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TITLE:
Bone metastases in the era of targeted treatments: insights from molecular biology.

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INTRODUCTION

Subsequent breakthroughs in oncology, including the use of more effective local and systemic treatment strategies have meaningfully improved patient outcomes for many cancer types, in particular when the tumour is still restricted to the primary site. However, despite these advances this outlook changes drastically when metastases develop. Bone metastases still are a common feature of advanced cancer such as lung, breast and prostate cancer, and remain generally incurable.

In this review we summarize the major mechanisms that drive physiological bone remodeling and metastatic bone disease from a molecular biology point of view highlighting the key pathways involved in these processes as we understand them today. In addition to these mechanistic insights, we evaluate the available clinical evidence for agents that target the discussed pathways.

THE VICIOUS CYCLE OF BONE METASTASIS

Bone is constantly being formed, broken down and renewed, with two specialized cell lines intricately working together to regulate and maintain bone mass. The osteoclast, which is derived from a hematopoietic lineage of cells, resorbs bone, while the osteoblast, whose origin is mesenchymal, synthesizes bone matrix.[1] The balance between the activity of both cell types is of extreme importance to maintain functional and healthy bone. Overactivation of osteoclasts results in fragile and brittle bone (e.g. osteoporosis), while lack of osteoclast function causes high bone density (e.g. osteopetrosis).

Bone resorption starts with the differentiation of osteoclastic hematopoietic precursors to mature osteoclasts that attach to the bone matrix. Podosomes, which contain actin and $\alpha_V\beta_3$ integrin, facilitate this adhesion and form a connection with the proteins osteopontin and vitronectin of the bone matrix. The Howship's lacuna is the region where bone resorption occurs and is closed-off where the cell adheres to bone, called the clear zones.[2] The resorptive surface of the osteoclasts excretes enzymes which digest the organic bone matrix (mostly consisting of collagen) and acidify the environment to dissolve the inorganic compound (hydroxyapatite) to phosphate, calcium and water. This acidic milieu is created by osteoclast expressed carbonic anhydrase 2 enzyme, which is present in the cytoplasm of the cell and generates H_2CO_3 from H_2O and CO_2. The spontaneous dissociation of carbonic acid results in the generation of a proton (H^+) and bicarbonate (HCO_3^-). While the proton is excreted at the resorptive surface by proton pump activity (H^+-ATPase), electroneutrality is preserved by a chloride channel that releases the bicarbonate on the basolateral membrane of the osteoclast preventing excess of bicarbonate to act as a limiting factor for the chemical reaction.[3]

Together with the dissolution of the inorganic bone matrix, the organic part of the bone is digested by the enzyme cathepsin K, which is a cysteine protease that targets the abundance of cysteine residues present in the structure of collagen. The primary degradation products of collagen are subsequently enclosed in vesicles containing the enzyme tartrate-resistant acid phosphatase (TRAP) which facilitates further degradation. These vesicles are then transported through the cytoplasm of the cell and released on the basolateral side.[4, 5]

The physiological process of bone resorption by osteoclasts is then followed by new bone formation by osteoblasts. When the bone microenvironment is invaded by metastatic tumourcells, this tightly controlled remodeling process becomes severely disturbed. The resulting dysregulation of bone homeostasis is termed the “vicious cycle” and starts with the secretion by the invading tumourcells of numerous proteins in the bone microenvironment (most importantly parathyroid hormone-related protein [PTHrP]) that promote osteoclast function and thus bone degradation. In addition, these growth factors will not only increase the recruitment, differentiation and activation of osteoclasts and osteoblasts, but will also severely disrupt the critical feedback mechanisms between these cells. The result is a positive feedback mechanism allowing tumourcells to proliferate by the release of growth factors and ionized calcium as result of excessive bone resorption by osteoclasts. In addition to tumourprogression, this process frequently exposes the patient to pain and bone complications such as fracture, spinal cord compression, and hypercalcemia. In reality, the vicious cycle consists of a vastly more complex network of mediators and interactions, with several drugs currently available that successfully target osteoclast formation and function to interrupt the vicious cycle and decrease skeletal complications in patients with metastatic bone disease as detailed in the following paragraphs.[6, 7]

PHARMACOLOGICAL TARGETS OF OSTEOCLAST FUNCTION

An overview of the discussed osteoclast targets is presented in Figure 2.

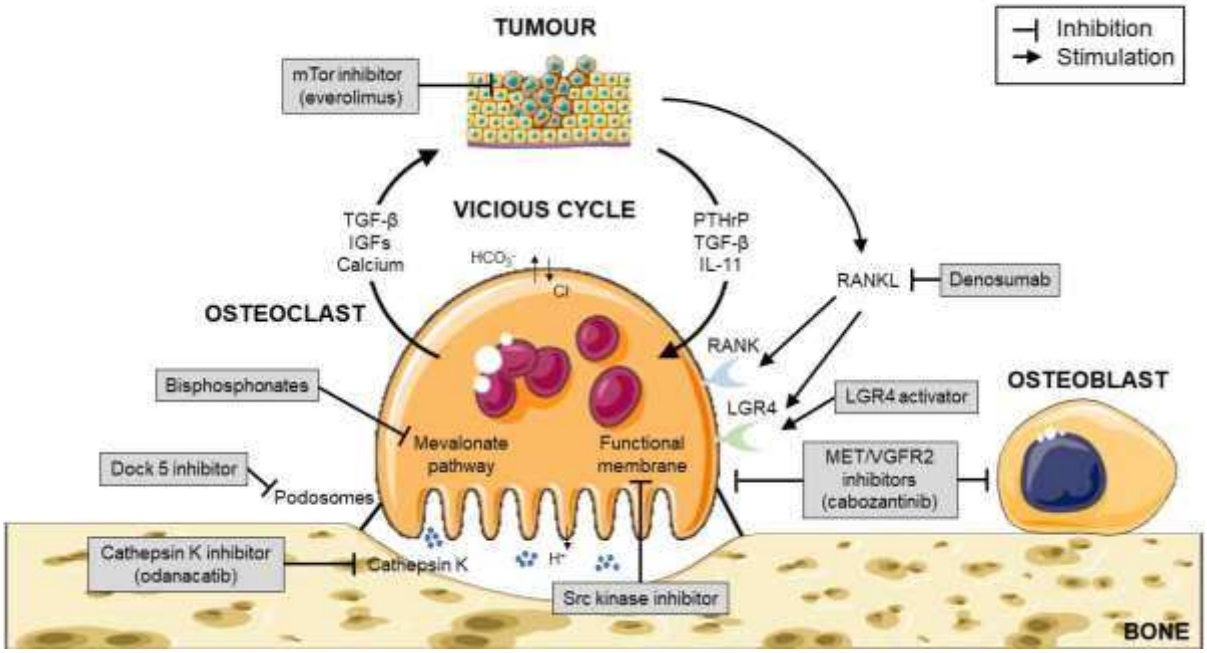

Figure 2: Summary of actionable targets in osteoclasts to inhibit bone resorption. (Figures creative commons attribution 3.0)

Bisphosphonates

Mechanism of action

Bisphosphonates were the first class of drugs that could successfully inhibit bone resorption by selective inhibition of osteoclasts, as first published by Herbert Fleisch[8] 50 years ago when they were still (incorrectly) referred to as diphosphonates. Two classes have subsequently been developed, with the older bisphosphonates (such as etidronate and clodronate) acting by reversing the pyrophosphorylytic reactions which are catalysed by aminoacyl-tRNA synthetases. This results in the generation of toxic non-hydrolysable bisphosphonate analogues of ATP (AppCp) which inhibit mitochondrial ADP/ATP translocase and induce osteoclast apoptosis.[9] However due to their low potency they have been superseded in clinical practice by newer generation bisphosphonates. Indeed, the current day clinically used nitrogen-containing bisphosphonates (N-BP) (e.g. pamidronate, risedronate, zoledronate) target the osteoclast farnesyl pyrophosphate synthetase (FPPS) and geranylgeranyl pyrophosphate synthase (GGPPS).[10] Once administered the N-BP, which has a high affinity for Ca^{2+} due to the two phosphonate groups, rapidly localizes to the bone mineral. When osteoclasts start resorbing this bisphosphonate saturated bone, the low pH in the Howship's lacuna protonates the phosphonate groups of the N-BP, releasing the N-BP from the bone mineral and dissolving them. The free bisphosphonate is then captured in vesicles and enters the osteoclast by pinocytosis. From the internalized vesicles the N-BP enters the cytosol after further protonation. In normal conditions FPPS and GGPPS, which are part of the mevalonate pathway, generate FPP and GPP. Both these compounds are important in the production of isoprenoids, which are lipids that are crucial for the post-translational prenylation of GTPases such as Ras and Rho. In addition, the accumulation of the substrates generates ApppI, a cytotoxic ATP analogue.[11] The N-BP however binds to the pocket of the FPPS enzyme and induces a conformational change, inhibiting the prenylation of GTPases and altering their intracellular localization and function.[12] While the mevalonate pathway is present in all cells of the human body, N-BP mainly affect osteoclasts because of their high affinity for bone mineral and the high endocytic activity of osteoclasts.[13, 14] The local effect of bisphosphonates in the microenvironment can be prolonged, as these molecules can reattach to the bone after dissolution.[15]

Effect of bisphosphonates on tumourgrowth

Next to the effect on osteoclasts, multiple preclinical studies and some clinical trials have demonstrated the positive effect of N-BP administration on the incidence of bone metastases and tumour progression in bone.[16] Taken together, evidence suggests the existence of direct and indirect anti-tumour effects of bisphosphonates and other bone modifying agents (Figure 1). First, indirect effects act through the inhibition of bone resorption which reduces the release and local availability of tumour growth factors (e.g. TGF-β) from bone matrix, interrupting the vicious cycle and making bone a less attractive environment for tumour cells.[16, 17] In addition, compelling in vivo data has demonstrated the direct targeting by N-BPs of cancer promoting pathways, including angiogenesis. This is mediated by inhibition of FPPS activity which disturbs prenylation signalling and leads to inhibition of endothelial cell adhesion, survival and migration.[16] N-BPs are also taken up by monocytes and dendritic cells, where

inhibition of the mevalonate pathway triggers elevated levels of the phosphor-antigen IPP, which is a strong activator of a specific subset of T lymphocytes (γδ T cells).[18] Together with presentation of the IPP antigen by cancer cells, the subsequent activation of γδ T cells leads to the release of interferon-γ and perforin, and cytotoxic tumour cell death. This immune system activating action of bisphosphonates has also been postulated to explain the flu-like symptoms that patients may experience after administration of these agents.

Next, preclinical data have also showed decreased tumour growth in bone in osteoclast deficient mice, suggesting a direct anti-tumour effect of N-BPs.[19] Further evidence for a direct anti-tumour effect comes from the observation that the administration of the highly bone-seeking phosphonocarboxylate analogue of risedronate resulted in reduced skeletal tumour growth even at doses that did not inhibit osteolysis.[20, 21] Furthermore, apoptosis was observed in a xenograft model of myeloma treated with clinically relevant doses of the N-BP zoledronic acid, where the presence of unprenylated Rap1A provided strong mechanistic evidence of inhibition of FPPS by zoledronic acid in these non-skeletal tumours.[22] Finally, tumour cell migration and invasion are also susceptible to N-BPs, with preliminary studies showing inhibited cellular invasion, which reduces metastatic outgrowth.[16]

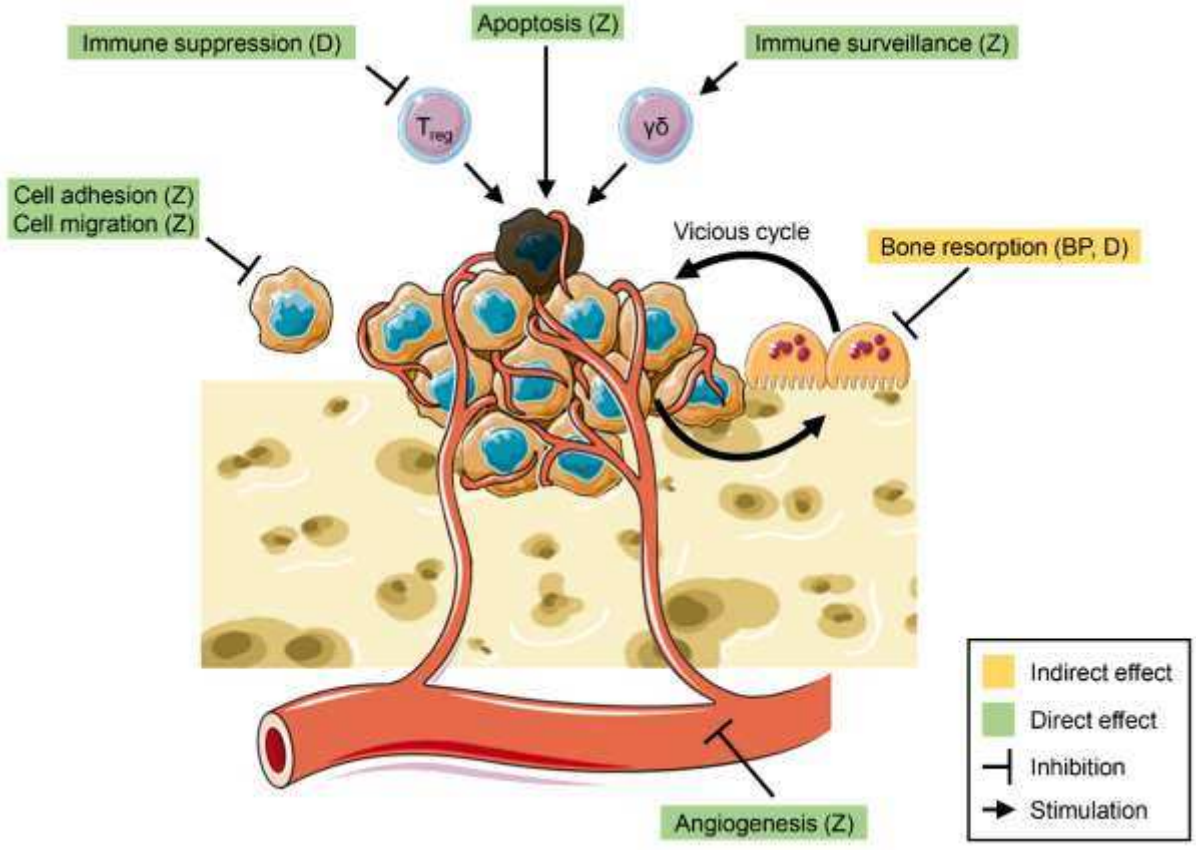

Figure 1: Direct and indirect effects of bone modifying agents in the bone tumour microenvironment. While indirect inhibition of tumour growth is mainly attributed to the interruption of the vicious cycle of bone resorption and growth factor release, direct anti-tumour effects through multiple mechanisms have been described for specific agents, including bisphosphonates (BP) (in particular zoledronic acid [Z]) and denosumab (D). (Figures creative commons attribution 3.0)

Receptor activator of nuclear factor-kB ligand (RANKL) inhibitors

Mechanism of action

The receptor activator of nuclear factor-kB ligand (RANKL) is a type 2 transmembrane protein from the TNF superfamily that exists in a membrane bound and soluble form that are both bioactive. The soluble form is generated by proteolytic cleavage of the membrane bound protein. In bone, RANKL is found on stromal/osteoblastic cells or exists in soluble form. It promotes the differentiation of osteoclastic precursor cells, activates mature osteoclasts and it prolongs osteoclast cell survival. The RANKL antagonist denosumab binds with high affinity to RANKL and blocks the binding with the RANK receptor and further downstream signaling. Osteoprotegerin (OPG) is the naturally occurring soluble decoy receptor for RANKL and binding with OPG results inhibition of RANKL-RANK signaling.[23]

Effect of RANKL inhibition on tumourgrowth

Preclinical studies have shown that RANKL-RANK inhibition reduces tumour-induced bone lesions, both those with osteolytic, osteoblastic or mixed phenotypes. This supports the notion that osteoclastic activity is a requisite element for osteolytic and osteoblastic lesions. Preclinical studies showed an important effect in tumour growth reduction as a consequence of interrupting the vicious cycle. Also, additive effects are observed when RANKL blockade is administered together with other therapies.[24] More recently, the complex interplay of RANK/RANKL signalling and tumourimmunology has generated a lot of interest. In particular, RANKL may be involved in another vicious cycle that involves T_{reg} lymphocytes and tumourassociated macrophages (TAMS) that have a high RANK expression and in which RANKL can act as a chemoattractant for these cells. Conditional on other factors, both TAMS and T_{regs} have the potential to promote tissue invasion and metastasis, and enhance the tumourpermissive properties of macrophages through RANK/RANKL signalling. The use of RANKL-targeted agents such as denosumab may therefore be a promising adjunct treatment strategy, especially in novel cancer immunotherapies.[25]

mTOR inhibitors

Mechanism of action

Phosphoinositide 3-kinase (PI3K) is an important pathway in different malignancies and dysregulation of the PI3K/AKT/mTOR pathway is a frequent observation in cancer.[26] Initiation of this pathway occurs through activation of receptor tyrosine kinases (RTK) by growth factors. In cancer cells upregulation or mutation of the RTK and/or their subunits p110α, p110β, p85α, p85β can occur. Additional activation is observed through the internal RAS and AKT (protein kinase B) pathway, whereas negative feedback mechanisms via PTEN and the TSC complex are frequently lost in malignancies. An important drug target in this pathway is the mechanistic target of rapamycin (mTOR), whose inhibition leads to a marked reduction in protein synthesis and cell growth. Two protein complexes of mTOR have been described (mTORC1 and mTORC2), and compounds that inhibit both complexes achieve superior inhibition of cell survival, proliferation and metabolism, compared to single complex inhibition alone, in preclinical studies.[27]

Working on a different target in the pathway are PI3K specific inhibitors, which can act on all or a specific subset of isoforms of PI3K. Normal activation of the PI3K pathway,

results in the activation of the RAS and AKT pathway promoting cell growth, differentiation, survival, proliferation and metabolism. The more specific inhibitors have a more narrow activity profile, a limitation that may be overcome by strict patient selection based on the analysis of mutational characteristics of the primary tumouror its metastases.

Compounds with both mTORC1/2 and PI3K inhibition have been generated as well, as these targets share structural domains belonging to the PI3K-related kinases superfamily (PIKK). This results in upstream and downstream AKT inhibition, avoiding the negative feedback activation loop, but at the cost of higher toxicity.

Drugs targeting AKT seem to be an interesting approach for tumourcells with loss of function mutations in the tumoursuppressor PTEN, as PTEN is a downstream complex of PI3K required for signaling of the AKT target.[27]

Effect of the mTOR inhibitor everolimus on bone
The effect of the mTOR inhibitor everolimus on osteoclastic and osteoblastic cells was evaluated in vitro. Osteoclastic precursor cells showed decreased cell viability with increasing everolimus concentrations, but more pronounced negative effects were observed on osteoclast formation and in mature osteoclasts. In human osteoblastic precursor cells the activity also decreased with incubation with everolimus. These findings established the importance of the mTOR pathway in bone biology. Interestingly, the RANK-RANKL signaling pathway for activating osteoclasts acts through mTOR signaling. This is supported by the observation that incubation with rapamycin, another mTOR inhibitor, results in an increase in OPG, an important decoy receptor for RANKL.[28, 29]

Preclinical in vivo experiments with everolimus in a murine ovariectomized model showed an increase in bone mineral density by 38%, and of total calculated bone volume by 37% in comparison with a control group. These results were confirmed by measurement of increased trabecular number and decreased trabecular separation. Additional histomorphology results showed a decrease in osteoclastic cell numbers by 25% with treatment. Everolimus could even partly reverse the bone remodeling rate by 41.5% in the same murine models. When a preclinical model of bone metastases was used, everolimus reduced bone lesions by 45%, increased bone mineral density and bone formation rate >50%, and decreased the total number of osteoclasts 43%.[30] All these data indicate a bone protective effect of everolimus.

MET and VEGFR2 inhibitors

Mechanism of action
Cabozantinib, a tyrosine kinase inhibitor (TKI), inhibits the phosphorylation of the tyrosine kinase receptors of mostly MET and VEGFR2, but also that of other receptors from the same family. The hepatocyte growth factor (HGF) is the only known ligand for the MET receptor and this signaling pathway is frequently dysregulated in different tumourtypes. Overexpression of both MET and VEGFR2 has been shown to induce metastases and tumorigenesis.[31]

While treatment with selective VEGFR inhibitors has demonstrated superior clinical outcomes, tumourrelapse or progression invariable occurs after some time.[32] The

resistance mechanism to the VEGFR inhibitor treatment is possibly upregulation of tyrosine kinase receptors (MET and VEGFR) caused by treatment induced hypoxia. In addition crosstalk between different pathways exist, providing the rationale for the development of TKIs with dual or broad spectrum activity.

In bone, treatment of osteoclasts with HGF results in increased cytosolic Ca^{2+} and Src kinase activity, both crucial for regulating the activity of osteoclasts. However, the increase in cell activity is not specific to osteoclasts and could be seen in other cell lines as well (epithelial cells, fibroblasts). In vitro motility experiments showed increased motility (chemotactic migration) of osteoclasts measured by the number of osteoclastic cells crossing a collagen filter. Increased DNA replication was observed as well after stimulation with HGF and osteoclasts are able to secrete HGF themselves. The only effect observed on osteoblastic cells was stimulation of the DNA synthesis.[33-35]

Effect of cabozantinib on bone metastases

Preclinical studies with cabozantinib in prostate cancer models have shown a better effect on osteoblastic metastases compared to osteolytic lesions. In particular cabozantinib reduced the size of osteoblastic lesions and reduced bone remodelling at the site of bone metastases. The effect of cabozantinib on osteoblast is biphasic, with induction of early osteoblast differentiation at low doses, and inhibition of osteoblast differentiation at higher doses, which may be due to the overall impact of cabozantinib on osteoblast viability. The reduction in bone resorption observed with cabozantinib treatment is primarily the result of a reduction in the numbers of osteoclasts, as opposed to the inhibition of the activity of individual mature osteoclasts.[35]

Cathepsin K inhibitors

Mechanism of action

Cathepsin K, mentioned above, is an enzyme that breaks down the organic phase of bone. Inhibitors of cathepsin K interfere with the catalytic characteristics of the enzyme due to covalent binding with amino acids of the functional region of the enzyme. Cathepsin K has been suggested to not only inhibit bone resorption but also stimulate bone formation by reducing the degradation of growth factors.[36] Unfortunately, concerns about off-target inhibition of cathepsins are rising and have led to the withdrawal of one such inhibitor in a phase 3 clinical trial due to skin adverse effects.[37]

Effect of cathepsin K inhibition on bone metastases

Preclinical in vivo metastatic breast cancer models show reduced osteolytic bone lesion formation and progression, resulting in less bone destruction with the administration of a cathepsin K inhibitor. Breast cancer cells express cathepsin K which feeds the seed and soil mechanism of the metastases theory. However the overall antitumoureffect is dominantly due to the drug’s antiresorptive effect. This hypothesis was confirmed since no effect was seen on soft tissue breast cancer tumours.[38]

Src inhibitors

Src is a non-receptor tyrosine kinase that is expressed in all tissue cells, yet the effect of src knockout in mice was only evident by the osteopetrotic phenotype in bone, due to the lack of osteoclast activity.[39] Not only do osteoclasts fail to form their functional resorptive membrane, but they also failed to migrate to the bone.[40]

c-Src is involved in numerous osteoclast functions, it is important in the assembly and disassembly of the podosomes, which are crucial for the attachment of mature osteoclasts to the resorptive surface of the bone.[41] Src is also an important product in the signalling cascade of RANK-RANKL, with a defect or absence in Src resulting in an interrupted pathway.[42] An additional mechanism of action in osteoclasts is the secretion of proteases and the regulation of vesicle transport. Importantly, other non-receptor kinases cannot replace the function of c-Src when it is lacking, highlighting its pivotal role in osteoclasts.[43]

Leucine rich repeat containing G-protein coupled receptor 4 (LGR4)

The leucine rich repeat containing G-protein coupled receptor 4 (LGR4) was identified as a new receptor for RANKL. RANKL was already known as the sole ligand of the RANK receptor and induces osteoclast synthesis and function, as discussed previously.

LGR4 competes with RANK for binding RANKL, and inhibits osteoclast differentiation by suppressing RANK-TRAF6 signalling and activating $G\alpha_q$ Ca^{2+} induced inhibition of NFATC1 during osteoclastogenesis. Moreover, osteoclast apoptosis is induced through Fas expression, effectively making LGR4 a key negative feedback mechanism in the RANK-RANKL signalling pathway that negatively regulates osteoclast differentiation and bone resorption.[44]

Dock5 inhibitors

Dock5 is a protein that is involved in intracellular signalling networks and acts as activator of small G proteins. The Dock5 inhibitor C21 inhibits the nucleotide guanine exchange on GTPase Rac1, predominantly by blocking the DHR2 catalytic and dimerization domain. Rac1 is needed for the formation of the osteoclast sealing zone and inhibition leads to a malfunction in the attachment of the osteoclast to the bone matrix.[45]

Preclinical in vivo data showed no impairment of bone formation with the use of C21 in contrast to bisphosphonate treatment. Also, in models of bone metastatic cancer a reduced tumour burden in the bone and increased bone volume was demonstrated. Again, these effects can be largely attributed to the shutdown of the vicious cycle in the bone. C21 has potential for clinical use in osteoporosis and rheumatoid arthritis as well, as benefits in bone volume could be seen in specific models for these conditions.[46]

PHARMACOLOGICAL TARGETS OF OSTEOBLAST FUNCTION

An overview of the discussed osteoblast targets is presented in Figure 3.

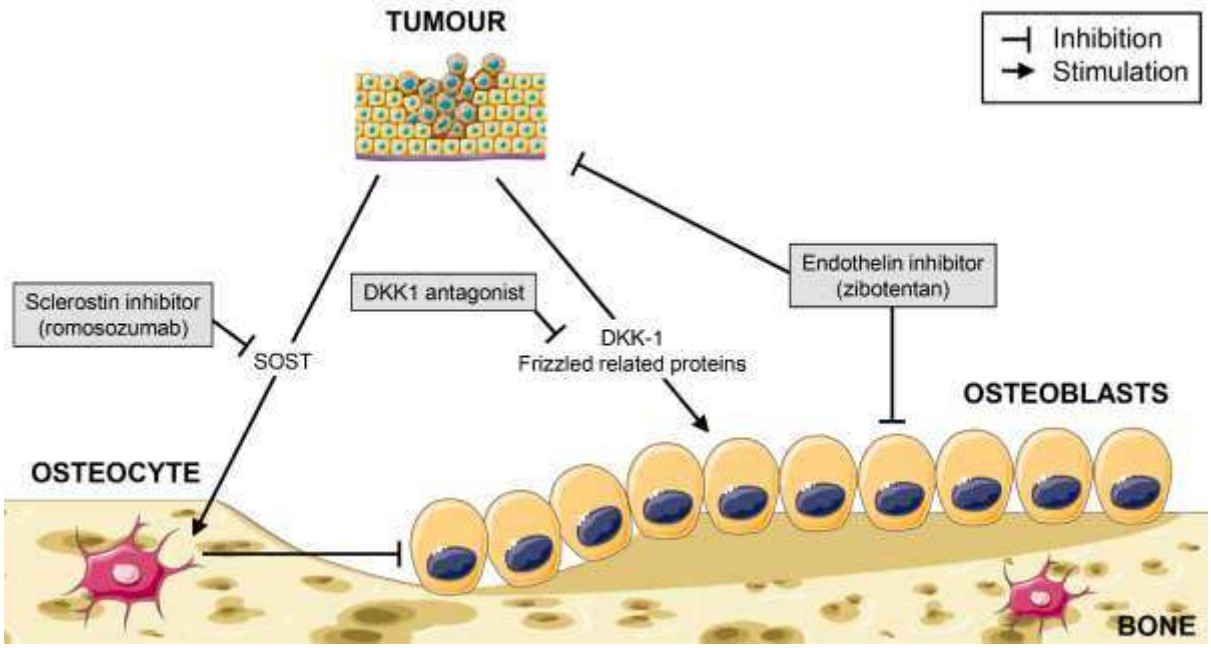

Figure 3: Summary of actionable targets in osteoblasts to promote bone formation. (Figures creative commons attribution 3.0)

Wnt inhibitors

Mechanism of action

The Wnt (Wingless-related integration site) pathway typically mediates close-range signalling between cells involving numerous processes in the body, including tissue shaping, proliferation, and metabolism. Signalling can occur through a canonical Wnt pathway (β-catenin dependent) or a non-canonical Wnt pathway (β-catenin independent). Activation of the β-catenin dependent pathway, by binding of Wnts to the Frizzled (FZD) receptors and the low density lipoprotein receptor related protein 5/6 (LRP5/6) coreceptors, results in the recruitment of dishevelled proteins (Dv1). This suppresses the β-catenin destruction complex and the stabilized β-catenin subsequently enters the cell nucleus. After binding to the TCF (T-cell factor) / LEF (lymphoid enhancer factor) transcription factor coactivator, the transcription of Wnt genes is initiated. The non-canonical pathway results in the activation of: Wnt/mTOR (as described above), Wnt/STOP (stabilization of proteins, resulting in activation of cellular processes), Wnt/JNK (leading to transcriptional activation), Wnt/PCP (important in alignment of cell polarity across the tissue), Wnt/Ca^{2+}(activation of canonical pathway or direct effect on transcription of Wnt genes) and Wnt/YAP/TAZ (activating transcription of Wnt genes).

Importantly, the Wnt pathway contributes to physiological bone mass regulation as well as many processes related to bone metastases (primary tumour dissemination, metastatic tumour dormancy, metastatic tumour outgrowth, tumour induced osteogenesis and tumour induced osteolysis). In the bone microenvironment the effects of Wnt on osteoblast function dominate, yet are complex and mediated via both canonical and non-canonical Wnt signalling. Activation of the Wnt pathway leads to bone formation, but the relative contribution of the canonical and non-canonical pathways to bone formation needs to be further elucidated. In contrast, in osteoclasts canonical signaling can either promote or inhibit osteoclast differentiation depending on the type of Wnt that serves as pathway activator, illustrating the complexity of the

role of Wnts in bone and their potential role as target for therapeutic intervention.[47-49]

Regulation of the Wnt pathway
The endogenous Wnt inhibitors Dickkopf-1 protein (DKK-1) and Sclerostin (SOST) bind to the LRP5/6 coreceptor preventing further signalling. While this coreceptor is predominantly present in canonical pathways, it also has importance in some non-canonical signalling. Secreted frizzled-related proteins (SFR) and Wnt inhibitory factor 1 (WIF1) bind directly to the Wnts, preventing binding of the ligand with the Wnt receptor. Both canonical and non-canonical pathways can be inhibited in this way.

Effect of DKK-1 and SOST on bone metastases
In preclinical models of metastatic breast cancer, DKK-1 can inhibit the growth of lung metastases. However, it also results in a 10-fold increase in the occurrence of bone metastases, without affecting primary tumour growth. This illustrates the dual role of DKK-1 in the breast cancer metastatic process. A possible mechanism may involve inhibition of Wnt/β-catenin signalling in osteoblasts resulting in a reduction of OPG secretion, the decoy receptor for RANKL. Without this decoy receptor RANKL can bind to RANK in an uninhibited way, resulting in uncontrolled bone resorption due to increased osteoclast differentiation and function.[50]
Sclerostin (SOST) expression occurs only on osteocytes and interfering with SOST produces effects that are limited to bone and does not affect tumour proliferation. Treatment with an anti-sclerostin antibody in a mouse model of multiple myeloma prevented myeloma induced bone loss, reduced osteolytic lesions, and increased bone strength and resistance to fracture. Furthermore, the use of the anti-sclerostin antibody showed a superior increase in bone strength in comparison to zoledronic acid.[51]

Endothelin inhibitors

Mechanism of action
The endothelin family consists of the small peptides ET-1, ET-2 and ET-3 (21 amino acids). ET-1 and ET-2 bind to the ET_A and ET_B receptor, while ET-3 only binds to the ET_B receptor. An increased expression of ET-1 and ET_A has been identified in renal, cervical, colon, lung, prostate, and ovarian tumours. Overall, activation of the ET pathway in cancer cells promotes tumour growth and progression, metastases, angiogenesis and proliferation. In the bone microenvironment the activation of ET-1/ ET_A results in increased osteoblast numbers and function, and a reduction in osteoclast activation and motility, which results in matrix remodelling and bone deposition.[52]

Effect of ET_A antagonists on bone metastases
Preclinical in vivo data showed reduced osteoblastic bone metastases with treatment of an ET_A antagonist, but no effects on osteolytic lesion inducing cancers were observed.[52] The effect of the ET_A antagonist zibotentan on overall survival was evaluated in a randomized controlled phase 3 trial (n=594) enrolling men with castration-resistant prostate cancer and bone metastases who were pain-free or mildly symptomatic for pain. Disappointingly, no benefit on overall survival or any secondary endpoints was observed in this study.[53]

THE EMERGING FIELD OF INNATE OSTEO-IMMUNITY AND BONE METASTASIS

The clinical observation of the sometimes long interval (up to decades) between primary cancer diagnosis and the occurrence of bone metastases has since long strengthened the hypothesis that disseminated tumourcells (DTC) can remain dormant in the bone microenvironment by means of complex interactions with the local host. The role of the innate immune system in this process has been intensively studied over the last years and these efforts may pave the way for effective immune therapies that harbor the power of the patient's own immune system to identify and clear DTCs or even bone metastases. However, current immunotherapies have only shown limited efficacy in bone probably because of low tumourimmunogenicity and tumourinduced tolerance, illustrating the need for a deeper understanding of local immune interactions in bone.[54-56]

Role of osteoblasts in tumour promotion

Intriguingly, some tumors can impact bone even without the presence of metastases. Two different in vivo mouse models of lung cancer showed increased osteoclast activity in different locations in the bones, without the presence of any metastases.[57] Further experiments also showed an increase in the number and activity of osteoblasts and neutrophils infiltrating the tumor. This was dependent on osteocalcin positive osteoblasts (Ocn^{+}) and indicates an effect of osteoblasts on the tumor. Of note, these neutrophils were $SiglecF^{high}$ (sialic acid binding immunoglobulin like lectin F) positive and express genes that promote amongst others angiogenesis, extracellular matrix remodeling, suppression of T-cell responses, tumourcell proliferation and growth.[57]

Immune checkpoints in cancer immunotherapy

The activation of T-cells requires two stimulatory signals: tumour antigen presentation by dendritic cells or macrophages, and co-stimulatory CD28 signalling on the T-cell surface by the antigen presenting cell through the CD80-86 receptors (Figure 4).[58] However, delivering the actual immune response after activation of the T-cell can be severely hampered by difficulties in infiltrating the site of bone metastasis. In addition, inhibitory cells trigger pathways after T-cell activation that can abrogate an effective anti-tumourimmune response. For example, upregulated CTLA-4 can bind with CD80-86 with a higher affinity than CD28 due to homologous resemblance, preventing the co-stimulatory signal.[59] Similarly, PD-1 is another immune checkpoint molecule that prevents over-action of the immune system by inducing apoptosis in the cells when it is activated by binding of its ligand. It is well-known that the PD-1 ligand is expressed by many cancer types and represents an important resistance pathway of tumour cells to immune mediated death. The recent clinical success of checkpoint inhibitor therapy in metastatic cancers (e.g. melanoma) using antibodies directed against CTLA-4 (e.g. ipilimumab) and PD-1 (e.g. pembrolizumab, nivolumab) receptors have showed the feasibility of these therapeutic approaches. Unfortunately not all patients will respond and responses are not always durable, suggesting that many other regulatory and escape pathways must exist. For this reason, combining multiple agents that target different checkpoints or pathways appears an attractive strategy to overcome resistance.[60]

For example, the anti-RANKL and anti-CTLA-4 antibodies have modest anti-metastatic effect when administered alone, but recent preclinical data suggests that

RANKL blockade improves the efficacy of anti-CTLA-4 antibodies against solid tumours and experimental metastases.[61] The same group also reported that RANKL blockade increases the anti-metastatic activity of antibodies targeting PD-1/PD-L1 in mouse models of melanoma, prostate and colon cancer.[62] While further clinical studies are required, a published case report of a woman with widespread bone metastatic melanoma achieving a remarkable response lasting 48 weeks with the combination treatment with denosumab (120 mg/ 4 weeks) and ipilimumab (3 mg/kg/ 3 weeks) appears to support these findings.[63]

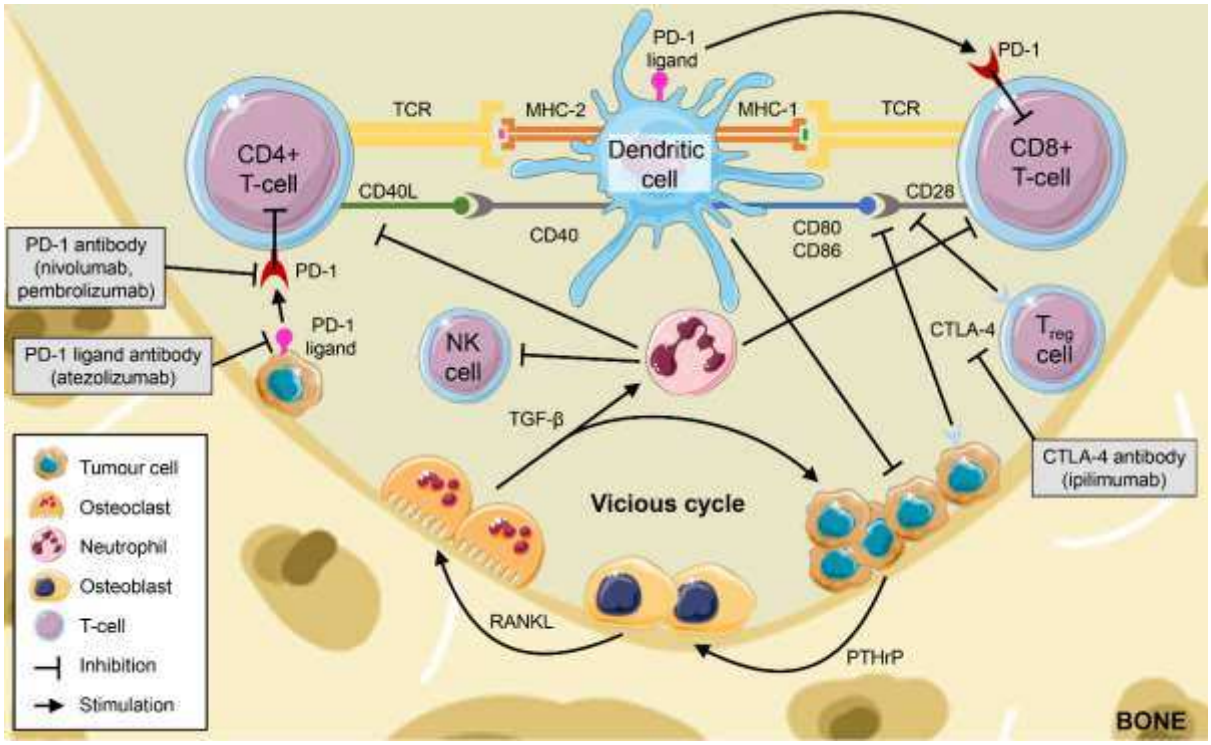

Figure 4: Overview of the complex interactions between immune cells, osteoblasts, osteoclasts, and tumour cells in the bone microenvironment and the targets for immunotherapies in cancer treatment. (Figures creative commons attribution 3.0)

TARGETING THE BONE MATRIX AND THE MICROENVIRONMENT

Nerve growth factor (NGF)

Bone pain is a common symptom in advanced cancer and can be challenging to treat. The nerve growth factor (NGF) is a member of the neurotrophin family and is important in the survival and maintenance of sensory and sympathetic neurons. The main receptor is tropomyosin receptor kinase A (TrkA) and binding of the ligand activates further intracellular signaling pathways.[64] Immunohistochemical analysis has demonstrated expression of the TrkA receptor by the majority of sensory and sympathetic nerve fibers in the bone environment and therefore makes it a potential target for treating bone pain.[64]

Indeed, in vivo preclinical breast cancer models have shown a significant reduction in induced pain and sprouting of TrkA+ and GAP43+ nerves after administration of an anti-NGF antibody, while normal nerve morphology was maintained. However, treatment with anti-NGF therapy did not impact disease progression or tumour induced bone remodelling.[65]

Transforming growth factor β (TGFβ)

TGFβ (transforming growth factor β) is important in the normal regulation of bone remodeling and maintains the balance between osteoblastic and osteoclastic activity. Cancer induced osteoclastic activity releases TGFβ in the bone environment which promotes the vicious cycle of tumor-induced bone destruction. It has been shown that increased concentrations of TGFβ are associated with poor disease prognosis, suggesting that targeting TGFβ may improve outcome.[7]

In preclinical mouse breast cancer models a decrease in tumour burden was observed in animals treated with an anti-TGFβ antibody. Furthermore, a reduction in osteoclast activity, osteolytic lesions and levels of PTHrP (parathyroid hormone related peptide) were observed as well. In contrast, the number of osteoblasts increased during treatment, supporting the ability of this treatment to abrogate the vicious cycle.[66]

Target	Blocking strategies
Osteoclast function	
Bisphosphonates	Small molecules
RANKL	Antibodies
mTOR	Small molecules
MET and VEGFR2	Tyrosine kinase inhibitors
Cathepsin K	Small molecules
Src	Tyrosine kinase inhibitors
LGR4	Antibodies
Dock5	Small molecules
Osteoblast function	
Wnt	Small molecules, antibodies
Endothelin	Small molecules, antibodies
Innate osteo-immunity	
CTLA-4	Antibodies
PD-1	Antibodies
PD-1 ligand	Antibodies
Bone matrix and micro-environment	
Nerve growth factor	Antibodies
Transforming growth factor β	Antibodies

Table 1: Summary pharmacological targets bone metastases.

CLINICAL RESULTS OF TARGETED PHARMACOLOGIC INTERVENTION IN BONE METASTASES

The previous paragraphs reviewed the different pathways and targets that are involved in normal and pathological bone remodeling and the interactions between bone and tumourcells (table 1). In the following sections a summary is provided of the main body of clinical evidence that provide the basis for the clinical use of agents that target these pathways.

Bisphosphonates

Prevention of skeletal related events (SRE) in bone metastatic disease

The benefits of bisphosphonate treatment has been evaluated in many malignancies, showing meaningful reductions in the risk of developing skeletal related events (SRE) in patients with multiple myeloma, or bone metastases from breast cancer, prostate cancer, and other solid tumors. While many bisphosphonates exist with some differences between their clinical use, zoledronic acid is probably the best studied molecule and carries the broadest label as it can be used for the prevention of SREs in all previously mentioned patient groups.

Prevention of disease progression and death

Based on tempting preclinical observations, zoledronic acid was also tested in an adjuvant setting in patients receiving curative treatment for breast cancer. Intriguingly, in post-menopausal women or when combined with ovarian suppression, the addition of zoledronic acid to endocrine treatment improved disease outcomes.[67, 68] In contrast, in patients receiving adjuvant chemotherapy after curative local treatment (AZURE trial), there was overall benefit in overall survival (HR 0.85; CI 95% 0.72-1.01), but only in the subgroup of post-menopausal women.[69] This suggests that the interplay between bone and cancer may be dependent on the endocrine milieu (and more particular estrogen levels), opening up an entirely new direction of research.

RANKL inhibitors

Prevention of skeletal related events (SRE) in bone metastatic disease

After the identification of RANKL as a potentially important therapeutic target in bone disease, the monoclonal anti-RANKL antibody denosumab was evaluated in a large number of clinical trials. In the setting of preventing SREs in bone metastatic disease denosumab has shown equal or superior efficacy compared to the bisphosphonate zoledronic acid. Firstly, a randomized control trial in advanced breast cancer patients (n=1026) comparing denosumab (120 mg SC/ 4 weeks) with zoledronic acid (4 mg IV/ 4 weeks) favoured denosumab, when comparing the time to first (HR 0.82; 95% CI 0.71-0.95) and subsequent (HR 0.77; 95% CI 0.66-0.89) SRE.[70] Also, in metastatic prostate cancer patients (n=1904) denosumab prolonged (HR 0.82; 95% CI 0.71-0.95) the time until first on study SRE compared with zoledronic acid.[71] Finally, in two randomized trials in multiple myeloma and solid tumours other than prostate and breast cancer, denosumab was found to be noninferior to zoledronic acid.[72, 73]

Prevention of disease progression and death

Having proven efficacy in the bone metastatic setting, several clinical trials have studied the potential of denosumab to prevent or delay the occurrence of bone metastases. In a large phase 3 randomized placebo controlled trial in men with castration resistant prostate cancer and unfavourable PSA kinetics (n=1432), denosumab (120 mg/ 4 weeks) increased bone metastasis free survival (HR 0.85; 95% CI 0.73-0.98) and delayed the time to first bone metastasis (HR 0.84; 95% CI 0.71-0.98) compared to placebo. However, these positive results have not led to registration for this indication because of concerns of the risk of developing osteonecrosis of the jaw.[74] In postmenopausal women with breast cancer (n=3420) receiving adjuvant denosumab (60 mg SC/ 6 months) not only delayed the time to first clinical fracture (HR 0.50; 95% CI 0.39-0.65), but also improved disease-free survival (HR 0.82; 95%

CI 0.69-0.98) compared with placebo, mirroring the results seen with zoledronic acid in this setting.[75, 76] Finally, the D-CARE study recruited women with early stage breast cancer and randomized between denosumab (120 mg SC/ 3-4 weeks 6 times + 120 mg SC/ 3 months for 54 months) and placebo given as adjuvant therapy together with chemotherapy. No benefit in the time to first bone metastases could be demonstrated with denosumab (HR 0.82; 95% CI 0.66-1.02), even though some secondary endpoints favoured the denosumab arm.[77]

mTOR inhibitors

Adding the mTOR inhibitor everolimus to the aromatase inhibitor exemestane in patients with advanced breast cancer improves outcomes compared to exemestane treatment alone. Interestingly, analyses of the profiles of bone turnover markers (e.g. bone-specific alkaline phosphatase, amino-terminal propeptide of type 1 collagen, and C-terminal cross-linking telopeptide of type 1 collagen) during treatment showed profound beneficial effects on disease progression in bone.[78] However, no further clinical development was performed specifically looking at the effects of everolimus on bone metastases in other cancers.

MET and VEGFR2 inhibitors

The results of an early phase II trial with cabozantinib in advanced prostate cancer looked extremely promising. Indeed, in a randomized controlled trial in castration-resistant prostate cancer patients (n=171) a prolonged progression free survival in the cabozantinib (100 mg/day) treatment arm was seen compared to placebo (HR 0.12; p<0.001).[79] Unfortunately these results could not be confirmed in the pivotal phase 3 trial (COMET-1) (n=1028) showing similar overall survival with cabozantinib or placebo (HR 0.90; 95% CI 0.76-1.06). Intriguingly, spectacular bone scan responses were seen with cabozantinib treatment (42% vs 3%, p= 0.001) as well as benefit in radiographic progression-free survival (HR 0.48; 95% CI 0.40-0.57).[80]

These conflicting results highlight the crucial importance of understanding the mechanism of action of novel targeted agents in order to correctly interpret the results of imaging techniques including bone scintigraphy. Indeed, the highly specific effects of these treatments can lead to unexpected behaviour. Given the observed discrepancies between scintigraphic and clinical response, it was hypothesized that cabozantinib could interfere with local bone metabolism and uncouple the tumourand bone interaction. This was indeed confirmed in an elegant preclinical study where fractures were imaged with ^{18}F-NaF-PET/CT and ^{99m}Tc-MDP scintigraphy after 7 days of treatment with cabozantinib. Even though no fracture healing was observed by that time, there was a greatly reduced uptake of the radiopharmaceutical probably by direct inhibition of osteoblast function, even though the mechanism of action needs further elucidation.[81]

Cathepsin K inhibitors

The available clinical data suggest that odanacatib has a similar efficacy to bisphosphonates in osteoporosis to increase bone mineral density and decrease the risk of fragility fractures.[82] However, concerns over an increased risk for cardiovascular events have led to the discontinuation of the clinical development of this molecule.

Src inhibitors

While dasatanib has been shown to have effects on bone metastases in patients with advanced breast cancer, this molecule has not been further studied in this setting. Indeed, in a small trial (n=25) patients were treated with dasatinib 100 mg daily and zoledronic acid 4mg IV at the start of the study. Partial response in bone was seen in 23% and the clinical benefit rate was 36%.[83]

Activin A inhibitors

Activin A is a regulator of bone remodelling and promotes osteoclast development and differentiation. Sotatercept is a decoy receptor that binds activin A with very high affinity preventing the continued loss of bone in myeloma patients with osteolytic lesions. In an early-phase clinical trial in multiple myeloma patients (n=30) sotatercept treatment resulted in an increase in bone alkaline phosphatase levels, suggesting increased osteoblastic activity. Nevertheless, these initial results need to be interpreted cautiously.[84]

CHALLENGES IN BONE METASTASES IMAGING AND TREATMENT RESPONSE

As discussed in the previous paragraphs, the mechanisms driving bone metastatic disease are numerous and complex. Progress in understanding these regulatory pathways has paved the way to new therapeutic targets in use today and many others still being actively researched. However, this mechanistic complexity also challenges the imaging methods used to assess response to these novel treatment candidates, as illustrated by the case of cabozantinib and bone scintigraphy. Together with historical insights regarding the specificity of imaging findings and the impact of confounding phenomena such as flare reaction when assessing response highlight the importance of continued validation of our techniques when new treatments are introduced. Currently, no single imaging technique is consistently superior for the assessment of metastatic bone disease across all tumour types and clinical scenarios. As will be discussed in the next papers in this special issue, both improvements in hardware and the development of new radiotracers will be required to solve the current hurdles in assessing metastatic bone disease.

CONCLUSIONS

Our understanding of the pathways driving bone remodeling in health and disease has increased considerably over the last decades, and new fields of study (e.g. osteo-immunology) are only on the verge of being discovered. These breakthroughs have already translated into meaningful new treatments for patients with bone metastatic cancer. However, important challenges remain before this condition will become curable. This is also mirrored by the challenges introduced by novel targeted agents on assessing response in bone using nuclear medicine imaging methods, which will benefit from the development of new radiotracers and techniques.

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