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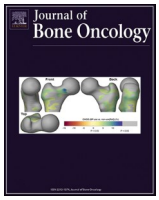

Macrophage heterogeneity in bone metastasis

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HIGHLIGHTS

- Metastasis alters bone tissue homeostasis.
- Heterogeneous macrophage populations play distinctive roles in bone metastasis.
- Promising advances were made recently in therapeutic strategies targeting metastasis associated macrophages.
- Major outstanding questions in the field of macrophages in bone metastasis call for further investigation.

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ABSTRACT

Previous studies illustrated that macrophage, a type of innate immune cell, plays critical roles in tumour progression and metastasis. Bone is the most frequent site of metastasis for several cancer types including breast, prostate, and lung. In bone metastasis, osteoclast, a macrophage subset specialized in bone resorption, was heavily investigated in the past. Recent studies illustrated that other macrophage subsets, e.g. monocyte-derived macrophages, and bone resident macrophages, promoted bone metastasis independent of osteoclast function. These novel mechanisms further improved our understanding of macrophage heterogeneity in the context of bone metastasis and illustrated new opportunities for future studies.

1. Introduction

Bone metastasis is a prominent feature in various types of cancer including prostate cancer (70–90 %), breast cancer (50–70 %), renal cancer (~30 %) and lung cancer (50–70 %), and represents the terminal stage of these malignancies [1–3]. Bone metastases frequently occur in specific regions such as spine, pelvis, and femur [4]. The pathology of bone metastasis of different primary tumours can be categorized as osteolytic, osteoblastic, or mixed. Bone lesions of breast and lung cancers predominantly display osteolytic features, while those of prostate cancer display osteoblastic or mixed features [5]. Following the onset of bone metastasis, there is a continuous process of osteolysis and expansion of tumour within bone tissue, resulting in the occurrence of skeletal-related events (SREs) including bone pain, fractures, and spinal cord compression [6]. These SREs greatly impact the life quality and survival of patients. Currently, bone metastasis remains an incurable condition, posing a significant clinical challenge. Gaining further understanding of bone metastasis is essential for the development of new therapeutic strategies.

Pioneering studies established *in vivo* bone metastasis models and illustrated the contribution of tumour cell heterogeneity in metastasis efficiency [7]. Studies in the past two decades prompt extensive research on the tumour microenvironment, i.e. stromal cells around tumour cells [8,9]. Recent studies strongly argue that intricate interactions between tumour cells and the supportive stroma play important roles in the development of bone metastasis [10–14]. For example, the osteogenic niche composed of bone-building osteoblasts was shown to be critical for the survival and colonization of disseminated tumour cells (DTCs) in the bone [12,15]. In recent decades, many studies unveiled the significant roles of macrophages in tumour cell proliferation, invasion, and metastatic progression across many cancer types [16,17]. Within the bone metastasis microenvironment, macrophages are also abundant and represent a major component of the immune system [18]. Previous bone metastasis studies largely focused on osteoclast, a macrophage subset specialized in bone resorption [19,20]. Although the accumulation of macrophages has been observed in patient samples of bone metastasis

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[21,22], their function and heterogeneity in this disease is just started to be unveiled. This review summarizes recent advances on the functions and subpopulations of macrophages in bone metastasis and discusses potential therapeutic approaches.

2. Bone marrow microenvironment

The bone tissue comprises cortical bone, cancellous bone, periosteum covering the bone surface and bone marrow. Periosteum contains a dense network of blood vessels and nerves, which allow tumour cells to enter bone marrow cavity via extravasation. Based on anatomic locations, two distinct niches hosting hematopoietic stem cells have been characterized: the *peri*-vascular niche consists primarily of bone marrow stromal cells (BMSCs) adjacent to blood vessels, and the endosteal niche consists of bone-lining, osteoblasts and osteoclasts close to bone matrix [23]. Interactions among these niche cells and hematopoietic stem cells regulate hematopoiesis bone and bone marrow homeostasis.

Upon infiltration of tumour cells, homeostasis of bone microenvironment is disrupted (Fig. 1). Tumour cells release factors, e.g. TGF-β, disturb bone reconstruction process and initiate a 'vicious circle' through the overactivation of osteoclasts to support tumour growth. Monocyte-macrophages recruited by CCL2-secreting tumour cells undergo polarization into metastasis-associated macrophages (MAMs) that promote tumour growth. G protein-coupled receptors (GPCRs) on the macrophage surface detect acidity, leading to an increased level of inducible cAMP early repressor (ICER), which in turn, increases the level of cAMP. This eventually induces macrophage polarization into an immunosuppressive status, enabling successful tumour immune evasion and metastatic growth [24,25]. Meanwhile, a high level of glycolysis in tumour cells induces alterations in Na^+ and H^+ concentrations inside and outside the cell membrane, resulting in an acidic microenvironment [26]. This together with the hypoxic environment in bone marrow may further facilitate tumour cell metastasis and osteolysis [27].

In summary, tumour cells cooperate with the tissue microenvironment in bone for survival and persistent growth following metastatic seeding. Dormancy, on the other hand, may largely be due to inefficiency in establishing this cooperation.

3. Macrophage heterogeneity in bone metastasis

Macrophages play critical roles in phagocytosis, inflammation induction, and antigen presentation, but also in inflammation resolution, immune suppression, and tissue homeostasis [28]. Tumour tissues contain macrophage subpopulations with various functions [29,30]. These macrophages, influenced by tumour-secreted cytokines, can transform into tumour-associated macrophages (TAMs) that possess protumoral and immunosuppressive characteristics [31]. Previous studies on TAMs were summarized in a comprehensive review recently [32].

In bone marrow, macrophages originate from two distinct sources. Traditionally, monocytes originated from bone marrow haematopoiesis and were believed to be the major source of macrophages across different tissues, including bone marrow. Previous studies identified a population of resident macrophages, referred to as osteomacs or bone resident macrophages, derived from embryonic progenitor cells, characterized by CD169 expression [33]. Osteomacs facilitate erythropoiesis within erythroblastic islands [33], maintenance of HSC within the perivascular niche [34] and osteoblast numbers within the endosteal niche [35]. A recent study indicated that osteoclasts can not only be derived from embryonic erythroid-myeloid progenitors and maintained through self-renewal, but also be replenished by fusion of monocytes [36].

To better understand the involvement of macrophages in different cancer types, recent studies in the past five years have utilized clinical human samples, mouse models, and relevant cell lines (summarized in Table 1). The following sections will discuss advances of major macrophage subpopulations in bone metastasis.

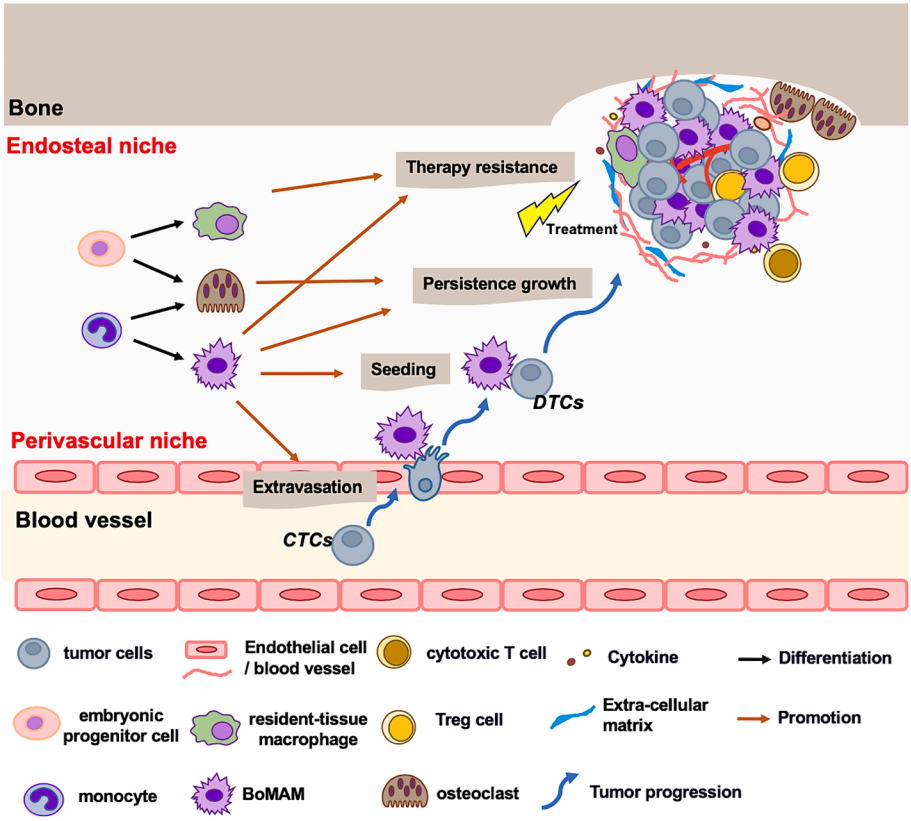

Fig. 1. Macrophage heterogeneity in bone metastasis. CTC: circulating tumour cells; DTC: disseminated tumour cells.

Table 1
Recent studies on macrophages in bone metastasis (2019–2023).

Tumor type	Model	References	Macrophages involved	Target/Drug
Mammary	Spontaneous metastasis (4T1FL cells S.Q. in BALB/c)	CD137 promotes bone metastasis of breast cancer by enhancing the migration and osteoclast differentiation of monocytes/macrophages [52]	Monocyte/ Macrophage/ Osteoclast	CD137L/CD137/Fra1; NPs-αCD137 Ab-F4/80
Mammary	Bone colonization (MCF-7, MDA-MB-231, BoM-1833 cells I.F. in CAnN.Cg-*Foxn1*^{nu} mice)	IL-11 is essential in promoting osteolysis in breast cancer bone metastasis via RANKL-independent activation of osteoclastogenesis [91]	Osteoclast	IL11/pSTAT3/c-fos/NFATc1
Mammary	Experimental metastasis (SCP2, SCP4 cell I. C. in BALB/c)	CST6 protein and peptides inhibit breast cancer bone metastasis by suppressing CTSB activity and osteoclastogenesis [92]	Osteoclasts	CTS6/CTSB/SPHK1; QLVAG-containing peptides DQ51, recombinant mutant CTS6
Mammary	Experimental metastasis (MCF7BoM2 cells I.C. in NU-*Foxn1*^{nu})	Exosomal miR-19a and IBSP cooperate to induce osteolytic bone metastasis of estrogen receptor-positive breast cancer [93]	Osteoclast	IBSP/integrin αVβ3/PTEN/ AKT; chlorogenic acid, IBSP receptor inhibitor
Mammary	Experimental metastasis (Py8119 or MDA231-BoM-1833 cells I.C. in C57BL/6J or NU(NCr)-*Foxn1*^{nu} mice)	Fam20C regulates bone resorption and breast cancer bone metastasis through Osteopontin and BMP4 [94]	Osteoclast	Fam20C/Osteopontin/BMP4
Mammary	Bone colonization (4 T1 cells I.T. in MRL/ MpJ-Fas lpr /J mice)	Osteoclast-derived apoptotic bodies inhibit naive CD8^{+} T cell activation via Siglec15, promoting breast cancer secondary metastasis [48]	Osteoclast	Siglec15/TLR2
Mammary	Experimental metastasis (MetBo2, 1833 cells I.C. in FVB/N or Il4ra$^{-/-}$;NU(NCr)-*Foxn1*^{nu} mice)	Monocyte-derived macrophages promote breast cancer bone metastasis outgrowth [22]	MDM; Bone RTM	CCR2, IL4R
Lung	Bone colonization (A549 cells I.T. in nude*)	Thrombospondin enhances RANKL-dependent osteoclastogenesis and facilitates lung cancer bone metastasis [95]	Macrophage	TSP-2/miR-486-3p/NFAT1; TSP-2 neutralizing antibody
Lung	Experimental metastasis (A549 cells I.C. in NU(NCr)-*Foxn1*^{nu} mice)	IL-20RB mediates tumoral response to osteoclastic niches and promotes bone metastasis of lung cancer [46]	Osteoclast	IL-19/JAK1/STAT3/IL-20RB
Prostate	Experimental metastasis (MycCaP-Bo cells I. C. in FVB/N mice)	Macrophages promote anti-androgen resistance in prostate cancer bone disease[21]	MDM; Bone RTM.	Activin-A/FN1/ITGA5/Src; eCF506, SRC inhibitor
Prostate	Experimental metastasis (PC3-Luc or PC3-GDF15-Luc cells I.C. in NU(NCr)-*Foxn1*^{nu} mice)	GDF15 promotes prostate cancer bone metastasis and colonization through osteoblastic CCL2 and RANKL activation[59]	Osteoclast	GDF15/GFRAL/RET/CCL2
Prostate	Bone colonization (PC3, C4, C4-2 cells I.T. in CAnN.Cg-*Foxn1*^{nu} mice)	Small extracellular vesicles deliver osteolytic effectors and mediate cancer-induced osteolysis in bone metastatic niche [96]	Macrophage	miR-152-3p/MAFB; antagomir-152-3p
Prostate	Bone colonization (PC3-Luc cells I.T in NOD/SCID mice)	CCL5 derived from tumor-associated macrophages promotes prostate cancer stem cells and metastasis via activating β-catenin/STAT3 signaling [56]	Macrophage.	CCL5/CCR5/β-catenin/ STAT3

S.Q.: subcutaneous injection; I.F.: intra-femoral injection; I.T.: intra-tibia injection; I.C.: intra-cardiac injection; MDM: monocyte-derived macrophage; RTM: resident-tissue macrophage.
*Strain unspecified.

4. Osteoclasts

In normal physiology, osteoclasts are coupled with osteoblasts to regulate the mineralization and remodelling of bone matrix. In this process, osteoclasts are in charge of bone resorption mainly through the secretion of hydrogen ions and protein hydrolases. This resorption, in turn, releases growth factors such as TGF-β and IGF-1 that stimulate the differentiation of mesenchymal cells into osteoblasts. Furthermore, osteoblasts secrete RANKL, a factor promoting osteoclastogenesis [37], which activates osteoclasts, prolongs their lifespan through cell division and generation of a distinct type of cells termed osteomorphs [38]. This cross-interaction is critical for healthy bone homeostasis.

In bone metastasis, the bone remodelling process is disrupted. In osteolytic bone lesions, tumour cells secrete numerous factors, including TGF-β, parathyroid hormone-related protein (PTHrP), interleukin-6 (IL-6), matrix metalloproteinases (MMPs), and exosomes. These factors stimulate osteoclast differentiation and activation. Consequently, the activated osteoclasts enhance bone absorption and release matrix-stored growth factors, such as bone morphogenetic proteins (BMPs), TGF-β, and insulin-like growth factor (IGF)-1. These growth factors support tumour growth and osteoblast activation, thereby promoting the secretion of more osteoclast-activation factors by both tumour cells and osteoblasts in a reciprocal manner [39]. In contrast, in osteoblastic bone metastasis, the balance shifts towards bone formation due to the secretion of osteoblast-promoting factors by tumour cells, including prostatic acid phosphatase and endothelin-1 [40]. However, osteoclast-mediated osteolysis remains essential for the enhanced osteogenic process even in osteoblastic lesions [41]. Depleting osteoclasts inhibits the development of osteoblastic lesions in LAPC-9 prostate cancer model [42] and delays the onset of bone metastases in patients [43]. Further studies demonstrated that osteoclasts in prostate cancer produce TGFβ, which leads to acetylation of Krüppel-like factor 5 (KLF5) in prostate cancer cells. This activation subsequently triggers CXCR4 expression on tumour cells, resulting in significant secretion of IL-11 in DU-145 and PC-3 models. Additionally, prostate cancer cells secrete Sonic Hedgehog (SHH), which induces peripheral osteoblasts to express high levels of IL-6. Consequently, this fosters osteoclastogenesis and further contributes to the development of bone metastatic lesions [44]. In a mouse xenograft bone tumour model with PhIP induced rat prostate adenocarcinoma tissue, osteoclasts were reported as a major source of MMP-9 that promoted angiogenesis and bone metastasis growth [45]. In bone metastasis model, A549 lung cancer cells secrete IL-20RB to stimulate osteoclasts, leading to the production of IL-19 which, in turn, promotes tumour cell proliferation by activating the JAK1/STAT3 signaling pathway [46]. Another reciprocal paracrine signaling is mediated by tumour-derived extracellular vesicles enriched with miR-378a-3p and osteoclast-derived ANGPTL2, which fosters bone growth of intra-tibially injected PC3 cells [47].

In addition to interacting with tumour cells, osteoclast-derived apoptotic bodies enriched in Siglec15 contributed to the

immunosuppression of TAMs by blocking the activation of sialylated Toll-like receptor (TLR2) on naïve $CD8^+$ T cells, which promotes secondary metastasis following bone metastasis in 4 T1 breast cancer model [48].

Furthermore, osteoclasts have been shown to contribute to bone pain by producing pronociceptive factors such as acid ion [49] and CCL2 [50], both of which activate local nociceptor terminals and cause nerve sensitization. Furthermore, activation of osteoclast leads to the destruction of bone matrix, enhancing SREs such as fractures and nerve compression. Indirectly, these events contribute to bone cancer-related pain [51].

5. Monocyte-derived macrophages

Using a novel breast cancer bone metastasis model, MetBo2, our recent study illustrated that $CD204^{high}IL4R^{high}$ macrophages, originating from $Ly6C^+CCR2^+$ inflammatory monocytes, are critical for bone metastasis outgrowth in an IL-4R dependent manner [22]. Another recent study illustrated that monocyte/macrophage- CD137 (4-1BB) signal upregulated Fra1 expression and promoted macrophage migration and differentiation into osteoclasts, which promoted osteolysis and metastatic growth of breast cancer cells 4T1FL in allograft model [52]. Additionally, bone marrow macrophages can promote dormancy of breast cancer cells in the bone marrow through exosomes. Specifically, M2-like macrophages inhibit the proliferation of MDA-MB-231 and T47D breast cancer cells and promote drug resistance through forming gap junction-mediated intercellular communication with cancer stem cells. While exosomes from M1-like macrophages are capable of reversing dormancy by activating p65-NFkB signaling of breast cancer cells [53]. However, another study revealed that peripheral blood monocytes with suppressed AMPK pathway activity could secrete factors such as MSN, ENO1, and PABPC1. These factors effectively inhibit bone metastasis growth and synergize with the anticancer drug Taxol in 231BC breast cancer subcutaneous and bone xenograft models [54].

In 2021, Kharchenko et al. published a single-cell RNA sequencing (scRNA-seq) analysis on prostate cancer bone metastasis samples obtained from patients suffering from spinal cord compression. Their data inferred that monocyte-derived TAMs express high levels of genes related to angiogenesis and immunosuppressive chemokines and low levels of inflammation related genes. Furthermore, these TAMs secreted chemokine CCL20 that interacted with CCR6 expressing-Treg and cytotoxic T cells, resulting in T cell exhaustion and promotion of bone metastases growth in an RM1 prostate cancer intracardiac injection model [55]. A separate study conducted by Xiang et al. demonstrated that TAM-derived cytokine CCL5 promotes self-renewal of prostate cancer tumour stem cells and bone metastasis through the β-catenin/STAT3 signaling pathway [56]. In a 3D co-culture model modified with basement membrane proteins, macrophages induced the production of sulfate esterase-1 (SULF1) by fibroblasts which led to a Wnt3A-driven progression of prostate cancer bone metastasis [57]. Furthermore, recent studies from our team generated a novel *in vivo* model of prostate cancer bone metastasis using MycCaP-Bo cells, a bone-homing subclones of MycCaP cells originated from Hi-Myc model. Using this model, we uncovered a novel mechanism that Activin A secreted by metastasis-associated macrophages upregulated integrin α 5 (ITGA5) and fibronectin (FN1) expression and SRC activation in tumour cells, leading to resistance toward Enzalutamide, an anti-androgen now widely used clinically [21].

6. Resident tissue macrophages (RTMs) in bone

An increasing number of studies have begun to reveal the function of resident tissue macrophages (RTMs) in bone metastasis. Previous studies reported that osteomacs accumulated in the lesions of prostate cancer and promoted woven bone formation [58]. Recent studies suggested a complicated network among prostate cancer cells, osteoblasts, and osteoclasts, in which prostate cancer cells secreted growth differentiation factor-15 (GDF-15) induced osteoblasts to produce CCL2 and RANKL. These cytokines then led to the recruitment of osteomacs and enhancement of osteolytic activity, which facilitated prostate cancer bone metastasis development [59].

Another well-characterized bone RTM is the $CD169^+$ macrophages [33,34]. Using a CD169-DTR mouse model that allows cell type specific ablation upon diphtherial toxin administration, our recent studies indicated that depletion of $CD169^+$ RTMs alone did not affect bone metastatic outgrowth of MetBo2 breast cancer model [22]. Consistently, in MycCap-Bo prostate cancer bone metastasis model, depletion of this population did not affect bone metastatic outgrowth. This indicated that this RTM population is dispensable for bone metastasis progression, at least in these models. Intriguingly, in the presence of enzalutamide treatment, depletion of CD169 RTM significantly inhibited resistance tumour growth, suggesting an important role of these cells in hormone therapy resistance [21].

7. Single cell heterogeneity of macrophages

Single-cell multi-omics technologies have provided unprecedented evidence of the cellular and molecular heterogeneity within cancer [60–62]. Some studies in the past few years have begun to use these technologies to characterize macrophage diversity in bone metastasis microenvironment. Using scRNA-seq, a recent study investigated the tumour heterogeneity of paired bone metastasis regions, involved biopsy samples and distal regions from 10 patients with prostate cancer. Unsupervised analysis showed 5 monocyte and macrophage sub-populations, including inflammatory monocytes and immunosuppressive MAMs (similar to Reg-TAMs) [55]. Using the same technology, our recent study identified 5 monocyte/macrophage sub-populations in the MycCap-Bo prostate cancer bone metastasis model, including 3 monocyte-derived macrophage (MDM) clusters, proliferating TAMs and RTMs. Notably, using genetically engineered mouse models targeting these macrophage sub-populations, our data indicated that MDMs were important for bone metastasis growth and anti-androgen resistance. In contrast, RTMs were dispensable during the metastasis process but are critical for resistance growth, which suggested an intriguing function switch of this macrophage sub-population upon treatment [21]. Furthermore, a recent paper performed single cell spatial proteomic analysis using GeoMx platform on matched primary tumour and bone metastasis samples from 21 ER + breast cancer patients, demonstrating that macrophages in bone metastasis exhibited diverse pro-inflammatory and immunosuppressive phenotypes. This study also identified a unique increase of a BMP-activated macrophage subset in bone, which was critical for bone metastasis growth in MMTV-PyMT model [63].

However, most single cell studies so far named each macrophage subcluster with one marker gene of choice, making it difficult to compare data from different studies. To address this difficulty, we recently conducted a comprehensive review and analysis of 66 single-cell multi-omics studies across 24 human cancer types with over 70 datasets containing high-quality single cell multi-omics data of TAMs. Based on this comprehensive analysis, seven sub-populations of TAMs with distinct molecular signatures were identified. Based on their molecular signature and experimentally validated characteristics by the field, we proposed a nomenclature for these TAM populations as interferon-primed TAMs (IFN-TAMs), inflammatory cytokine-enriched TAMs (Inflam-TAMs), lipid-associated TAMs (LA-TAMs), angiogenesis-associated TAMs (Angio-TAMs), immune regulatory TAMs (Reg-TAMs), proliferating TAMs (Prolif-TAMs), and tissue resident TAMs (RTM-TAMs) (Fig. 2). This could hopefully provide a framework for the field to compare future single cell data and identify new TAM populations [64] in tumour microenvironment including bone metastasis.

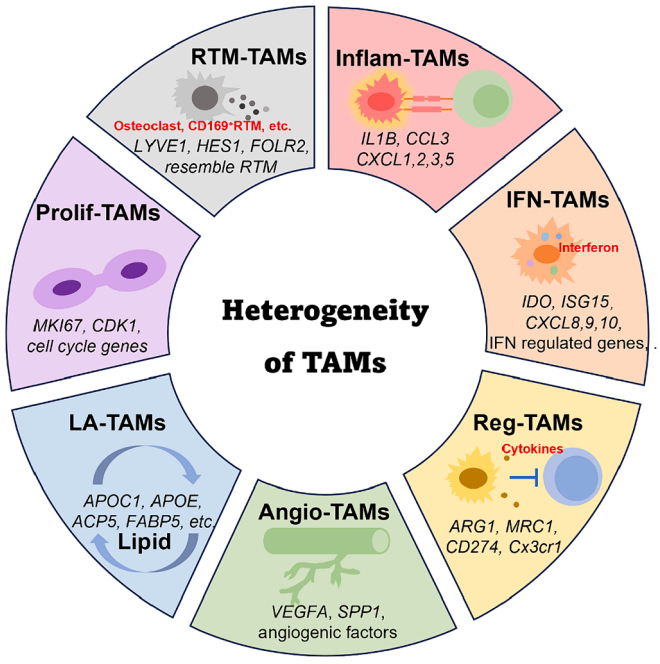

Fig. 2. Macrophage subpopulations in bone metastasis. RTM-TAMs: tissue resident TAMs; Inflam-TAMs: inflammatory cytokine-enriched TAMs; IFN-TAMs; interferon-primed TAMs; Reg-TAMs: immune regulatory TAMs; Angio-TAMs: angiogenesis-associated TAMs; LA-TAMs: lipid-associated TAMs; Prolif-TAMs: proliferating TAMs.

8. Macrophage-targeting therapies against bone metastasis

Clinical treatments for cancer bone metastases encompass a range of modalities including surgery, radiation therapy, chemotherapy, endocrine therapy, and immunotherapy [5]. Macrophage-targeting drugs, such as Denosumab targeting osteoclasts, have been used in combination to enhance therapeutic efficacy and reduce SREs [65]. As of now, macrophage-targeting therapies can be classified into several approaches aimed at decreasing monocyte recruitment and macrophage differentiation, depleting macrophages, enhancing macrophage phagocytosis, reprogramming TAMs into anti-tumour phenotype, and engineering macrophages to attack tumour [66].

Our recent studies indicated that CCL2-CCR2 mediated monocyte recruitment is critical for bone metastasis outgrowth and therapy resistance [21,22]. Pre-clinical studies highlighted the crucial role of Polycomb Repressor Complex-1 (PRC1) in inducing CCL2 secretion by tumour cells [67]. CCL2 antibodies (CNTO888), CCR2 inhibitors (MLN1202), and the small molecule PRC1 inhibitor, GW-516, are effective in diminishing monocyte recruitment and impairing bone metastasis [67–69].

CSF-1-CSF-1R and RANK-RANKL are key signaling pathways for macrophage survival and osteoclast differentiation. FDA-approved Pexidartinib (PLX3397) attenuates the growth of prostate cancer cells in bone and pathological bone remodelling by effectively targeting CSF-1R [70,71]. However, a phase II trial using Pexidartinib in late-stage castration resistance bone metastatic prostate cancer (NCT0149904) was stopped due to a lack of patient enrolment. Further clinical trials are required to confirm its *in vivo* efficacy. Denosumab is employed for its ability to impede the RANK-RANKL pathway, thus influencing the generation of osteoclasts and delaying the onset of skeletal related events caused by bone metastases [72]. Additionally, studies by the CAPIETTO group indicated that combined elimination of granulocytes and osteoclasts was necessary for the removal of established bone allograft metastases of both LLC-Fl cells and 4 T1-Fl cells [73].

Clodronate undergoes intracellular metabolism to yield non-hydrolyzable ATP analogues, exerting inhibitory effects on cellular mitochondrial respiration. Liposomal formulations of clodronate could be phagocytosed by macrophages, resulting in irreversible damage and effective clearance [74]. This drug has undergone standardization for clinical adjuvant therapy for bone metastasis [75].

Several macrophage related targets can be harnessed to treat bone metastases. The ‘don’t eat me’ signaling is mediated by CD47, which exhibits high expression on tumour cells and interacts with signal-regulated protein-alpha (SIRP-α) on macrophages, inhibiting their phagocytic activity towards these cells [76,77]. Similarly, the presence of a CD24-Siglec-10 axis between tumour cells and macrophages contributes to tumour immune evasion [78–80]. The use of neutralizing antibodies against these targets proved to be effective in enhancing phagocytosis and elimination of tumour cells by macrophages. Clinically approved or under development CD47-neutralizing antibodies include Hu5F9-G4, CC-90002, CD24 fully human monoclonal antibody BCG002, and IMM4701, a specific dual-targeting molecule for CD47 and CD24 based on the ‘mAb-Trap’ dual-antibody platform. In 2019, Philip Owens’ group conducted immunohistochemistry and digital spatial profiling (DSP) of the tumour microenvironment on patient samples with bone metastases from prostate cancer. They identified multiple immune checkpoints, including PD-L1, B7-H4, OX40L, and IDO-1, present at osteogenic lesions [81]. These key findings provide new opportunities for immunotherapeutic approaches against bone metastases.

Recent research continues to develop new therapy based on newly identified mechanisms. Reagents such as anti-CD137 nano-antibody, Fra1 inhibitors [52] and SRC inhibitors [21] have shown promise in mitigating bone metastasis or inhibiting therapy resistance. The use of a Siglec-15-specific neutralizing monoclonal antibody (S15NAB) in 4 T1 model has demonstrated improvements in binding osteoclast-derived apoptotic bodies and a reduced likelihood of further metastatic spread from bone lesions [48].

Other attractive approaches include reprogramming pro-tumour macrophages into anti-tumour macrophages through intrinsic immune domestication [82] and chimeric antigen receptor engineered macrophage (CAR-M) [83]. In a recent study, researchers constructed a bone-targeting immunostimulatory-loaded metal–organic framework (MOF) nanoparticle to repolarize bone marrow macrophages into pro-inflammatory phenotype, which significantly inhibited bone metastasis growth of MDA-MB-231 breast cancer model [84]. Meanwhile, bone-targeting nanoparticles can be delivered to the bone preferentially, which effectively reduces the side effects of the drug [85]. Although the *in vitro* and *in vivo* data are promising, these approaches have not been applied to clinical trials of treating bone metastasis yet.

Furthermore, the high infiltration of macrophages inside tumour makes them an attractive vehicle for drug delivery. A near-infrared light-activated all-in-one macrophage containing Oxaliplatin was developed for chemo-photodynamic combination therapy in 4 T1 and EMT6 breast cancer models, providing a therapeutic platform for bone metastasis treatment in situ [86]. Zhang’ s group conjugated zoledronic acid into mesoporous silica nanoparticles encapsulated with gold nanorods to achieve bone targeting and used photothermal therapy to treat MDA-MB-231 bone xenograft model [87]. Macrophages or osteoclasts are also able to efficiently phagocytose Ibandronate-loaded nanohorns and release them in the lysosomal acidic environment, thereby killing themselves and potentially facilitating the treatment of bone metastatic lesions [88]. Although these strategies using advanced materials could improve drug efficacy, their application in the clinic requires further verification.

9. Conclusion and discussion

Despite many progresses in research and clinical practice, bone metastasis remains a major challenge to patients. Recent studies employing several novel *in vivo* models illustrated new disease mechanisms involving sub-populations of macrophage (Table 1)

[21,22,89,90]. Future studies call for the employment of new tools and technologies, e.g. spatial omics, intra-vital imaging etc., and more comprehensive analysis of patient samples to better understand the function and mechanism of action of these macrophage sub-populations in human disease. This may eventually lead to effective treatment against bone metastasis.

Outstanding Questions:

1. How do macrophage subpopulations play specific roles in bone metastases and drug resistance?
2. How to target specific macrophage subpopulations to improve clinical management of bone metastases?
3. Can macrophage subtype classification be utilized for early detection of bone metastases?

CRediT authorship contribution statement

Jingxuan Guo: Conceptualization. **Ruo-Yu Ma:** Writing – original draft. **Bin-Zhi Qian:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Bin-Zhi Qian reports a relationship with Hanall Biopharma Co Ltd that includes: consulting or advisory. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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