used by haematopoietic stem cells (HSCs) homing to bone<sup>33–36</sup> (FIG. 2). Osteoblasts and bone marrow stromal cells attract and regulate HSCs, and provide a niche through protein interactions that include integrins, such as  $\alpha 4\beta 1$ -vascular cell adhesion molecule 1 (VCAM1); chemokines, such as CXCL12 (also known as SDF1)-CXCR4; BMPs, Notch, nestin and OPN<sup>33,34,37–43</sup>. Preclinical studies demonstrate that bone resorption can also regulate HSC mobilization and homing<sup>44,45</sup>. Metastatic prostate cancer and probably other

cancers directly compete for the occupancy of the HSC niche during localization to the marrow. Once in the niche, they begin a process of either evicting HSCs into the peripheral blood or driving them into progenitor pools<sup>46</sup>.

CXCL12 is expressed at high levels by osteoblasts and bone marrow stromal cells, and expression of its receptor, CXCR4, on cancer cells has an important role in tumour cell homing to bone<sup>35,47-50</sup>. CXCL12 expression from bone marrow endothelial monolayers has been demonstrated to promote prostate cancer cell migration and upregulation of both MMP9 and  $\alpha\beta3$  on prostate cancer cells<sup>48,51</sup>. Kang and colleagues<sup>35</sup> found that CXCR4 was highly overexpressed in subpopulations of a serially selected and highly metastatic human breast cancer cell line. The overexpression of CXCR4 along with other bone metastasis signature genes, such as *IL11*, connective tissue growth factor (*CTGF*) and *MMP1*, in the parental breast cancer cell lines increased their capacity to metastasize to bone<sup>35</sup>. IL-11 and MMP1 stimulate bone resorption by increasing osteoblast production of RANKL, and CTGF can stimulate osteoblast proliferation, as well as neoangiogenesis. When expressed together, these proteins can act cooperatively to cause osteolytic metastasis, but overexpression of individual proteins was insufficient to accelerate bone metastases<sup>35</sup>. Several groups have also demonstrated a direct role for CXCR4–CXCL12 in breast and prostate cancer cell proliferation<sup>50,52-54</sup>, suggesting that this pathway might also be required for tumour colonization in bone.

Tumour cell surface integrins interact with extracellular matrix (ECM) proteins that are expressed in the bone microenvironment. The  $\alpha\beta3$  integrin interacts with bone-derived OPN, fibronectin and vitronectin, and expression of  $\alpha\beta3$  by breast and prostate cancer cells is associated with higher rates of bone metastasis, tumour-associated osteolysis and colonization in bone<sup>55,56</sup>. Tumour cell expression of the  $\beta1$  integrin family members  $\alpha5\beta1$ ,  $\alpha2\beta1$  and  $\alpha4\beta1$  — which are receptors for fibronectin, collagen I and VCAM1, respectively — has been implicated in the interactions of leukaemia, myeloma, prostate and breast cancer cells with bone marrow stroma, and can result in enhanced colonization and survival in bone<sup>57-64</sup>.

CXCR4 ligation increases  $\alpha\beta3$  expression on prostate cancer cells and  $\alpha4\beta1$  on myeloma cells, suggesting a crosstalk between CXCR4 and integrin expression that could both promote tumour cell recruitment to bone and colonization<sup>48,49,65</sup>. Definition of the temporal, anatomical and spatial pathways for tumour cell homing to and colonization in bone will be necessary to develop anti-homing and anti-dormancy therapies.

### Invading bone

The types of cancer that are commonly associated with profound osteolysis include breast, lung and renal cancer, as well as multiple myeloma and adult T cell leukaemia. Clinical and experimental evidence indicates that bone resorption is also increased in osteoblastic metastases. Indeed, concentration of the bone resorption marker, N-telopeptide (NTX), is high in patients with prostate cancer with osteoblastic disease and is a strong predictor of morbidity and mortality<sup>66</sup>. Tumour-derived PTHRP was one of the first characterized mediators of local bone destruction to be associated with bone metastases. In mice with an absence of hypercalcaemia or detectable circulating levels of PTHRP, neutralizing antibodies to PTHRP could block breast tumour-associated bone loss and tumour growth in bone<sup>14</sup>. In patients with metastatic breast cancer, PTHRP expression levels are increased in bone metastases compared with the primary tumour<sup>67</sup>. Yin *et al.*<sup>68</sup> showed that TGF $\beta$  in the bone microenvironment induced tumoural PTHRP production that resulted in enhanced bone resorption<sup>68</sup>. Subsequent studies, described below, showed that TGF $\beta$  in bone modulates many other pro-metastatic and osteolytic factors.

MMPs have been implicated in the general metastatic cascade, and more specifically in bone invasion and bone metastases through an increase in locally active RANKL. The effects can be direct, as exemplified by MMP7 cleavage of RANKL in prostate cancer<sup>30</sup>, or indirect, as shown by MMP1 and a disintegrin-like and metalloproteinase with thrombospondin motifs 1 (ADAMTS1), which proteolytically cleave epidermal growth factor (EGF)-like ligands to decrease osteoblast-derived OPG and hence favour osteoclastogenesis<sup>69</sup>. Moreover, MMP13 can activate MMP9 and TGF $\beta$  to increase local expression of RANKL at the breast cancer–bone interface<sup>70</sup>.

Other autocrine–paracrine mechanisms that promote tumour osteolysis involve the Jagged 1–Notch signalling pathway. Jagged 1 expressed in breast cancer cells mediates bone metastasis by activating the Notch pathway in bone cells resulting in increased IL-6, which confers a growth advantage to tumour cells.  $\gamma$ -secretase inhibitors reduce Jagged 1-mediated bone metastasis by disrupting the Notch pathway in stromal bone cells and so provide the rationale for targeting this pathway to treat bone metastases<sup>71</sup>.

Transcription factors, such as GLI2, RUNX2 and hypoxia-induced growth factor 1 $\alpha$  (HIF1 $\alpha$ ) in tumour cells have been implicated in promoting tumour osteolysis. The Hedgehog signalling molecule GLI2 induces PTHRP expression and resultant osteolysis in metastatic human breast cancer cells<sup>72</sup>. GLI2 is also involved in TGF $\beta$ -mediated melanoma metastasis to bone<sup>73</sup>. The osteoblast transcription factor RUNX2 regulates MMP9 in bone metastatic cancer cells and controls cell invasion<sup>74</sup>. In support of this, impaired intranuclear trafficking of RUNX2 in breast cancer cells inhibits osteolysis *in vivo*<sup>75</sup>. Tumour expression of HIF1 $\alpha$  inhibits osteoblast differentiation and promotes osteoclast differentiation, supporting HIF1 $\alpha$  as a factor that promotes tumour osteolysis and tumour growth in bone<sup>76,77</sup>.

### Bone matrix and tumour growth

Beyond encountering cells that are resident in the bone marrow, there is an exchange of factors from the bone matrix that are released during resorption, the most notable being TGF $\beta$ , which affect tumour localization and growth. Calcium, which is abundant in the bone matrix, has a profound effect on tumour cells. Breast and prostate cancer cells express the calcium-sensing receptor (CASR) and respond to ionized calcium<sup>78</sup> (FIG. 4). Calcium stimulation of these cells leads to an inhibition of apoptosis and a stimulation of proliferation<sup>79</sup>. In addition, ionized calcium leads to increased PTHRP secretion by tumour cells and hence induces further resorption and calcium release<sup>80,81</sup>. The CASR has been shown to be central to prostate cancer skeletal metastasis, as short hairpin RNA knockdown of this receptor in prostate cancer cells reduced tumour localization in bone<sup>82</sup>. Ionized calcium can be a potent chemoattractant to breast cancer cells and could support bone localization in addition to tumour cell proliferation<sup>83</sup>. Calcilytics, which are antagonists of the CASR, are under intensive investigation for the treatment of autosomal dominant hypocalcaemia, which results from activating mutations in the CASR<sup>84</sup>. Agents such as these could represent a strategy to restrict cancer growth in bone.

Bone matricellular proteins that could affect tumour localization include OPN, which is also released by stromal and tumour cells, secreted protein acidic and rich in cysteine (SPARC), periostin, bone sialoprotein, dentin matrix acidic phosphoprotein 1 (DMP1), syndecan 1 and decorin. SPARC, which is produced by osteoblasts, leukocytes and cancer cells, induces cancer cell migration by interacting with  $\alpha$ v $\beta$ 5 (REF. 85) (FIG. 4). OPN, which is derived from bone matrix, stromal and tumour cells, has an important role in tumour metastasis. Experiments in mice deficient for OPN or overexpressing OPN revealed that levels of OPN correlate with skeletal metastatic potential<sup>86</sup>. Periostin, which is increased during the initial response of the bone marrow stroma to a tumour, has been shown to promote the growth of



metastases in mouse models<sup>87</sup>. Bone sialoprotein has also been associated, at least in part, with increased migration and cell survival in breast and prostate cancer metastasis<sup>88,89</sup>. Differential expression of OPN and bone sialoprotein in breast and prostate cancers has been proposed as a potential switch between osteolytic versus osteoblastic presentation<sup>90</sup>. Other proteins of the ECM, including the proteoglycan syndecan 1, dentin sialophosphoprotein (DSPP) and DMP1 have been shown to be associated with breast and/or prostate cancer progression in bone<sup>91-93</sup>. By contrast, decorin suppresses bone metastasis in breast cancer models<sup>94</sup>.

TGF $\beta$  that is derived from resorbed bone matrix has a central role in most of the events leading to bone metastases and tumour expansion in bone through the regulation of osteolytic and pro-metastatic factors<sup>95</sup> (FIGS 3, 4). Human breast cancer bone metastases show active TGF $\beta$  signalling by nuclear accumulation of phosphoSMAD2 (REF. 96). TGF $\beta$  signalling blockade by the stable expression of a dominant-negative TGF $\beta$  receptor 2 (DNT $\beta$ R2) in MDA-MB-231 breast cancer cells inhibited TGF $\beta$ -induced tumour expression of PTHRP and suppressed bone metastasis in a mouse model<sup>68</sup>. Knockdown of SMAD4 also inhibited the development and the formation of bone metastases in a similar model<sup>96</sup>. In turn, overexpression of the inhibitory SMAD7 in 1205Lu melanoma cells reduced the formation of osteolytic lesions<sup>97</sup>.

TGF $\beta$  may interact with other microenvironmental factors in bone, such as hypoxia, to promote tumour growth. The bone marrow is a hypoxic microenvironment (with 1–7% O<sub>2</sub>) that enhances tumour metastasis and growth<sup>76</sup> (FIG. 2). Interactions between HIF1 $\alpha$  and TGF $\beta$  signalling pathways in breast cancer cells have additive responses in inducing the expression of vascular endothelial growth factor (VEGF) and CXCR4 *in vitro* and *in vivo*<sup>77</sup>. In a mouse model, the inhibition of either pathway in the tumour cells through mRNA knockdown decreased bone metastasis, and the loss of both did not have additional effects. By contrast, combined treatment with pharmacological pathway inhibitors decreased bone metastases more than either treatment alone, resulting in less osteoclastic bone resorption and tumour growth. These data indicate that hypoxia and TGF $\beta$  signalling drive, in parallel, bone metastases and also that they regulate a common set of tumour genes; small-molecule inhibitors by acting on both tumour cells and the bone microenvironment, thus additively decrease tumour burden<sup>77</sup>. Of all the growth factors that are present in mineralized bone matrix, the TGF $\beta$  pathway dominates and promotes bone metastases of many different tumour types through the mechanisms described above. Abundant preclinical evidence supports blocking the TGF $\beta$  pathway to treat bone metastases and that the major source of TGF $\beta$  in bone metastases is that released from the bone matrix as a consequence of osteoclastic bone resorption<sup>98</sup>.

### Resorption, formation and metastatic growth

Tumour metastasis to bone can alter normal bone physiology, resulting in uncoupled bone remodelling (FIG. 5). Enhanced osteoclast activity exacerbates the growth and progression of bone metastases (FIGS 4, 5), and preclinical data suggest that modulation of bone resorption prevents the development of bone metastases. Mice with increased osteoclast activity that is induced through vitamin D deficiency, oestrogen or androgen deprivation, or through the administration of granulocyte colony stimulating factor (G-CSF), GM-CSF or parathyroid hormone (PTH), develop increased osteolytic tumour burden<sup>99-102</sup>. Furthermore, *Cxcr4*<sup>-/-</sup> mice, which have enhanced osteoclast activity, have a higher tumour burden in bone<sup>103</sup>. By contrast, the administration of osteoclast inhibitors, such as bisphosphonates, OPG, RANKL antagonists and  $\beta$ 3 integrin antagonists<sup>104</sup>, before tumour inoculation diminishes the growth of solid tumour bone metastases and myeloma tumour burden in bone<sup>105-107</sup>. Likewise, mice with genetically defective osteoclast function as a result of *Src*<sup>-/-</sup>, *Cd47*<sup>-/-</sup>, *Opn*<sup>-/-</sup> or *Itgb3*<sup>-/-</sup> deletion have reduced skeletal tumour

burden<sup>108–110</sup>. Thus, osteoclastic changes are essential for both tumour cell colonization and growth in bone. Together, these data indicate that the state of the bone microenvironment before bone metastasis can modulate tumour growth and the subsequent behaviour of tumours in bone and thus it represents an important target for metastasis prevention.

Interestingly, reports suggest that cancer cells can fuse with macrophages<sup>111</sup> or can induce the fusion of osteoclast precursors to promote multinucleated giant cells<sup>112</sup>, leading to mature osteoclasts with tumour cell nuclei and increased function<sup>113–116</sup>. This identification of tumour cells with osteoclast properties shows that tumour cells are able to behave like bone cells in some circumstances, and this is known as osteomimicry. How widespread such osteomimicry is in skeletal metastasis and how this can be optimized for therapeutic advantage has yet to be determined. Recent work in mouse models of breast cancer has shown that progesterone receptor-positive epithelial cancer cells express RANKL and that treatment with RANKL inhibitors inhibits their growth, which highlights their potential for inhibition through targeted anti-RANKL strategies<sup>117</sup>.

Another form of osteomimicry involving osteoblasts has been identified in breast and prostate cancer cells. The osteoblastic nature of prostate cancer skeletal metastases (FIGS 3, 5) results in bone that is of a poor quality, immature and woven type<sup>118</sup>. Various mechanisms for a tumour-associated increase in bone tissue have been proposed. Tumour cells in the metastatic prostate lesion may transdifferentiate to become mesenchymal cells that are capable of osteoblastic activity, cancer cells may induce resident cells in the marrow microenvironment to enter the osteoblast lineage, and prostate cancer cells may induce the proliferation and/or differentiation of osteoblast lineage cells. The ability of prostate cancer to undergo epithelial–mesenchymal transition (EMT) and hence osteomimicry has been substantiated in animal models and humans<sup>119–122</sup>. RUNX2 has been implicated in the osteomimicry that is attributed to breast and prostate skeletal metastasis<sup>123–125</sup>. Osteoblasts are a vital component in certain aspects of tumour localization in bone. Tumours that display the phenotypic ‘osteoblastic’ response are thought to be dependent on osteoblasts in the bone microenvironment for their continued growth and survival, resulting in a co-dependent cycle<sup>126,127</sup>.

## Other cell types that prepare and feed the soil

### Bone marrow endothelial and haematopoietic cells

Cancer cells preferentially adhere to bone marrow endothelial cells rather than to endothelial cells that are derived from other organs<sup>128</sup>. Such adherent cancer cells may extravasate through the bone marrow endothelium to take up residence in the bone microenvironment. Subsequently, tumour neovascularization is essential for establishing micrometastases and for enabling their expansion in the bone. Bone metastasis and bone-residing tumours, such as myeloma, modify and recruit endothelial cells to enhance neoangiogenesis<sup>129</sup>. Targeting of stromal and endothelial cells in the bone marrow with the multitargeted tyrosine kinase inhibitor sunitinib resulted in bone marrow vessel leakage, stromal apoptosis and disrupted lung cancer cell colonization and osteolysis<sup>210</sup>. Increasing evidence supports myeloid cell participation in angiogenesis, but the ability to effectively measure the vascular supply to a tumour in bone is challenging<sup>130</sup>. Haematopoietic cells that reside in the marrow influence cell communication by releasing pro-angiogenic factors or by creating permissive conditions in the tumour microenvironment that favour the growth of blood vessels<sup>131</sup>. The haemangioblast is the progenitor cell for endothelial and haematopoietic cells and has been reported to express CD34 and CD133. Beyond their role during development, these cells continue to exist in adults in the bone marrow and peripheral blood as stem-like cells, which could be implicated in tumour vasculogenesis. Endothelial progenitor cells (EPCs) have

been implicated in numerous different tumours, but their identification and precise functions remain elusive (FIGS 2, 6).

### Adipose stem cells

Recently, adipose stem cells that are present in the bone marrow microenvironment, which develop from the same MSC lineage as osteoblasts, were shown to promote prostate tumour growth<sup>132</sup>. Bone marrow-derived MSCs have also been implicated in tumour growth and metastasis through their ability to promote and protect tumour-initiating cancer stem cells<sup>133</sup>. Interestingly, the potential of MSCs to interact with tumour cells has prompted investigations into whether MSCs can be used for the targeted delivery of antitumour agents to cancer cells<sup>134</sup>. More work is clearly needed in this area and is limited at least in part by difficulties in the standardization of MSC identification<sup>135</sup>.

### Myeloid and immune cells

Bone marrow-derived myeloid cells (including, macrophages, monocytes, myeloid-derived suppressor cells, myeloid dendritic cells and osteoclasts) and lymphocytes are recruited to tumours and areas of hypoxia and neoangiogenesis and can either promote tumour growth or enhance antitumour immune responses<sup>136–138</sup> (FIGS 2, 6). Recent evidence suggests that tumour-associated myeloid cells can be influenced by anti-resorptive targeted therapies (through either direct or indirect mechanisms), which could represent an osteoclast-independent mechanism of action of the anti-resorptives.

Myeloid-derived suppressor cells (MDSCs) are a subpopulation of immature myeloid cells that are characterized by GR1 expression and by the  $\alpha$ M $\beta$ 2 (CD11b) integrin adhesion marker<sup>139</sup>. MDSCs from myeloma-bearing mice had a greater capacity to become bone-resorbing cells compared with MDSCs from control mice<sup>136</sup>. Recent reports that mice with osteoclast defects and immune defects were protected from tumour-associated bone loss but did not have decreased tumour burden in bone<sup>140</sup> highlight the role of immune cells in bone metastasis. Bisphosphonates decrease MDSC numbers and reduce MMP secretion, which may represent an osteoclast-independent mechanism of action<sup>141</sup>. Indeed, zoledronic acid decreased tumour burden in murine bone with severely defective osteoclasts<sup>142</sup>. The regulation of MDSC differentiation, recruitment from the bone marrow to tumour sites and MDSC function in tumour biology is under intensive investigation.

Although T lymphocytes have a key role in influencing general tumour growth<sup>143</sup> and bone remodelling<sup>144</sup>, many animal models of bone metastasis are carried out in immunocompromised mice that lack T cells, thus their role in regulating tumour growth within the bone microenvironment remains largely unstudied. Of note, pro-inflammatory CD4<sup>+</sup> T<sub>H</sub>17 cells secrete RANKL, tumour necrosis factor (TNF) and TGF $\beta$ , all of which activate osteoclasts and promote bone resorption<sup>144,145</sup> and thus may enhance tumour growth in bone through the enhancement of the vicious cycle. It is anticipated that future investigations will reveal an important role for T cell subsets in regulating bone metastasis.

### Platelets

In addition to effects on haemostasis, activated platelets are an important source of pro-angiogenic (VEGF) and anti-angiogenic (thrombospondin 1 (TSP1)) factors, the aggregation of which at tumour sites affects tumour growth<sup>146</sup> (FIGS 2, 6). Tumour cells engineered to respond to platelet-derived lysophosphatidic acid (LPA) have enhanced bone metastatic potential in mice partly through platelet-derived LPA-mediated induction of osteolytic factors such as IL-6 and IL-8 (REF. 147). Likewise, genetic and pharmacological inhibition of platelet-specific integrins ( $\alpha$ IIb $\beta$ 3) or platelet activation decreased osteolytic bone metastases in mice<sup>110,148</sup>. Targeting platelets is a promising therapeutic approach for



inhibiting bone metastasis, in particular to prevent metastasis or to decrease tumour burden in bone.

Conversely, bone marrow megakaryocytes, from which platelets are derived, inhibit prostate cancer tumour growth in bone<sup>149</sup> and also inhibit bone resorption by inhibiting osteoclast formation<sup>150</sup>. The negative effect of megakaryocytes on bone resorption might be partly mediated through the production of the osteoclast inhibitory factor OPG<sup>151</sup>. Megakaryocytes might also influence bone remodelling and resorption through effects on osteoblast proliferation that are mediated by the  $\alpha 3\beta 1$ ,  $\alpha 5\beta 1$  and glycoprotein IIb integrins<sup>152</sup>. As mature megakaryocytes are located at vascular sinusoids, they are also among the first cells to encounter cancer cells as they enter the bone marrow environment, so direct action involving integrin-mediated signal transduction could be involved. Interestingly, bisphosphonates increase megakaryocyte proliferation and increase the platelet concentration of the anti-angiogenic integrin ligand TSP1 (REFS 153,154), which suggests that bisphosphonates could have non-osteoclast mechanisms that decrease tumour growth in bone. Thus, platelets and their megakaryocytic precursors interact with cancer cells before, during and after metastasis to bone.

## Tumour cell dormancy

Paradoxically, metastasis is an inefficient process with only 0.001–0.02% of cancer cells that are experimentally introduced into the circulation actually forming metastatic foci<sup>99,155,156</sup>. Preclinical evidence suggests that metastatic tumour cells can home to and localize in the HSC niche and survive in a dormant state. The skeleton is the preferred site for many tumour cells to reside, and they can remain there in a dormant state for long periods of time.

The presence of disseminated tumour cells (DTCs) in the bone marrow of patients with primary cancer reveals the housing potential of the skeleton (FIGS 1, 5). Patients with bone marrow DTCs at diagnosis are at a higher risk of both skeletal and extraskeletal metastasis. Evidence exists that DTCs can persist in the bone marrow for years in a quiescent state, and are resistant to cancer therapies<sup>8,55,157</sup>. Myeloma cells interact with bone marrow stroma partly through integrin  $\alpha 4$  (also known as VLA4) and VCAM1 to facilitate quiescence and protection from apoptosis, providing resistance to chemotherapy<sup>158</sup>. Of patients with prostate cancer who have had a radical prostatectomy, 72% have DTCs in the bone marrow<sup>159</sup>; and 30% of patients with localized breast cancer have bone marrow DTCs at diagnosis<sup>9,160</sup>. These DTCs are strong predictors of biochemical recurrence of disease and do not bode well for outcomes<sup>161</sup>. Molecular characterization of disseminated breast cancer cells in the bone marrow has revealed the specific expression of a subgroup of transcripts, including the metastasis regulator *TWIST1*, and SRC activation<sup>162,163</sup>. Breast cancer cells that have active SRC are associated with an increased risk of bone metastases in humans<sup>163</sup>. This was attributed to a cell-autonomous pro-survival role of SRC, and was linked to the pro-survival effects of CXCL12, as well as resistance to the pro-apoptotic effects of TRAIL. Increasing clinical evidence suggests that DTCs are influenced by the bone microenvironment. The treatment of women with localized breast cancer with the bisphosphonate zoledronic acid modulated the presence of DTCs in the bone marrow<sup>8,164</sup>.

Tumour cells might mimic HSC cells, and evidence for factors that induce HSC dormancy could explain tumour cell dormancy in bone marrow niches. Prostate cancer cells bind to osteoblast annexin II, which induces the expression of growth arrest-specific 6 (GAS6) receptors. GAS6 receptors are well-known inducers of dormancy in HSCs and were found to reduce cell cycle progression in prostate cancer cells, thus implicating osteoblasts as facilitators for tumour dormancy in bone<sup>165</sup>. Understanding tumour cell dormancy within

the bone marrow is probably key to inducing long-term remissions and overcoming resistance to cancer therapies.

## Therapeutic targeting of bone metastases

### Targeting osteoclasts

Osteoclastic bone resorption inhibitors are the standard of care for patients with established bone metastases, and the therapeutic targeting of osteoclasts is an area of intense clinical investigation (FIGS 4, 6). Bone matrix-targeted bisphosphonates, such as pamidronate, zoledronic acid and ibandronate, are amino-bisphosphonates that block farnesyl pyrophosphate synthase and that disrupt protein prenylation<sup>166</sup>. In addition to the effects on bone resorption by osteoclasts, amino-bisphosphonates can affect other cell types that promote bone metastases, including  $\gamma\delta$ -T cells, monocytes, MDSCs and endothelial cells, as well as tumour cells. Clinical data suggest that bisphosphonates can limit the progression of breast cancer both in bone and in other tissues<sup>8,167</sup>. The therapeutic disruption of RANKL with a subcutaneously administered humanized neutralizing antibody, denosumab, results in significant decreases in skeletal complications and reduces bone pain<sup>168</sup>. In addition, denosumab may be effective in directly targeting subtypes of breast and prostate cancers that express RANKL<sup>117</sup>. Other osteoclast-targeted therapies under clinical evaluation for the treatment of bone metastasis include cathepsin K inhibitors, SRC inhibitors and  $\alpha\nu\beta 3$  inhibitors<sup>169–171</sup>; interestingly, these therapies also affect tumour cells and stromal components that participate in bone metastases (FIG. 6).

### Targeting TGF $\beta$ and bone matrix proteins

Several strategies to inhibit TGF $\beta$  signalling are being applied to cancer<sup>172</sup>. The different classes of TGF $\beta$  inhibitors that have been used in preclinical models and clinical trials of bone metastases include monoclonal neutralizing TGF $\beta$  antibodies that prevent TGF $\beta$  ligand–receptor interactions; small molecules that inhibit T $\beta$ RI (and T $\beta$ RI II) kinase activity, preventing the activation TGF $\beta$  R-SMADs; and a natural product derivative, halofuginone (HFG), which inhibits TGF $\beta$ , although the exact mechanism remains to be investigated (FIGS 4, 6).

Although the effects of TGF $\beta$  on osteoclasts and osteoblasts are dose- and context-dependent *in vitro*, genetically modified mice showed that increased levels of TGF $\beta$  in bone promoted osteoclastogenesis and bone resorption<sup>173</sup>. In support of this, systemic TGF $\beta$  inhibition with an orally active T $\beta$ RI kinase inhibitor SD-208, or a pan-neutralizing TGF $\beta$  antibody, causes increased bone mass and increased bone mineralization by inhibiting osteoclastic bone resorption and stimulating new bone formation<sup>174,175</sup>. BMP7, an inhibitor of TGF $\beta$  signalling, inhibits the formation of bone metastases in preclinical models<sup>176,177</sup> and is a potent inducer of bone formation; it is currently approved for clinical orthopaedic fracture treatments<sup>178</sup>.

As a result of its wide variety of effects on numerous cell types and pathways, blockade of TGF $\beta$  or its downstream signalling could have undesirable consequences for wound healing and immune function<sup>179</sup>. However, human dose escalation studies with the small molecule TGF $\beta$  inhibitor, LY2157299, for patients with advanced metastatic melanoma, pheochromocytoma, and colon, prostate and breast cancer demonstrated that the drug is well tolerated with minimal toxicity.

Other bone matrix proteins that are released during osteoclastic resorption include insulin-like growth factor 1 (IGF1), platelet-derived growth factors (PDGFs) and BMPs, as well as calcium. These proteins and mineral elements have been shown to increase the growth of certain tumour types, and represent druggable (and in most cases currently available) anti-

bone metastasis targets. Bisphosphonates and other diphosphonate molecules have strong avidity to bone matrix with long bone half-lives and can be used to target compounds to the bone matrix.

### Targeting osteoblasts

One of the promising osteoblast targets investigated in preclinical and clinical trials has been endothelin 1. Endothelin 1 is a vasoconstrictive agent that stimulates osteoblast proliferation and enhances osteoblast differentiation<sup>180,181</sup>. Strong support of its ability to target prostate cancer skeletal metastases in animal models promoted its investigation in human clinical trials<sup>128,182,183</sup>. Atrasentan was the first endothelin receptor subtype A antagonist to be investigated in patients with prostate cancer, and zibotentan is a more selective and promising inhibitor that is currently in clinical trials<sup>184</sup> (FIGS 3, 6).

The bone stroma and osteoblast compartments can interact with tumour cells to promote resistance to cytotoxic chemotherapy. An exciting new approach takes advantage of the fact that cancer cells use CXCR4 and VLA4 to home to and engraft in the marrow. Cancer cells in the bone marrow are often resistant to chemotherapy because they are held in G0 phase of the cell cycle by contact with bone marrow stromal cells. HSC mobilizing agents such as AMD3100 and anti-VLA4-targeted agents mobilize leukaemia and myeloma cells into the blood, which leads to increased sensitivity to chemotherapy in mice<sup>185,186</sup>. This approach is now being tested in clinical trials in the treatment of acute myeloid leukaemia and multiple myeloma.

### Targeting neoangiogenesis

Anti-angiogenic therapies have been of intense interest and promise in cancer therapy in general, but of less focus in skeletal metastasis. Cutting the blood supply to a tumour regardless of its location has clear merit; however, the feasibility and strategies needed to achieve this in the vascular-rich site of the bone marrow are challenging and the biology is poorly understood. In a model of skeletal metastasis, the anti-angiogenic agent avastin was found to inhibit tumour growth, albeit through the indirect targeting of osteoclasts<sup>187</sup>. It is not clear what the exact target of anti-angiogenic therapy is, as the actions of such agents on circulating endothelial progenitor cells have been inconclusive<sup>188</sup>. Combining an anti-angiogenic agent with cytotoxic chemotherapy produced promising results in an animal model of breast cancer skeletal metastasis<sup>189</sup>.

Bone marrow-derived endothelial progenitors are under investigation as potential targets for anti-angiogenic strategies<sup>190</sup>. Experimental strategies using endothelial progenitor cell markers such as ID1 are emerging, and they provide valuable new avenues for therapeutic development<sup>191</sup>. Difficulty in this area has stemmed from the lack of a reliable endothelial progenitor cell marker that can be used in the differing locations that these cells reside in, such as the bone marrow, peripheral blood and tumours.

Metastasis-associated protein 1 (MTA1) is a pro-angiogenic factor that is associated with prostate cancer progression<sup>192</sup>. Silencing MTA1 effectively limited the expression of pro-angiogenic factors such as VEGF by prostate cancer cells and thus represents a potential new target of angiogenic events in skeletal metastases in patients with prostate cancer.

### Treating bone pain

Cancer-associated bone pain is extremely difficult to treat and is often fairly resistant to opioids<sup>193</sup>. Bone is densely innervated by primary sensory afferent and sympathetic neurons that are located in the periosteum and the intramedullary bone<sup>194</sup>. Bone resorption inhibitors substantially decrease pain that is produced by the growth of bone metastases<sup>161</sup>. Honore *et*

*al.*<sup>195</sup> found that the acidic environment that is produced by resorbing osteoclasts activates the acid-sensing transient receptor potential vanilloid type 1 (TRPV1) pain neurons<sup>195</sup> (FIG. 2). The RANKL antagonist OPG successfully prevented activation of pain fibres and decreased pain behaviour in mice with bone metastases. Nakanishi *et al.*<sup>196</sup> demonstrated that acid activation of TRPV1 neurons upregulates calcitonin gene-related peptide in the dorsal root ganglia and is associated with inflammatory-mediated pain<sup>196</sup>. Likewise, bone-residing tumour cells secrete factors, such as nerve growth factor (NGF) and endothelin 1, that activate pain neurons. Invasively expanding tumours in bone may also compress or directly induce nerve injury. Inhibitors of bone resorption, TRPV1, endothelin A receptor and NGF all represent therapeutic targets for cancer-induced bone pain<sup>194</sup>. Another approach to pain relief in patients with bone metastases has been the use of radiopharmaceuticals. Strontium, samarium and radium have strong avidity to the calcified matrix of bone. These agents can exert a small antitumour effect through localized radiation, but have substantial effects on bone pain and are primarily indicated to provide pain relief<sup>197</sup>.

## Future directions

Bone metastases are common and have devastating effects on cancer patients. The bone microenvironment is a unique and fertile soil for cancer metastasis, and tumour cells modify the bone microenvironment during cancer invasion and expansion through the recruitment and modulation of osteoclasts, osteoblasts, immune cells, vascular elements, bone matrix and neuronal processes. Better characterization of this tumour-modified stroma and the identification of the molecules that affect tumour expansion and dormancy in bone is essential moving forwards. In particular, the role of immune cells (T cells, natural killer cells, macrophages, MDSCs and dendritic cells) is crucial for understanding tumour expansion in bone and represents an area of great therapeutic promise. Metastasis to bone has proved a challenging process to model *in vitro* and in mice because various cell types are required in distinct temporal stages. Current animal models are appropriate for evaluating tumour expansion in bone using aggressive tumour cell lines. However, these models use highly unstable and selected cell lines developed in different strains or species inoculated into immunocompromised (or non-strain matched) animals, thus it is unclear how representative these models are of physiological bone metastases. Also, the effects of immune responses on all stages of the metastatic process are difficult to evaluate. Current animal models of cancer develop spontaneous metastasis to bone too infrequently to readily facilitate the study of tumour homing to and dormancy within bone. Animal models of osteoblastic metastases are also challenging. There is a need to develop better animal models to study tumour localization and tumour cell dormancy in bone. The enigma of why some tumour cells lie dormant in bone for decades before progressing to symptomatic disease requires comprehensive and detailed clarification.

Massively parallel DNA sequencing technologies have the potential to transform our understanding of cancer biology. Beyond the somatic mutations in cancer cells, germline DNA variants can affect any stromal cell element and can probably contribute to cancer metastasis in ways that will be uncovered as we turn our attention away from the fractured and unstable cancer genomes and towards the analysis of stromal cell genomes. Genetically unstable tumour cells are exposed to clonal selection during the colonization of distant sites<sup>198</sup>, but cells of the host tumour microenvironment represent underused, genetically stable therapeutic targets. Great efforts are underway to better understand the epigenome of cancer cells, including the function of microRNAs; however, epigenetic changes in tumour stromal cells probably have important roles in cancer biology and may be amenable to therapeutic intervention. The identification and characterization of genetic and epigenetic changes in the key stromal elements that are involved in skeletal metastasis could lead to

valuable and wide-reaching therapeutics for preventing the fatal attraction and devastating consequences of skeletal metastasis.

Powerful imaging technologies are rapidly evolving, and it will be of great interest to use these technologies to evaluate how the different stromal cell elements that are involved in bone metastasis may be visualized in live animals. Nanotechnology applications in medicine are in their infancy, and efforts are underway to use our knowledge of the bone microenvironment to target nanoparticles to tumour sites within bone. Stromal-targeted nanoparticles can have applications that are diagnostic, therapeutic or both: such agents have been dubbed 'theranostics'.

We hope that advances in understanding the basic biology of bone remodelling, biomechanics and haematopoiesis, coupled with the advances in cancer genetics, will continue to yield new and exciting therapeutic targets and insights into cancer metastasis in bone.

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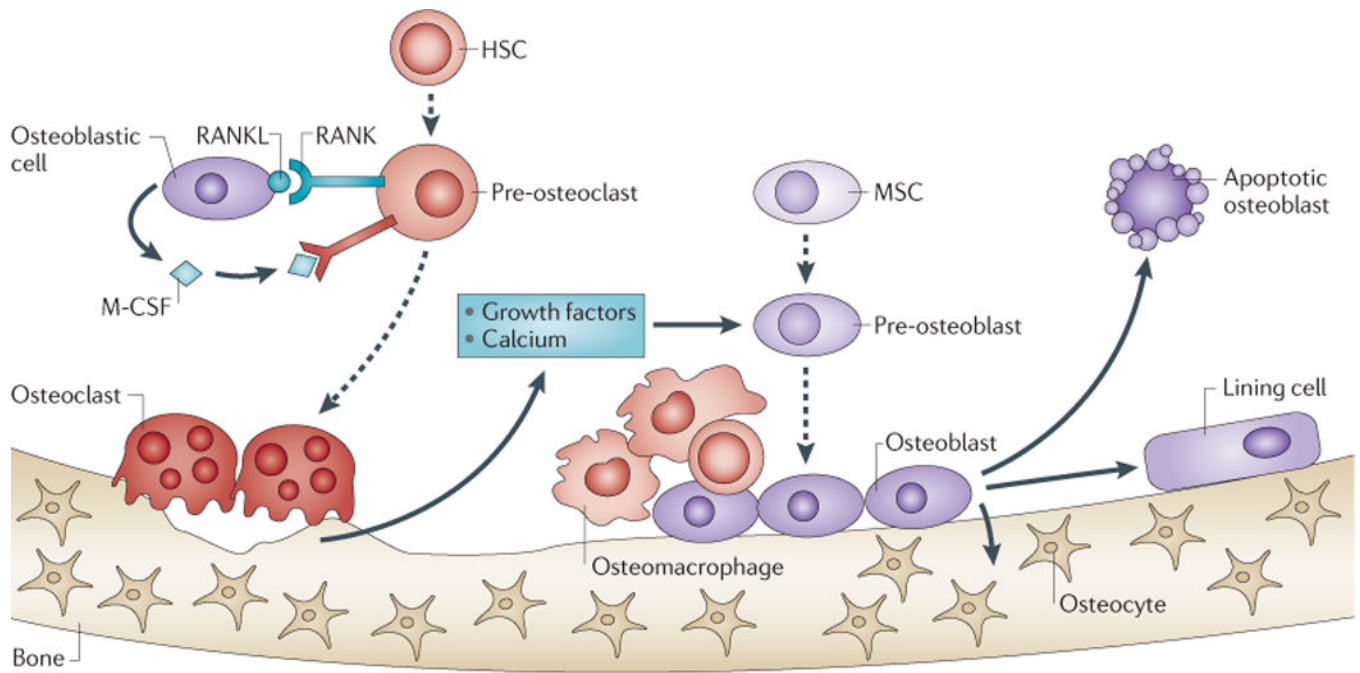
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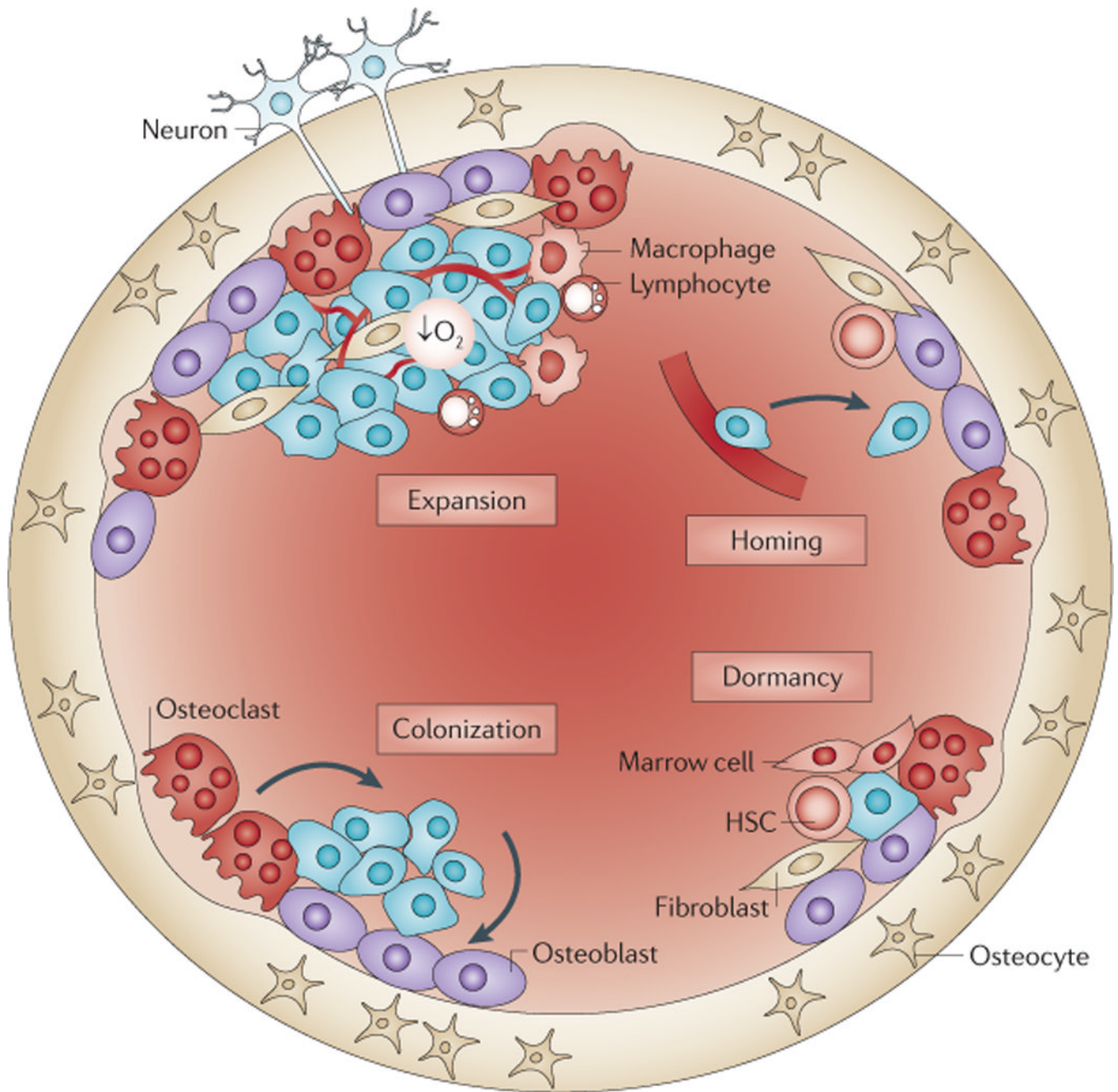
### At a glance

- Bone metastases are a common complication of cancer and are generally incurable. They cause considerable pain, pathological bone fractures and hypercalcaemia. Up to 50% of patients prescribed anti-resorptive drugs to treat bone metastases develop new bone metastases, skeletal complications and disease progression, emphasizing the need for new therapies.
- Tumour invasion into bone is associated with osteoclast and osteoblast recruitment. Osteoclasts secrete acid, collagenases and proteases that demineralize the bone matrix and degrade matricellular proteins. Macrophage colony stimulating factor and receptor activator of NF- $\kappa$ B ligand (RANKL) are important growth factors that support osteoclastogenesis, and they are primarily produced by osteoblasts. Osteoprotegerin is an endogenous decoy receptor of RANKL that inhibits osteoclastogenesis.
- Bone marrow mesenchymal stem cells are directed along the osteoblast lineage through local factors, such as transforming growth factor- $\beta$  (TGF $\beta$ ), bone morphogenetic proteins (BMPs) and WNT proteins. These pathways lead to the expression of three key transcriptional regulators of osteoblast function, including RUNX2. The osteoblast-stimulating activity of metastatic tumour cells is thought to be due to the ability of these cells to express many of the factors that can drive osteoblast formation.
- Osteoblasts and bone marrow stromal cells may attract metastatic tumour cells to bone and provide a niche through protein interactions that include integrins, such as  $\alpha$ 4 $\beta$ 1-vascular cell adhesion molecule 1; chemokines, such as CXCL12-CXCR4; BMPs; Notch; nestin; and osteopontin. These mechanisms are similar to the physiological recruitment of haematopoietic stem cells.
- The invasion and growth of metastatic tumour cells in the bone involves the modulation of a large number of genes and proteins that include matrix metalloproteinases, parathyroid hormone-related protein, TGF $\beta$ , interleukin-6, Jagged 1-Notch, GLI2, RUNX2, hypoxia-induced growth factor 1 $\alpha$ , calcium and the calcium-sensing receptor.
- Beyond the effects on osteoclasts and osteoblasts, tumours in the bone microenvironment recruit and modulate the function of platelets, myeloid cells, immune cells and nerve cells, and induce the formation of new blood vessels. These changes all help to ensure the growth and survival of metastatic tumour cells in bone and represent important therapeutic targets.
- Drugs, such as bisphosphonates or RANKL antibodies, that target osteoclastogenesis decrease the incidence of skeletal complications and are the current standard of care for patients with bone metastases. These anti-resorptive agents might also have direct antitumour effects.
- Advances in our understanding of the basic biology of bone remodelling, biomechanics and haematopoiesis, coupled with the advances in cancer genetics and tumour imaging should yield new therapeutic targets and insights into cancer metastasis in bone.



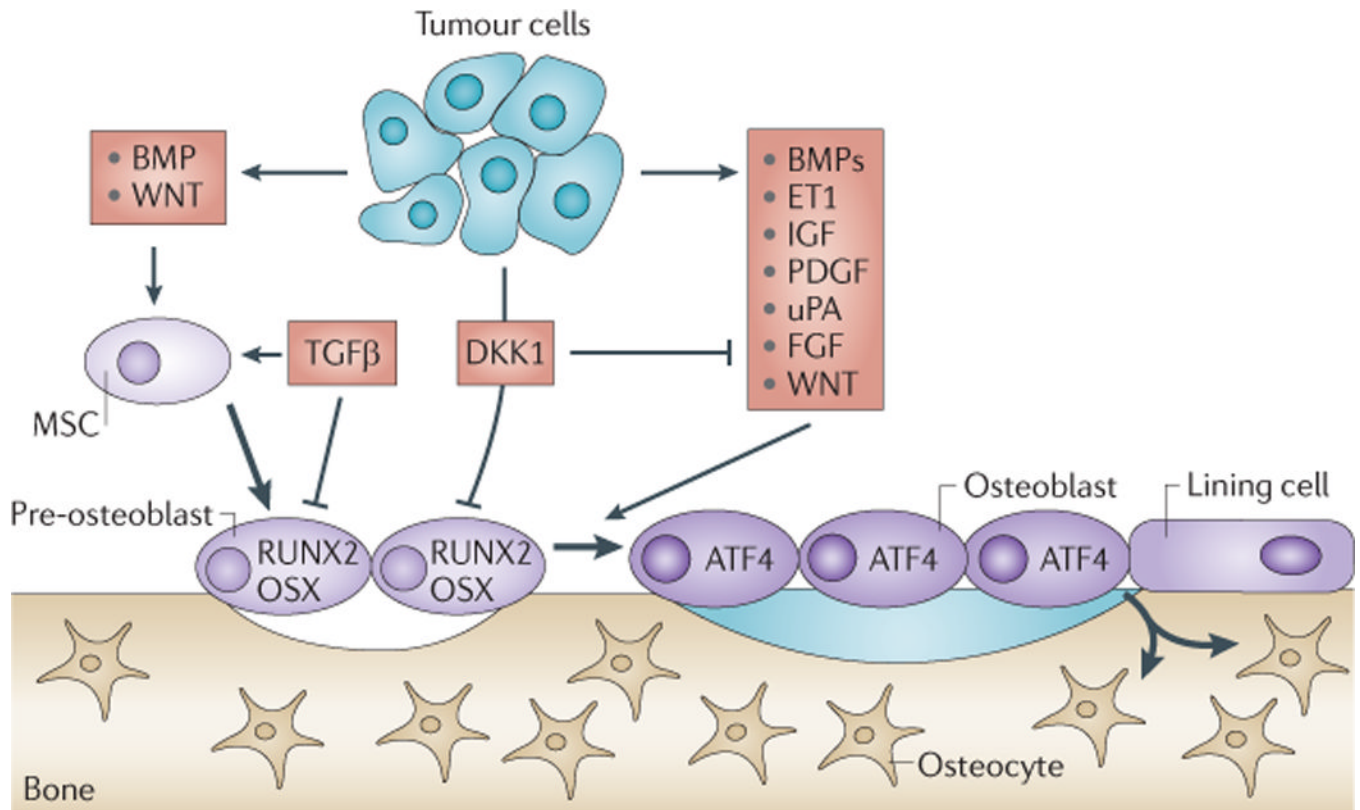
### Figure 1. Bone remodelling

The bone is a dynamic hard tissue that undergoes a continuous remodelling process to maintain skeletal strength and integrity, with 10% of the skeleton being replaced annually. In a finely balanced, coupled and sequential process (indicated by the dashed arrows), haematopoietic stem cell (HSC)-derived osteoclasts resorb bone (releasing growth factors and calcium) and mesenchymal stem cell (MSC)-derived osteoblasts replace the voids with new bone, a process that is dependent on osteoblast commitment, proliferation and differentiation coupled with osteoblast production of type I collagen and its subsequent mineralization to form the calcified matrix of bone. Osteocytes, which are terminally differentiated osteoblasts that are embedded in bone, sense mechanical strain, signal to osteoclasts and osteoblasts, and participate in the remodelling process<sup>206</sup>. Bone lining cells are osteoblastic in origin and have been proposed to form both a canopy over remodelling sites and a layer over bone surfaces, as well as a conduit to communicate with osteocytes<sup>207</sup>. The endosteum and periosteum (the lining on the inner and outer bone surfaces) contain a population of tissue macrophages, termed osteomacs, which are likely to have important roles in bone remodelling<sup>208</sup>. M-CSF, macrophage colony stimulating factor; RANK, receptor activator of NF- $\kappa$ B; RANKL, RANK ligand.



**Figure 2. Cross-section of bone depicting stages of bone metastases**

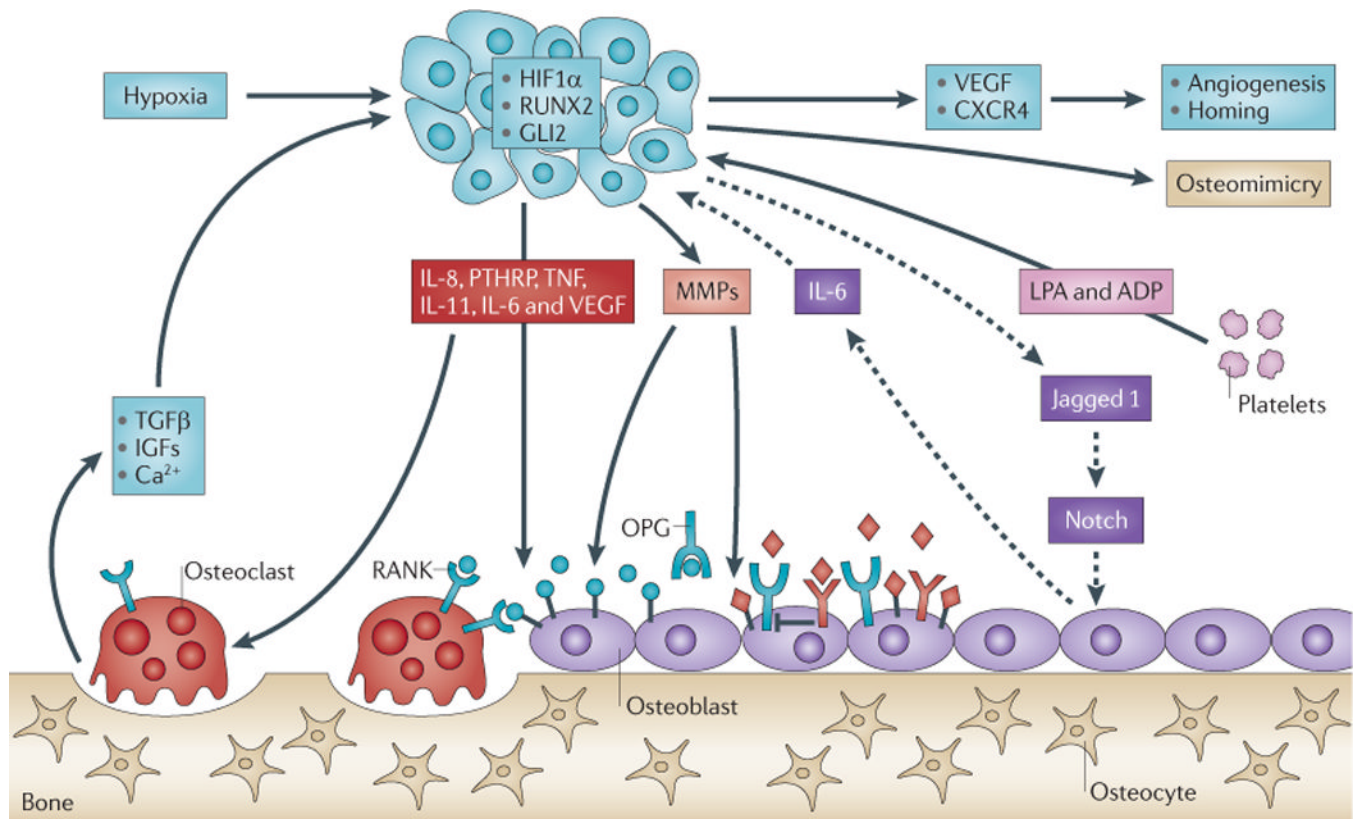
Schematic representation of tumour cell interactions within the bone microenvironment during stages of tumour metastasis to bone — tumour cell homing, dormancy, colonization and expansion. Tumour cells home to and enter the bone marrow cavity and either remain quiescent or dormant or begin growth and colonization. Tumour-mediated recruitment and modulation of bone-residing cells (osteoclasts, osteoblasts, fibroblasts, blood vessels, mesenchymal stem cells, haematopoietic stem cells (HSCs), lymphocytes, macrophages, platelets, neurons and osteocytes) and bone matrix modifications alter the bone environment thus favouring tumour growth and invasion and resulting in pain, fracture and further tumour dissemination.



**Figure 3. Tumour–osteoblast interactions**

Tumours produce various factors that regulate bone formation at different levels of osteoblast development. Bone morphogenetic proteins (BMPs), WNTs and transforming growth factor- $\beta$  (TGF $\beta$ ) provide signals to mesenchymal stem cells (MSCs) to move to areas of bone formation and to differentiate to the osteoblast lineage. Osteoblast progenitors and pre-osteoblastic cells respond to positive osteoblastic factors that are produced by tumour cells, such as BMPs, endothelin 1 (ET1), insulin-like growth factors (IGFs), platelet-derived growth factor (PDGF), urinary plasminogen activator (uPA) and fibroblast growth factors (FGFs), as well as the negative regulator dickkopf 1 (DKK1). Osteoblast-associated transcription factors include RUNX2, osterix (OSX) and activating transcription factor 4 (ATF4). Once osteoblasts produce and mineralize a collagen matrix (shown in blue) they may undergo apoptosis, become lining cells or be sequestered in the bone matrix as terminally differentiated osteocytes. TGF $\beta$  can function at multiple stages that include recruiting stem cells and promoting stem cell renewal, coupling osteoclastic bone resorption to bone formation and inhibiting osteoblast differentiation. The BMP inhibitor, noggin, as well as endothelin A receptor antagonists, can block osteoblastic metastases. Little is known of the potential interactions between tumours and osteocytes.

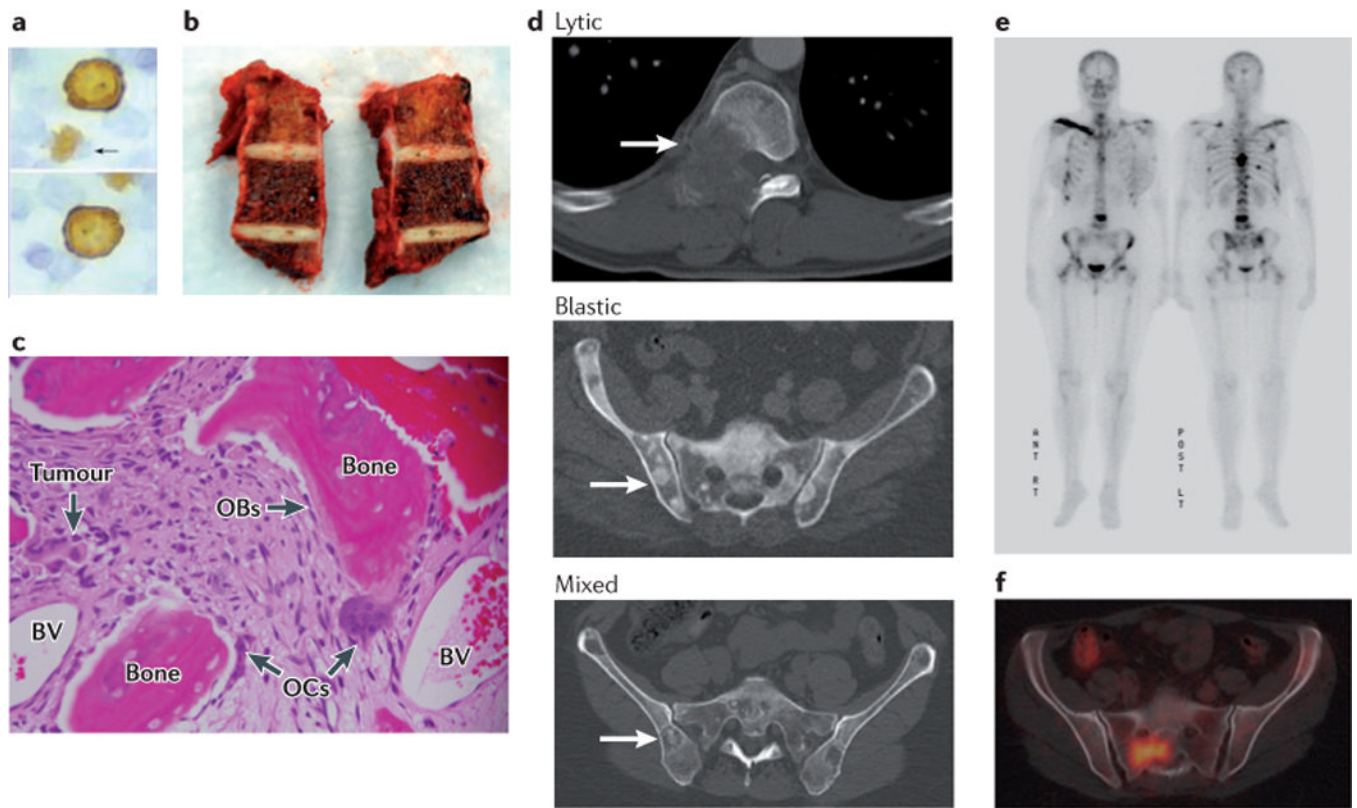




#### Figure 4. Mechanisms of tumour-associated osteolysis

Tumours secrete osteolytic factors (such as, parathyroid hormone-related protein (PTHrP), interleukin-11 (IL-11), IL-6, IL-8, vascular endothelial growth factor (VEGF), tumour necrosis factor (TNF), Jagged 1 and epidermal growth factor (EGF)-like ligands) that stimulate osteoclastic bone resorption either directly (indicated by solid arrows) or indirectly (indicated by dashed arrows) by increasing the ratio of receptor activator of NF- $\kappa$ B ligand (RANKL) to osteoprotegerin (OPG). Osteoclastic bone resorption causes the release and activation of growth factors (transforming growth factor- $\beta$  (TGF $\beta$ ) and insulin-like growth factor- $\beta$  (IGF $\beta$ )) and ions (calcium) that are stored in mineralized bone matrix to further enhance the local milieu. Tumour-associated hypoxia and hypoxia-inducible factor 1 $\alpha$  (HIF1 $\alpha$ ) in conjunction with TGF $\beta$  can increase tumour production of VEGF and the chemokine CXCR4 to increase angiogenesis and tumour homing. Tumour-produced matrix metalloproteinases (MMPs) can cleave membrane-bound RANKL (blue balls) or EGF-like growth factors (red diamonds), which can increase the ratio of RANKL to OPG to favour osteoclastogenesis. Platelet-derived lysophosphatidic acid (LPA) and ADP act on tumour cells to induce growth and the release of osteolytic factors IL-8 and IL-6.

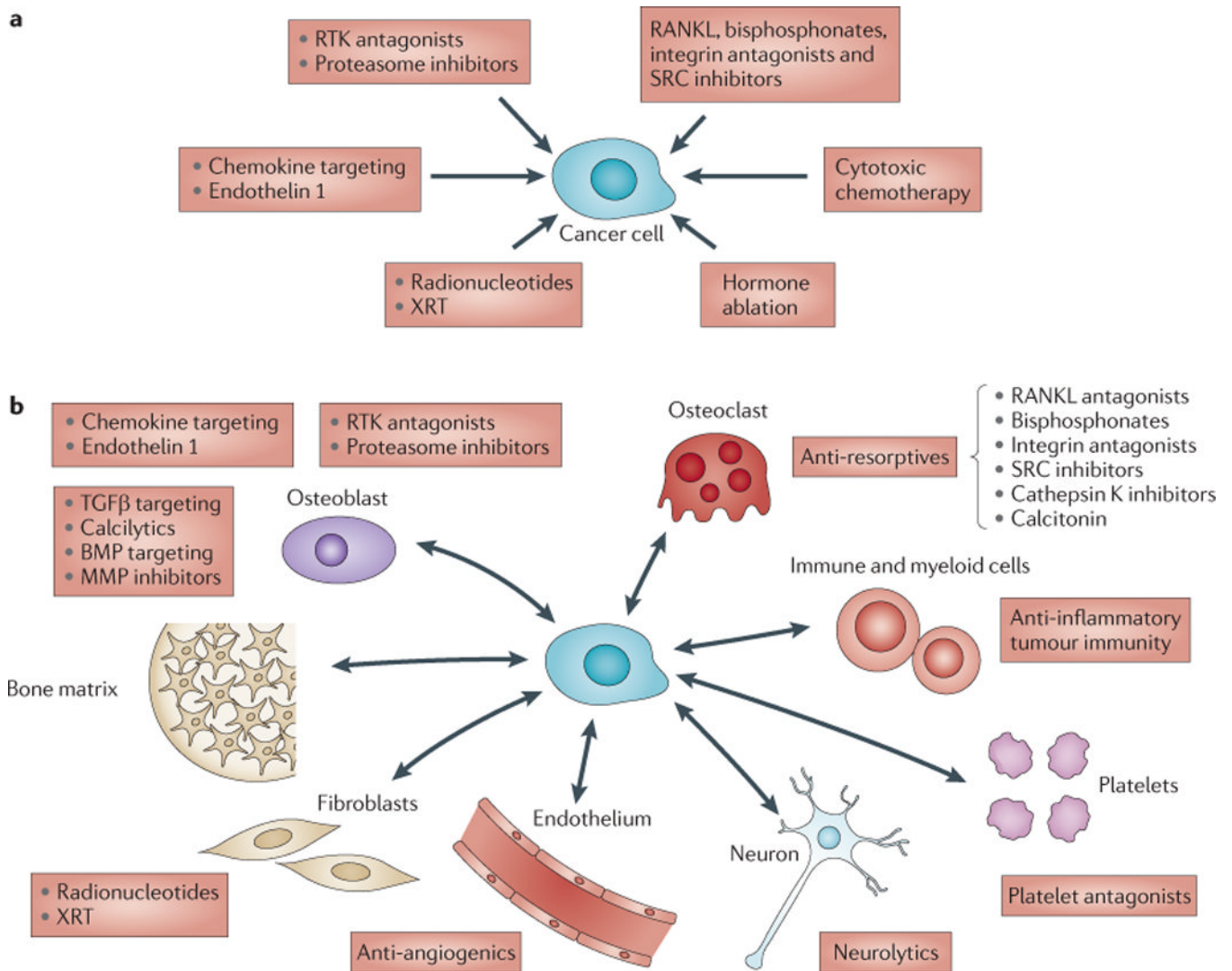




#### Figure 5. Clinical presentations of bone metastases

Bone metastases can be detected or indicated by various approaches. The presence of disseminated tumour cells in the bone marrow (shown in part **a** by immunohistochemical staining for cytokeratin in a bone marrow smear taken from a patient with breast cancer) is associated with an increased risk of bone metastasis. Post-mortem examination (part **b**) also clearly shows osteoblastic lesions, in this example in the vertebral bodies from a patient who died of metastatic prostate cancer. A bone biopsy (part **c**) stained with haematoxylin and eosin from a patient with metastatic breast cancer clearly shows the invasion of the tumour cells into the bone and the presence of osteoclasts (OCs) and osteoblasts (OBs).

Computerized tomography (CT) scans (part **d**) can clearly show the different types of bone lesions: a lytic metastasis present in a vertebral body from a patient with metastatic lung cancer; a blastic metastasis (deposition of new bone) in the pelvis of a patient with metastatic prostate cancer; and a scan that shows a patient with metastatic breast cancer who has both lytic and blastic metastases in the pelvis. Bone metastases can be extensive as indicated by the full-body bone scans (part **e**) from a patient with metastatic breast cancer. Metastases are clearly present in the skull, ribs, clavicles, spine, pelvis and the tops of the femurs. Positron emission tomography using radiolabelled [18F]-2-fluoro-deoxy-D-glucose combined with CT (part **f**) also clearly shows active bone metastases, in this case a sacral metastasis in a patient with metastatic renal cancer. BV, blood vessel. Part **a** courtesy of R. Aft, Washington University School of Medicine, USA. Part **b** courtesy of K. Pienta, University of Michigan School of Medicine, USA. Part **c** courtesy of D. Novack, Washington University School of Medicine, USA. Images in parts **d–f** courtesy of V. Reichert and J. Burkett, Washington University School of Medicine, USA.



**Figure 6. Overlapping benefits of targeting tumour and stromal cells for bone metastases**

The growth and survival of metastatic cancer cells in bone require the support of numerous cells and molecules within the bone microenvironment. Many of the therapies targeted to cancer cell signalling pathways (part **a**) have overlapping effects on bone stromal components (part **b**), which can enhance the antitumour effects. Likewise, stromal-targeted therapies can also target tumour cells. Many therapies target more than these two cell types, such as receptor tyrosine kinase (RTK) inhibitors. Anti-resorptive therapies target genes and proteins that are involved in functional osteoclast development from pre-osteoclasts and haematopoietic progenitor cells, but most of these therapies also have direct antitumour effects. Targeting pro-tumour and antitumour immunity that is mediated by myeloid cells and lymphocytes and the proteins that they secrete can disrupt tumour growth in bone and in other sites. Inhibition of platelet aggregation and activation can limit the release of platelet-derived pro-tumour and pro-angiogenic molecules and can disrupt tumour–platelet aggregates, reducing adhesion to bone vessels. Targeting acid-sensing and sympathetic neurons can decrease bone pain. A reduction in pain levels can improve quality of life, enhance mobility (which can strengthen bones and prevent fracture) and improve nutrition, which can promote improved survival. Anti-angiogenic therapies decrease tumour burden in bone and potential metastasis of metastases. Bone-targeted radiation not only targets tumour

cells but can also disrupt fibroblast–stromal support of tumour cells. Numerous protein factors (such as transforming growth factor- $\beta$  (TGF $\beta$ ) and bone morphogenetic proteins (BMPs)) that are stored within the bone matrix, as well as calcium, are released during bone metastases and can enhance tumour growth and promote the activation and release of other bone-derived, pro-tumour growth factors. Targeting of osteoblasts through endothelin 1, chemokine disruption, anabolic effects of proteasomes or RTK and SRC inhibition can decrease fractures and decrease the release of tumour chemoattractants and osteoclast growth factors. MMP, matrix metalloproteinase; XRT, radiotherapy.