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Review

Osteoimmunology in bone malignancies: a symphony with evil

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ABSTRACT

Bone marrow is pivotal for normal hematopoiesis and immune responses, yet it is often compromised by malignancies. The bone microenvironment (BME), composed of bone and immune cells, maintains skeletal integrity and blood production. The emergence of primary or metastatic tumors in the skeletal system results in severe complications and contributes significantly to cancer-related mortality. These tumors set off a series of interactions among cancer, bone, and immune cells, and disrupt the BME locally or distantly. However, the drivers, participants, and underlying molecules of these interactions are not fully understood. This review explores the crosstalk between bone metabolism and immune responses, synthesizing current knowledge on the intersection of cancer and osteoimmune biology. It outlines how bone marrow immune cells can either facilitate or hinder tumor progression by interacting with bone cells and pinpoints the molecules responsible for immunosuppression within bone tumors. Moreover, it discusses how primary tumors remotely alter the BME, leading to systemic immune suppression in cancer patients. This knowledge provides critical rationales for emerging immunotherapies in the treatment of bone-related tumors. Taken together, by summarizing the intricate relationship between tumor cells and the BME, this review aims to deepen the understanding of the diversity, complexity, and dynamics at play during bone tumor progression. Ultimately, it highlights the potential of targeting bone-tumor interactions to correct aberrant immune functions, thereby inhibiting tumor growth and metastasis.

1. Introduction

Cancer remains a major global health threat and the second leading cause of death.[1] In addition to genetic mechanisms, the interaction between cancer cells and their surrounding microenvironment is critical for disease progression.[2] The tumoral microecosystem comprises an amalgamation of malignant and non-malignant cells, such as fibroblasts, endothelial cells, and immune cells. The immune compartment, notable for its diverse composition and functional plasticity, can act as either a "fertilizer" or "herbicide" of tumor cells depending on the context. Therapies leveraging the immune system have emerged as a revolutionary advancement for the clinical management of cancers. Yet, some patients remain unresponsive or develop resistance, indicating the urgent need to comprehend the intricacies of tumor-immune crosstalk.

Hematopoietic stem cells (HSCs) sit at the apex of hematopoiesis, giving rise to all the immune and blood cells in the human body. Hematopoiesis begins in the yolk sac during embryonic development and transitions to the bone marrow after birth. In adults, bone marrow houses and sustains most HSCs, and therefore plays a crucial role in immune cell development and regulations (Fig. 1).[3] Bone marrow HSCs occupy specialized niches—endosteal or perivascular—that regulate HSC functions through various communication mechanisms.[4,5] Growing evidence indicates that dynamic remodeling of these niches can significantly influence HSC behavior under both normal and stress conditions.[6] Furthermore, the interaction between bone and immune system is bidirectional, with aberrant immune responses leading to pathological bone remodeling.[3,7] Thus, the intertwining of bone and immune biology is fundamental in maintaining tissue homeostasis and influencing disease progression locally and systemically.

Interestingly, skeletons are also susceptible to primary malignant bone neoplasms and metastatic disease. Primary malignant bone tumors mainly affect adolescents and young adults,[1,8] accounting for about 1% of all cancer cases worldwide. Though primary bone cancers are relatively rare, tumors arising from breast, prostate, lung and kidney fre-

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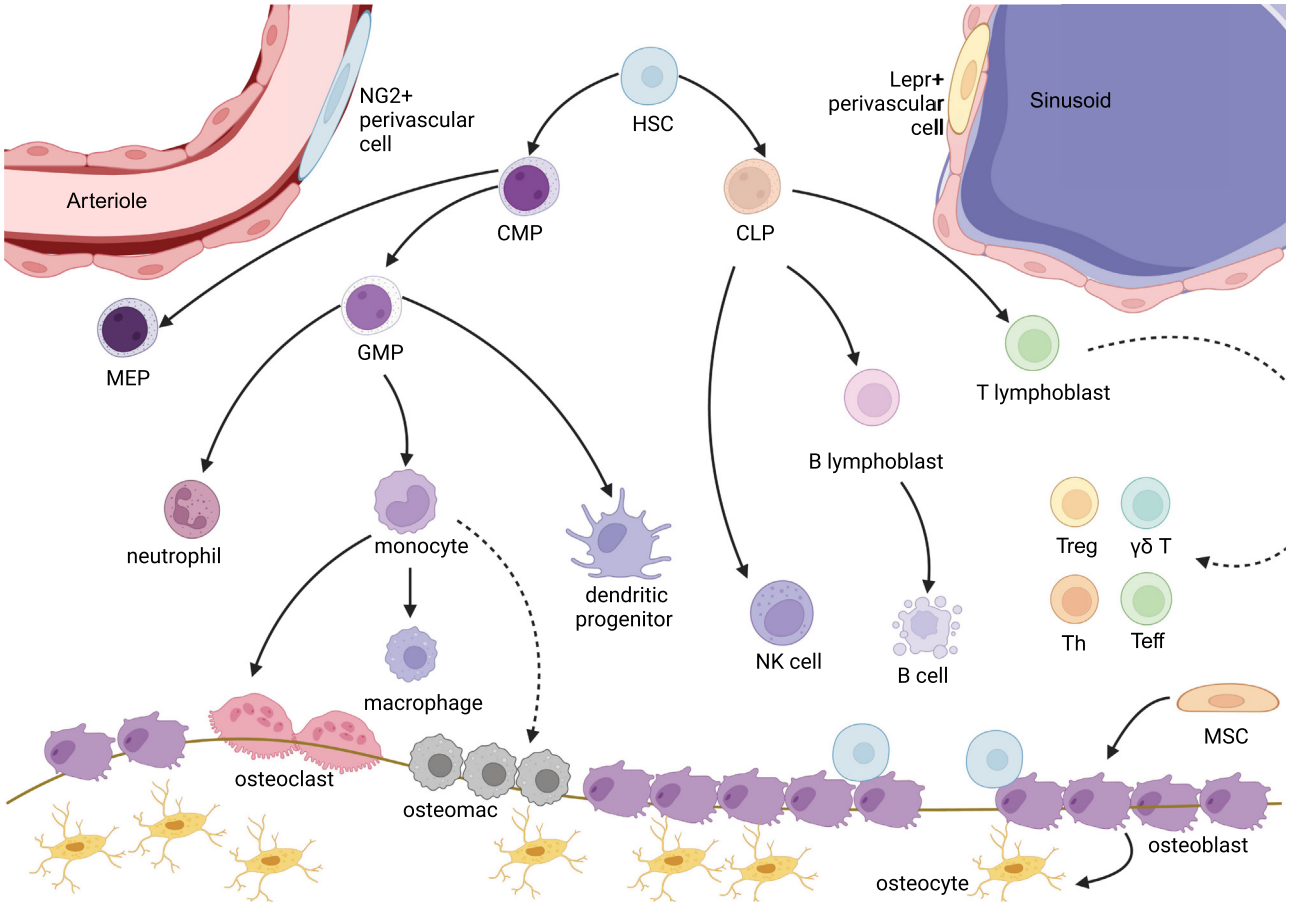

Fig. 1. Schematic of bone marrow hematopoiesis at steady state. HSCs residing in the bone marrow's specialized microenvironments, are influenced by various bone marrow cells. HSCs differentiate into CMPs and CLPs, marking the start of a differentiation cascade that forms diverse bone marrow cell types. CMPs develop into GMPs and MEPs, where GMPs differentiate into neutrophils, dendritic progenitors, and monocytes — the latter maturing into osteoclasts and macrophages. Meanwhile, CLPs evolve into NK cells, B lymphoblasts, and T lymphoblasts, with B lymphoblasts becoming B cells within the bone marrow. The lymphoid progenitors then migrate to the thymus for maturation into T cells, including effector, helper, and $\gamma\delta$ T cells, which subsequently recirculate to peripheral tissues, including the bone marrow. MSCs, precursors to osteogenic, adipogenic, and chondrogenic lineages, are found in the bone marrow's perivascular stromal fraction. They play key roles in regulating HSCs and hematopoiesis. MSC-derived osteoblasts, alongside HSC-derived osteoclasts, maintain bone remodeling balance. This dynamic interplay between skeletal and immune systems underlines the critical aspects of bone immunology. CLPs, common lymphoid progenitor cells; CMPs, common myeloid progenitor cells; GMPs, granulocyte-macrophage progenitor cells; HSCs, hematopoietic stem cells; MEPs, megakaryocyte-erythroid progenitor cells; MSCs, mesenchymal stem cells; NK, natural killer.

quently spread to the bones, making them the most preferred site for metastatic diseases.[9,10] Any bone can be susceptible to bone tumors, coming with serious symptoms like bone pain and fractures, and require systemic treatments for optimal clinical management.[9] While the majority of patients with primary bone cancers can survive over five years with proper treatments, therapeutic approaches for bone metastasis have seen limited progress over the past few decades.

Significant interconnections exist between bone biology, immunology, and cancer biology. Understanding how skeletal homeostasis and pathology influence the onset of bone tumors, including the spatial-temporal changes in immune and bone cells during bone tumor development and their impact on tumor growth, is crucial for devising effective treatments for bone-related tumors. In recent years, approximately 4000 articles exploring the interaction between the bone and immune systems have been published annually, marking a fourfold increase since the early 2000s (Fig. 2). Similar trends are observed in publications on bone malignancies and studies of the bone microenvironment. This surge indicates the growing interest and evolving nature of these fields over the past decades. These studies provide critical insights into how immune cells can either promote or inhibit tumor progression within the bone microenvironment. In this review, we aim to offer a concise overview of the immunoregulation of bone homeostasis and malignancy. We start by outlining the mutual regulatory mechanisms between bone and immune cells, followed by discussing the most recent laboratory and clinical efforts in investigating the roles of key immune populations in bone-related cancers. We especially emphasize the remote crosstalk between bone and tumors of other tissues. Lastly, we briefly explore current therapies and emerging strategies to leverage the immune system in combating bone malignancies.

2. Bone-immune crosstalk at steady state

The skeleton is an integral part of the human body, playing a pivotal role in movement support, organ protection, mineral homeostasis, and the production of blood and immune cells.[11] Bone tissue comprises extracellular matrix, mainly collagen and calcium hydroxyapatite, along with osteoblasts, osteoclasts, and osteocytes. Meanwhile, bone marrow within bone cavities is filled by hematopoietic and stromal components. The hematopoietic compartments include hematopoietic stem and progenitor cells, as well as various mature immune cells. The bone marrow stroma consists of endothelial cells and cells derived from mesenchymal progenitor cells, including osteoblasts, adipocytes and chondrocytes. Given the shared signaling networks of cytokines and chemokines between the bone and immune compartments,[12] close interactions exist

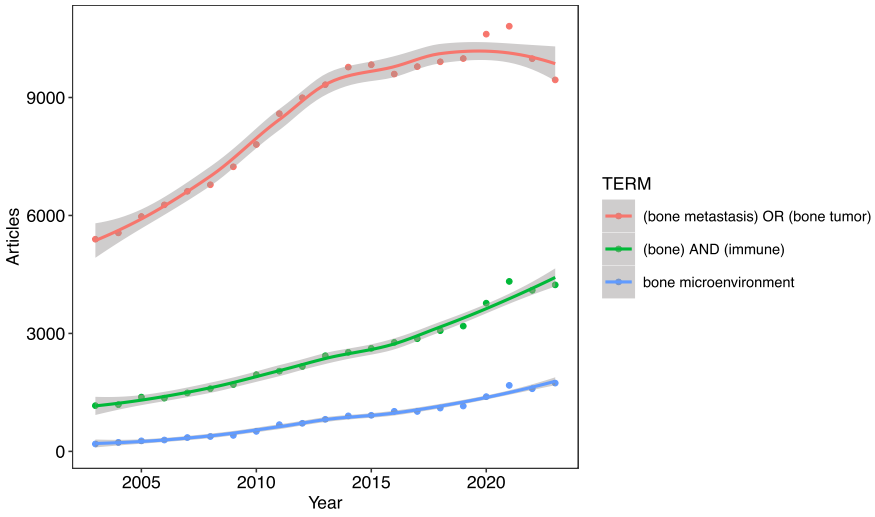

Fig. 2. Annual number of articles including relevant terms published from 2003 to 2023. This graph illustrates the annual number of articles published on various bone-related research topics, including 'bone metastasis' or 'bone tumor', 'bone' and 'immune', and 'bone microenvironment', over the period from 2003 to 2023. Data were retrieved from PubMed (https://www.ncbi.nlm.nih.gov/pubmed/).

between two parts and shape a dynamic bone environment.[3] This concept, known as 'osteoimmunology', was introduced in 2000 to describe the immunoregulation of bone pathology[13] and has since expanded to encompass reciprocal influences between the bone and immune systems in both health and disease.

2.1. Skeletal regulations of immune system

A stable bone microenvironment is essential for immune system functions. Bone cells contribute to immune cell production, maturation, trafficking, and the regulation of immune tolerance. On top of these functions, bone marrow microenvironments actively regulate HSCs. The hematopoietic stem and progenitor cells (HSPCs) are not randomly distributed in the bone marrow but reside in specialized compartments, such as endosteal regions or areas adjacent to bone marrow vessels. Endosteal niches, mainly composed of osteoblasts, and perivascular niches, consisting of endothelial cells and surrounding mesenchymal stem cells (MSCs), maintain HSCs through various molecules like BMPs, Jagged1, SCF1, and CXCL12[6]. Alterations in niche components directly or indirectly influence the HSC pool size and have varying impacts on mature blood cells. For example, selective blockade of BMP signal by inactivation of BMP receptor type IA in Mx1-cre labeled lineage significantly increases osteoblast numbers in trabecular bone regions and doubles c-Kit$^+$ HSPCs in bone marrow, while shows minimal effects on differentiated cell lineages.[14] In contrast, conditional ablation of developing osteoblasts by ganciclovir in Col2.3ΔTK mouse models results in a notable loss of HSCs and their progeny cells.[15,16]

Bone cells can also directly regulate the proliferation and maturation of distinct immune cell lineages. For instance, IL-19 is primarily secreted by osteocytes in bone tissues, which is essential for granulopoiesis and neutrophil development. Activation of mTORC1 selectively in osteocytes significantly boosts IL-19 secretion, promoting neutrophil formation through IL-20Rβ/STAT3 signaling without impacting other hematopoietic lineages.[17] Interestingly, Yu et al. reported that Ocn+ osteoblasts are pivotal for lymphoid cell maturation by producing Notch ligand DLL4, which activates Notch signaling in T cell precursors that guide their thymic homing.[18] Selective depletion of DLL4 in Ocn+ cells did not affect the normal thymic function but reduced the number of T cell precursors in the thymus and mature T cells in peripheral blood. B cell maturation has also been found to be impaired in osteoblast-deficient bone marrow,[18,19] supporting the critical role of bone homeostasis in immune cell functions.

Bone microenvironment also maintains physiological immune tolerance and restricts pathological immune responses. During infection or inflammation, cytokines produced by stromal cells increase significantly and cause profound remodeling of the bone microenvironment,[20] leading to diseases such as osteoporosis. Regulatory T cells (Tregs), which constitute about 30% of bone marrow CD4+ *T* cells, protect the structural and functional integrity of the bone and bone marrow through controlling inflammation and other non-immune functions.[21] Bone marrow resident Tregs have been suggested to exhibit distinct phenotypic features as compared to their counterparts in other tissues. They are marked by expression of HSC marker CD150 and often found in close proximity to HSCs in bone marrow.[22] The traffic of bone marrow Tregs relies on the chemokine CXCL12,[23] secreted primarily by LepR+ mesenchymal stromal cells.[24] Intriguingly, IL-10 secreted by Tregs directly acts on the IL-10 receptor (IL-10R) on MSCs and alters their phenotypes to support HSPC expansion under stress conditions,[25] indicating a regulatory loop between Tregs and MSCs in the bone marrow. Acute immune reactions, such as those seen in systemic infections like sepsis, significantly deplete osteoblasts.[26] This depletion, driven by elevated G-CSF levels during sepsis, diminishes IL-7 production and lymphoid progenitors in the marrow, contributing to sepsis-induced lymphopenia and its associated high mortality rate.[26] However, treatment with osteoblast-stimulating parathyroid hormone can replenish osteoblasts and IL-7 levels, ameliorating lymphopenia and extending survival in septic mice. These observations underscore the bone microenvironment's critical role in ensuring effective immune responses during pathological conditions.

2.2. Immunoregulation of bone development and homeostasis

Bones are continuously remodeled throughout life. This dynamic process involves the removal of old or damaged bone and the formation of new bone to maintain skeletal integrity and mineral homeostasis. Although there are debates on defining the exact steps of the bone remodeling cycle, it generally entails the resorption phase in which osteoclasts break down bones, followed by a formation phase in which osteogenic cells deposit new bone on the resorbed surface. The osteoclastic and osteoblastic activities are tightly coupled by various local and systemic factors during the bone remodeling cycle. Immune cells influence bone health primarily through their involvement in this remodeling process.

As the primary cells responsible for bone resorption, osteoclasts are derived from the monocytic lineage of hematopoietic cells in the bone marrow.[27] Therefore, they share common origins and regulatory signals with certain immune cells. For instance, pro-inflammatory cytokines like IL-1, IL-6, and TNF-α from macrophages or other immune cells can activate downstream signaling pathways like Akt, MAPK and JNK in osteoclast progenitor cells to promote osteoclast maturation.[4] In autoimmune arthritis, engagement of antigen receptor in activated T cells also induces the secretion of receptor activator of nuclear factor kappa-B ligand (RANKL),[28] which is a central molecule for osteoclast differentiation and activation. Conversely, other immune cytokines like interferons

(IFNs), IL-3, IL-4, and IL-12 inhibit osteoclastogenesis.[29] Interestingly, activated T cells also secrete IFN-γ and IL-3, exerting a Janus-faced role in bone remodeling regulation.[30] The lineage heterogeneity or dynamics of immune cells may contribute to their diverse functions in regulating bone remodeling. For instance, in mouse model of palatal expansion, an initial increase in type 1 helper T (Th1) cells and type 17 helper T (Th17) cells promotes osteoclastogenesis, followed by the recruitment of Tregs, which inhibits Th1 and Th17 cells and indirectly down-regulates osteoclast activities.[31]

Osteogenic lineages originate from MSCs in the bone marrow. Despite ongoing debates regarding the specific markers of osteogenic MSCs, they are generally believed being enriched in the perivascular stromal fraction.[32,33] Inside the bone remodeling compartment, angiogenesis actively occurs and enables the recruitment of these osteogenic progenitors, thereby creating a milieu for direct interactions between immune cells and osteogenic cells. Previous studies have highlighted the effects of cytokines released by immune cells on osteoblastic cells. For example, macrophages promote MSC differentiation or osteoblast proliferation by releasing factors such as PGE2, oncostatin M, BMP2, BMP4 and TGF-β, and consequently enhance bone mineralization.[34,35]

The balance of bone remodeling is disrupted by abnormal immune cell activities in certain inflammatory conditions and infections. In rheumatoid arthritis (RA), cytokines from Th17 cells either directly target monocytes or induce the synovial fibroblasts to secrete RANKL, both actions promoting osteoclastogenesis and resulting in inflammation-associated bone destruction.[36,37] Additionally, these cytokines stimulate the formation of neutrophil extracellular traps (NETs) that further intensify the inflammatory response within RA tissues.[38,39] Thus, within the bone marrow, the activities of immune cells and bone cells are closely tied in most scenarios, which also holds true for cancers that directly impact the bones.

3. Osteoimmunomodulation of primary bone cancers

Primary bone cancer is relatively rare, with osteosarcoma being the most prevalent form. This cancer typically arises from the bone-forming cells and is most often found at the ends of long bones in children and teenagers.[8] Tumor-infiltrating immune cells significantly impact the development and progression of osteosarcoma and other primary bone tumors.[40] In the following section, we will discuss the immunoregulatory mechanisms of primary bone cancer and focus particularly on osteosarcoma due to its unique position among primary bone malignancies.

The infiltration and activation of cytotoxic lymphocytes are pivotal in anti-tumor immune responses. Accumulating evidence suggests that T lymphocyte infiltration is higher in osteosarcoma samples than in normal tissues.[41] In some patient cohorts, increased infiltration of cytotoxic T cells is associated with improved clinical outcomes.[42,43] However, the relationship between $CD8^+$ *T* cell infiltration and clinical outcomes in high-risk osteosarcoma patients remains controversial,[44] pointing to potentially distinct functional states of tumor-infiltrating T cells in these patients. Studies have shown that the infiltration of lymphocytes correlates with the expression of exhaustion markers, such as PD1 and PD-L1, as well as reduced T cell clonality within osteosarcoma microenvironment.[41,45] In line with the exhausted phenotype, $CD8^+$ *T* cells infiltrating osteosarcoma exhibit diminished cytotoxic cytokine secretion,[43,45] supporting their defective cytotoxic response. Exhaustion signatures in infiltrating lymphocytes are significantly higher in recurrent chordoma, a rare bone cancer, compared to primary tumors.[40] This suggests a progressively deteriorating adaptive immune response against tumor cells as the disease advances. Meanwhile, the presence and suppressive functions of Tregs are elevated in osteosarcomas.[46,47] Consequently, it has been suggested that the decreased ratio of $CD8^+$ *T* cells to Tregs, rather than the infiltration of either population alone, may better predict clinical outcomes in osteosarcomas.[42,46] Exosomes from osteosarcoma cells contain higher levels of immunosuppressive proteins than those from normal bone-forming cells and are more potent in inhibiting the proliferation of effector T cells and inducing regulatory T cell phenotypes,[48] providing a potential mechanistic explanation to the above clinical observations.

The importance of natural killer (NK) cells in controlling osteosarcoma progression and metastasis is increasingly recognized. NK cells are consistently present in osteosarcomas, and their infiltration correlates with tumoral expression of PD-L1.[49] Unlike T lymphocytes, the osteosarcoma-infiltrating NK cells appear to retain intact function, making them promising candidates for immunotherapy against osteosarcoma.[50,51] Nonetheless, osteosarcomas have developed various strategies to evade NK cell-mediated immunosurveillance. For example, TGF-β inhibits NK cell activity,[52] and osteosarcoma can secrete TGF-β directly[53] or release it from the bone matrix when tumors degrade bone.[54] Furthermore, osteosarcomas express higher level of a soluble NKG2D ligand, MHC class I chain-related molecule A (MICA), than benign tumors or healthy bone tissues.[55] Soluble MICA decreases NKG2D expression on NK cells[56] and likely enables osteosarcoma cells to escape NK cell-mediated killing. Restoring these compromised recognition mechanisms on NK cells could improve their therapeutic potential against osteosarcoma.

To date, research on the role of B cells in osteosarcoma is limited. In patient samples, an increase in B cell infiltration has been unexpectedly linked to osteosarcoma progression,[57] challenging the traditional belief that B cells primarily support T cell-mediated tumor cell killing. This association is further supported by the correlation between expression of B cell lineage marker MEF2C and poorer prognosis in TCGA osteosarcoma patient cohort.[58] Interestingly, the same study also identified two other B cell markers associated with improved clinical outcomes, indicating a multifaceted role of B cells within the osteosarcoma microenvironment. More in-depth investigations are needed to clarify the precise contributions of B cells to osteosarcoma pathogenesis.

Macrophages are the most abundant immune cells in the osteosarcoma microenvironment. Analogous to tumor associated macrophages (TAMs) in other cancers, osteosarcoma-infiltrating macrophages are traditionally classified into two distinct polarization states: the anti-tumor M1 and the pro-tumor M2 phenotypes. Emille Buddingh and colleagues noted an upregulation of macrophage-related genes in osteosarcoma patients without metastasis compared to those with,[59] indicating a potential anti-metastasis function of TAMs in osteosarcomas. Their analysis indicated both M1 and M2 macrophages were present in these samples, but surprisingly, the total count of TAMs was associated with the favorable clinical outcomes, challenging a universally pro-metastatic role of TAMs across cancers. Conversely, other studies argue against the antimetastatic role of TAMs in osteosarcoma. Increased TAM infiltration has been observed in lung metastasis compared to primary osteosarcomas in both preclinical models and patient samples and demonstrated to promote osteosarcoma metastasis through multiple modes of action.[60–62] Specifically, TAM secretome enhances the migration and invasion capacities of osteosarcoma cells by activating COX2 and STAT3 pathways.[60] Additionally, osteosarcoma cells can induce a metastasis-promoting M2-like phenotype in TAMs[61,62] which in turn enhance tumor angiogenesis and T cell suppression. Furthermore, adjuvant chemotherapies may reprogram osteosarcoma-infiltrating macrophages to secrete more IL18, facilitating immune evasion through the LAT2-mTORC1-CD47 axis.[63] These findings highlight the complex and diverse functions of TAMs in osteosarcoma.

In addition to TAMs, myeloid-derived suppressor cells (MDSCs) are critical in restricting anti-tumor immune responses in osteosarcoma. MDSCs represent a heterogeneous population of immature bone marrow-derived myeloid cells that can give rise to monocytes, macrophages, neutrophils, mast cells, and others. While differentiated myeloid lineages have context-dependent roles in most cancers, MDSCs uniformly exert immunosuppressive effects by inhibiting T cell proliferation and function, primarily by producing reactive oxygen species (ROS) or nitric oxide (NO) and increasing arginase activity.[64] MDSCs are

divided into two morphologically and phenotypically distinguishable subsets: monocytic (M-MDSCs) and polymorphonuclear (PMN-MDSCs), each utilizing distinct mechanisms to suppress effector T cell activity.[65] In preclinical models and patient samples, osteosarcomas are highly infiltrated with MDSCs, and their infiltration is associated with worse clinical outcomes.[43,66] To date, the osteosarcoma-specific mechanisms driving MDSC expansion and activity are insufficiently studied. Emerging research from preclinical models suggests that dysregulation of IL-1 family cytokines, including IL1β and IL-18, may underlie the accumulation and suppressive properties of MDSCs in osteosarcomas.[67,68]

The involvement of other mature myeloid cells, including neutrophils, monocytes, dendritic cells, and mast cells, in osteosarcoma development is debated, with evidence for both pro-tumor and anti-tumor effects. On one hand, elevated neutrophil-to-lymphocyte ratio or monocyte-to-lymphocyte ratio is generally predictive of worse clinical outcomes in osteosarcoma patients,[69,70] implying these cells may favor tumor progression. Similarly, regulatory dendritic cells are enriched in osteosarcoma and associated with decreased survival rates,[49] suggesting their pro-tumoral function. On the other hand, innate immune cells are known to possess anti-tumor capabilities. The antitumor function of neutrophils is well-documented in various cancer types.[71,72] However, their role in osteosarcomas remains less clear. Dendritic cells, known for their effectiveness in antigen presentation, are being actively evaluated for developing osteosarcomas vaccines.[73,74] Resolving these controversies requires further studies into the dynamics and interactions of immune cells within the osteosarcoma microenvironment.

4. Immunoregulation of bone metastasis

The skeleton is a common site for metastasis in multiple types of cancers.[75,76] Bone metastasis is particularly prevalent in breast and prostate cancers, accounting for about 60% and 90% of metastatic cases,[77] respectively, and thus surpassing metastasis to any other organ. Despite clinical progress, bone metastasis is still incurable and represents a leading cause of mortality in patients, causing severe skeletal-related events like fractures, bone pain, and hypercalcemia.[77] Moreover, bone metastasis is linked to an increased risk of subsequent metastasis to visceral organs.[77,78] Our recent studies indicate that the bone microenvironment reprograms disseminated tumor cells (DTCs) to enhance their metastatic potential and resistance to therapies, and promote further spread to other tissues.[79,80] Therefore, delineating the crosstalk between metastatic tumor cells and bone microenvironment is imperative, which will lay the foundation for developing new therapies for bone metastasis.

Like metastasis to other organs, metastasis to bones involves a sequence of biological processes: tumor invasion and intravasation into the bloodstream, circulation, extravasation into the bone marrow parenchyma, and secondary tumor outgrowth within the bone.[76] Once disseminated tumor cells seed in the bone, they must adapt to diverse microenvironmental challenges to survive and colonize in bones, sometimes entering a dormant state or remaining as micrometastases for prolonged periods before evolving into clinically detectable metastases. Early-stage bone metastases are influenced by various bone marrow components, including endothelial cells, perivascular MSCs, osteoblasts, bone matrix, etc.[81–86] In the advanced stage, a forward loop is established between tumor cells, osteoblasts, and osteoclasts, known as the vicious cycle of bone metastasis, which fuels the tumor growth and disrupts bone homeostasis.[87] Emerging evidence pinpoints a critical role of immune cells in modulating bone metastasis, which will be summarized in the subsequent sections.

4.1. Lymphocytes

Lymphocytes exhibit a dual-faced role in the development of bone metastasis. In breast cancer patients, analysis of bone marrow immune cells reveals a decrease in total T cells but an increase in activated T cells and NK cells as well as their memory fractions in metastatic cancer patients.[88] This suggests a dynamic shift in the composition and function of bone marrow lymphocyte as bone metastasis progresses. The anti-bone metastasis functions of lymphoid cells primarily involve direct elimination of tumor cells and can be influenced by other bone microenvironment components. An excellent example is that osteoclasts release TGF-β from the bone matrix, which can suppress the proliferation and function of T and NK cells.[52,89] In ER positive breast cancer, tumor-derived SCUBE2 promotes the formation of osteoblastic niches and protects tumor cells from NK cell-mediated cytotoxic activities by enhancing collagen deposition.[90] Thus, targeting these non-immune components could theoretically potentiate lymphocytes to eradicate bone metastases. However, the pro-bone metastasis role of lymphocytes has also been implicated in previous studies, mainly through their effects on bone remodeling. For example, T cell activation in arthritis is known to increase its production of RANKL that directly binds RANK on osteoclast precursors to activate osteoclast differentiation and cause bone loss.[91] This T cell-dependent osteoclastogenesis has also been reported in bone metastasis.[92,93] Yet, cytokines secreted by activated T cells like IFN-γ inhibit osteoclast differentiation and prevent bone loss caused by metastatic tumors.[94,95] Intriguingly, a recent report by Danna Arellano et al. suggests that suppressed T cells, rather than activated ones, promote osteoclast formation and bone metastasis.[96] Further studies are needed to clarify the precise roles of bone metastases-infiltrating T cells.

Regulatory T cells contribute to the pro-metastatic functions of lymphocytes in bone metastasis. Although they represent a small fraction of $CD4^+$ *T* cells, Tregs are significantly increased in bone metastases from prostate and breast cancer.[97,98] Beyond their established immunosuppressive effects, Tregs also secrete large amounts of RANKL to enhance the metastatic potential of breast cancer cells.[99] Given the potent effect of RANKL in osteoclastogenesis, it is perceivable that increased Treg infiltration in bone metastasis might support the osteoclast-driven vicious cycle by secreting RANKL. However, a previous study proposed that Tregs can inhibit T-cell-driven osteoclastogenesis and contribute instead to the osteoblastic phenotype of prostate bone metastasis.[97] Whether these seemingly contradictory findings is due to differences in experimental models or specific Treg subpopulations remains to be determined.

Other T cell subsets also take part in creating the immunosuppressive milieu within bone metastases. For example, Th17 subsets are prevalent in bone metastases of castration-resistant prostate cancers (CRPC).[100] In soft tissues, immune checkpoint therapies expand Th1 cells to achieve an optimal response. However, in CRPC bone metastases, Th1 cells are absent and the numbers of Th17 cells increase further upon such therapy, leading to diminished clinical responses.[100] Correspondingly, elevated levels of IL-17, mainly produced by Th17 cells, have been detected in the bone marrow and are associated with increased bone metastasis in arthritis mouse models with mammary carcinoma.[101] Tumor-derived factors and other tumor-infiltrating cells promote IL17 and Th17 cell proliferation,[102] which counteracts the effects of IFN-γ and stimulates osteoclastogenesis,[103] indicating Th17-IL17 axis is a promising immunotherapeutic target for bone metastasis treatment.

4.2. MDSCs

Immunosuppressive mechanisms mediated through MDSCs are instrumental in cancer metastasis. Their accumulation has been observed in bone metastases of breast cancer, prostate cancer, and multiple myeloma.[104–108] Soluble factors released by bone metastatic tumor cells, such as G-CSF, VEGF, IL6, and TNFα, are known to attract and expand MDSC populations. Specifically, tumors with a propensity for bone metastasis secret higher levels of G-CSF and thus recruit more MDSCs compared to non-bone metastatic tumors.[109] Additionally, the activities of other non-tumoral cells within bone microenvironment influence MDSC expansion. For instance, CXCL5 from bone marrow macrophages

and DKK1 from osteoblastic cells have been shown to enhance MDSC recruitment or accumulation in bone metastases.[108,110]

MDSCs exert their pro-bone metastasis functions through multiple mechanisms. They suppress both innate and adaptive immune responses, which has been excellently reviewed by many other groups.[111–113] For sake of brevity, we will not elaborate this topic herein. Evidence from previous studies using immune-deficient models shows that the growth of xenograft tumors including bone metastasis is reduced following MDSC depletion, highlighting the immunosuppression-independent, pro-metastatic functions in MDSCs.[114,115] Further evidence found that secreted factors from MDSCs directly promote bone metastasis, as the proliferation of tumor cells is enhanced *in vitro* when co-cultured with MDSCs.[104,116] Additionally, MDSCs enhance osteolytic activity, which is crucial for sustaining the vicious cycle of bone metastasis. For example, bone metastasis-infiltrating MDSCs produce two-fold more levels of TGF-β than their counterparts in healthy subjects, which upregulates the expression levels of PTHrP and Gli2 in tumor cells to drive osteoclastogenesis and the osteolytic cycle.[115] Additionally, lineage tracing and *in vitro* differentiation assays have demonstrated that MDSCs can differentiate into mature osteoclasts within bone metastases and hence directly support the degradation of bones.[106,117]

4.3. Macrophages

Macrophages exhibit remarkable phenotypic plasticity in different microenvironments, and their complexity is further compounded by their origins.[118,119] They can be classified based on their location into bone marrow macrophages, which resemble monocyte-derived macrophages found in other organs, and bone resident macrophages, or osteomacs, which are identified by specific markers such as CD166 and M-CSFR alongside canonical markers.[120,121] So far, it is still unclear about the developmental origins of osteomacs, but they are known to interact with osteoclasts, megakaryocytes, and osteoblasts within the bone and form a niche complex that regulates HSC functions.[120,122,123] Despite the shared monocytic lineage, osteomacs differ morphologically from osteoclasts and do not express osteoclast markers. Yet, there are presumptions that osteomacs may link to osteoclastogenesis and consequently promote bone metastasis. Importantly, osteomacs are found in proximity to mature osteoclasts and can differentiate into osteoclast-like cells *in vitro*, suggesting they may be osteoclast precursors *in vivo*.[124,125] Osteomacs also support the mineralization deposition of osteoblasts, and their depletion significantly delays bone healing, mirroring the coordinated behaviors of osteoclasts in bone homeostasis.[126–128] However, a recent study reports that depleting $CD169^+$ macrophages, which specifically marks osteomacs in mice, impairs bone repair without affecting the number of monocytes or osteoclasts,[127] indicating an osteoclast-independent function of osteomacs in bone remodeling and pathology.

The role of bone marrow macrophages in bone metastasis is poorly understood, characterized by both scant research and contradictory findings. These macrophages are found in greater number in bone metastases than in primary tumors in both murine models and human patients.[129,130] Genetic ablation of bone metastasis-infiltrating macrophages but not $CD169^+$ osteomacs significantly reduces metastatic growth in mice, supporting their role in promoting bone metastasis.[130] In prostate cancer, these infiltrating macrophages induce a wound-healing-like response in cancer cells, thereby rendering tumor cells resistant to androgen deprivation therapies.[129] Furthermore, these macrophages are reported to display a distinct activation state that is characterized by increased expression of IL4R and CD204.[130] Macrophage-specific deletion of IL4R significantly reduces bone metastasis in murine models.[130] In addition, the CCL2-CCR2 chemokine axis is another key regulator of bone metastasis-associated macrophages.[131,132] Bone metastatic tumor cells highly express CCL2, enhancing the infiltration of macrophages in a CCR2-dependent manner.[132,133] Neutralizing antibodies against CCL2 or CCR2 have demonstrated effectiveness in slowing bone metastasis progression in both mice[130] and human trials,[134] suggesting a viable therapeutic avenue for bone metastasis treatment.

The polarization of bone metastasis-associated macrophages, particularly into the M2-like phenotype, has also been reported.[107,130] For example, in prostate cancer bone metastases, the infiltrating macrophages express anti-inflammatory cytokines as the M2-like macrophages do.[107] This M2-like polarization is triggered by tumor-derived cytokines, including CSF-1 and M-CSF,[135] as well as through the efferocytosis of apoptotic cells. Ingesting apoptotic cancer cells or neutrophils by macrophages enhances their secretion of M2-related factors like MFG-E8, CXCL5, TGF-β, PGE2, and PAF2.[108,136–138] These cytokines and growth factors amplify M2-like phenotypes and accelerate bone metastasis progression. Yet, it remains unclear whether such polarization is elicited by the efferocytotic activity itself or by specific apoptotic components. Blocking these activities could offer a strategy to reverse macrophages' immunosuppressive phenotypes and mitigate bone metastasis.

4.4. Other immune cells

Neutrophils serve diverse functions in bone metastasis, shifting between tumor-inhibitory N1 and tumor-promoting N2 phenotypes. TGF-β is known to promote the N2 phenotype of tumor-associated neutrophils (TANs).[139,140] Blocking TGF-β enhances the expression of proinflammatory cytokines and cytotoxic activity of TANs, thereby inhibiting tumor growth.[139] Recent evidence suggests that TANs promote cancer metastasis through the formation of NETs, or NETosis. NETs are reported to contribute to multiple critical metastatic steps, such as enhancing angiogenesis,[141] facilitating cancer cell migration and invasion,[142] attracting tumor cells to distant organs,[143] aiding the initial seeding of CTCs,[144] protecting tumor cells from adjuvant therapies,[145] breaking down growth-inhibitory proteins,[146] and remodeling ECM to awaken dormant tumor cells.[147] Such extensive involvement of NETs substantially supports their functional role in bone metastasis, even though most of NET functions are studied only in lung or liver metastasis models.

Emerging evidence highlights the involvement of dendritic cells in bone metastasis. In early-stage breast cancer, the presence of $CD1a^+$ and $CD83^+$ dendritic cells within the stroma is associated with a lower risk of bone metastasis and improved disease-free survival.[148] Interestingly, while CD83 is a known marker of DC activation, CD1a expression varies among DC subsets. $CD1a^+$ and $CD83^+$ DCs show low concordance in patient samples, suggesting the existence of distinct DC populations within bone metastases.[148] The specific functions of these DC subsets remain unexplored. In breast cancer murine models, an increased infiltration of $B220^+CD11c^+$ plasmacytoid DC (pDCs) was observed in bone metastases.[149] These pDCs promote an immunosuppressive Th2 response that is accompanied with secretion of osteolytic cytokines, yet their depletion reversed these effects and effectively eliminated bone metastasis and associated bone damage.[149] Additionally, it is reported that splenic $CD11c^+$ DCs exposed to the tumor secretome can transform into multinucleated giant cells with osteoclastic activity.[150] These DC-derived osteoclasts migrate to pre-metastatic bone marrow and collaborate with RANKL-secreting Th17 cells to intensify osteolytic damage prior to tumor cell arrival, a phenomenon that will be discussed in the following section.

In summary, the discussed content summurizes the immunoregulatory mechanisms involved in bone-related tumors and highlights the critical interactions with immune cells that orchestrate disease progression (Fig. 3).

5. Remote remodeling of osteo-immune crosstalk by distant tumors

It is well established that primary tumors can exert influence over distant organs. Consequently, the osteo-immune microenvironment can

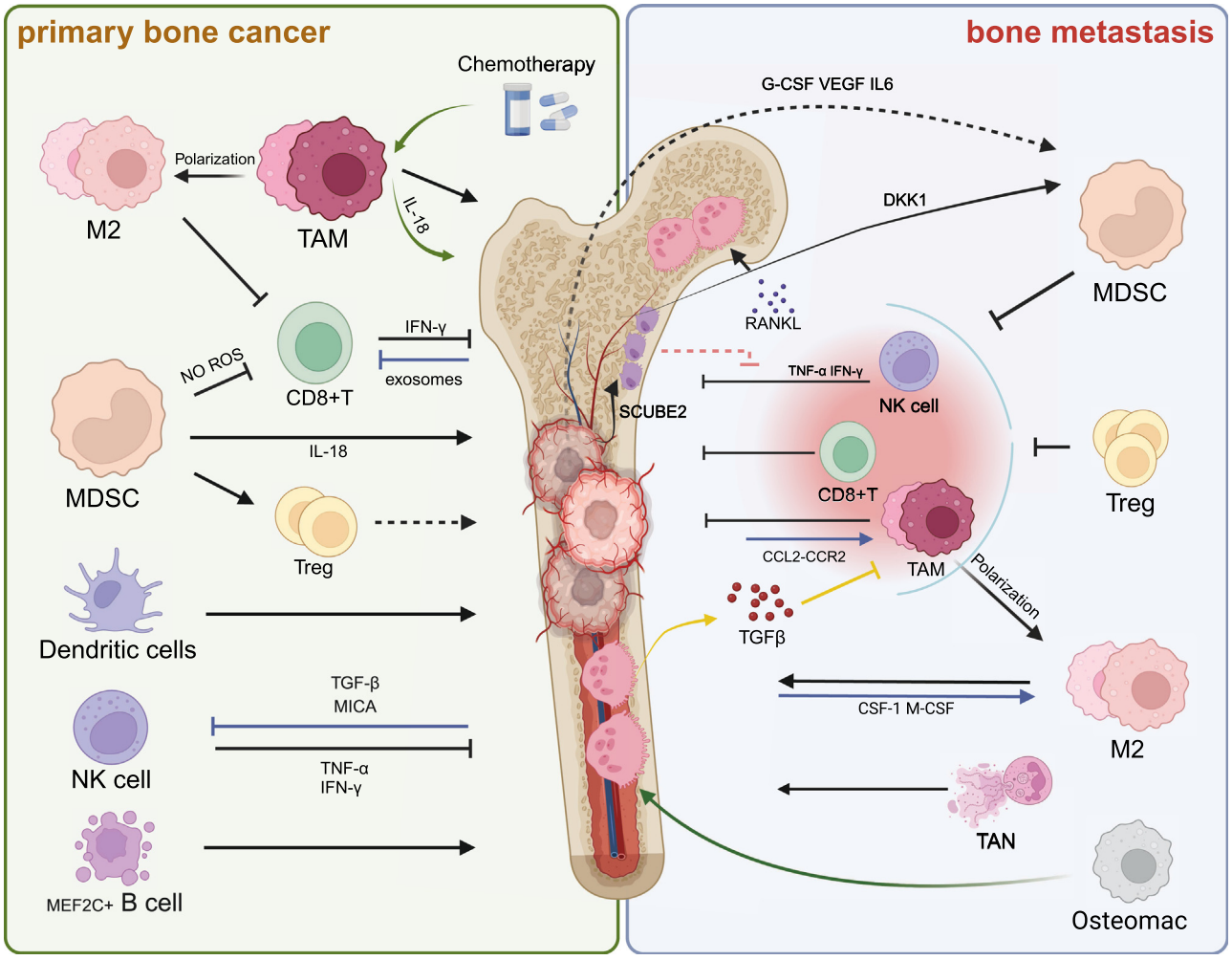

Fig. 3. Immunoregulators of bone-related tumors. The microenvironment in bone cancers, both primary and metastatic tumors, is defined by the intricate interactions between immune cells, tumor cells, and bone cells. These interactions significantly impact tumor progression. Immune cells, displaying dual roles, can promote or inhibit tumor growth. In osteosarcoma, TAMs and M2-like macrophages release cytokines like IL-18 and hinder $CD8^+$ *T* cells, facilitating tumor progression. Tumor cells contribute by secreting exosomes that impair $CD8^+$ *T* cell function, while MDSCs and $MEF2C^+$ B cells promote tumor growth through cytokine secretion and immune suppression. Infiltration of DCs subsets is linked to poorer outcomes, whereas NK cells offer anti-tumor activity that tumors can suppress. In the context of bone metastasis, the regulatory roles of osteoblasts and osteoclasts are emphasized. Osteoclasts, by enhancing osteolytic damage and releasing TGF-β, inhibit the anti-tumor functions of T and NK cells. Activated T cells and Tregs influence osteoclast differentiation through RANKL secretion, with Tregs also capable of suppressing osteoclastogenesis. MDSCs promote osteolysis and osteoclastogenesis, while both MDSCs and osteomacs can differentiate into osteoclasts. Osteoblasts affect metastasis progression; for example, tumor-derived SCUBE2 inhibits NK cell activity, and DKK1 secretion by osteoblasts enhances MDSC's tumor-promoting functions. Neutrophils also facilitate metastasis via the formation of neutrophil extracellular traps. DCs, dendritic cells; MDSCs, myeloid-derived suppressor cells; NK, natural killer; TAMs, tumor-associated macrophages; TAN, tumor-associated neutrophil; Tregs, regulatory T cells.

be remotely remodeled by tumor-derived factors. In a pioneering work, Rosandra Kaplan and colleagues reported that tumor-derived factors can mobilize bone marrow hematopoietic progenitor cells to predetermined sites, thereby creating supportive niches for impending metastatic tumor cells.[151] This groundbreaking finding led to the introduction of the concept of 'premetastatic niche' (PMN). Further studies have elucidated how primary tumors remotely prepare the ground for future metastases by orchestrating the formation of PMNs across various organs, including bones.[152–154] As a specialized microenvironment conducive to cancer cell colonization and proliferation, PMNs consist of tumor-derived factors, an accumulation of immune cells, and modified extracellular matrix. Importantly, PMNs are not merely static receivers but are actively shaped to prime them for the arrival of metastatic cells.

Among various mechanisms, bone marrow acts as the primary responder for many tumors to regulate PMN formation. Primary tumors release soluble factors, including growth factors and exosomes, to mobilize immunosuppressive cells from the bone marrow to target organs and thus alter the distant 'soil' to support cancer cells. Notably, VEGFR1+ bone marrow derived cells (BMDCs) that express integrin $\alpha 4\beta 1$ are instrumental in metastasis-promoting functions of PMNs. Their interaction with fibronectin on resident fibroblasts enhances MMP9 and CXCL12 production, facilitating tumor cell adherence and proliferation in PMNs[152]. The augmented presence of BMDCs and MMP9 at premetastatic sites, such as lungs, promotes metastatic outgrowth by stimulating neoangiogenesis or recruiting MDSCs.[155–157] MDSCs accumulated in PMNs can suppress the cytotoxic activity of $CD8^+$ *T* cells and NK cells, aiding in the immune evasion of metastasizing cells.[158] In contrast, neutrophils drawn to premetastatic organs by tumor-secreted CCL2 are reported to impede metastatic outgrowth.[159] However, as previously discussed, neutrophil recruitment and subsequent NETosis in premetastatic tissues have also been linked to pro-metastatic functions, raising questions about the precise role of neutrophils in metastasis progression. One possible explanation for this ambiguity is the varied

nomenclature and surface markers for immune cells involved in PMNs, which may have complicated data interpretation.

Alteration in bone remodeling are early indicators of impending bone metastasis, with both osteogenic and osteolytic phenotypes being observed in cancer patients and mouse models.[90,160–162] In mouse models, exposure to breast cancer cell-derived condition media leads to increased collagen deposition by osteoblasts in metaphyseal bones, where are also susceptible to bone metastasis.[163] Similarly, activation of osteoblasts is noted in patients and animal models with localized lung cancer.[164] Our research, along with others, has shown that early-stage bone metastasis benefits from such osteogenic microenvironment for cell proliferation and survival upon adjuvant therapies.[82,85,165,166] Furthermore, increased collagen deposition creates an immunosuppressive milieu by engaging the LAIR1 inhibitory receptor on NK cells.[90] Activated osteoblasts have also been reported to promote the expansion of SiglecF^{high} neutrophils in the bone marrow, which circulate to the lung and enhance the growth of primary lung cancers.[160] Conversely, breast cancer cells are known to induce osteolytic bone lesions via exosome and RANKL secretion, acting as skeletal PMNs.[161,162,167] Specifically, exosomes containing miR-21 from bone-metastatic cells enhance osteoclast differentiation and activity by downregulating PDCD4, and thereby establish osteolytic regions for colonizing tumors.[161] Additionally, the activation of T cells by tumor antigens, together with the involvement of splenic cDCs and bone marrow B cells, may cause premetastatic bone loss by releasing cytokines that promote osteoclatogenesis.[150,168] These seemingly contradictory osteogenic or osteolytic PMNs may reflect the dynamic and heterogeneous nature of the premetastatic bone microenvironment.

The crosstalk between primary tumors and bones underpins the emergence of systemic immunosuppression observed in cancer patients. A key mechanism involves the accumulation of MDSCs, resulted from the skewed myeloid differentiation of bone marrow progenitor cells.[169] Granulocyte–monocyte progenitors (GMPs) in the bone marrow that give rise to most MDSCs have been implicated in this process. Previous studies have noted the increase of circulating GMPs and its association to shorter progression-free survival and higher metastasis rates in cancer patients.[170,171] Throughout tumor progression, the quantity and frequency of GMPs in the bone marrow rise, accompanied by increased levels of cytokines such as GM-CSF, G-CSF and IL-6.[171–173] These cytokines are critical in promoting the proliferation and differentiation of MDSC progenitors into monocytic and granulocytic lineages, thereby augmenting MDSC production in the bone marrow.

Primary tumors also modify osteoimmune interactions to perpetuate the biased myeloid hematopoiesis. Our recent research has shown that extracellular vesicles from distant tumors carry HTRA1 to the bone marrow and trigger osteoprogenitor (OP) expansion through MMP13 upregulation,[174] aligning with the osteogenic bone phenotype seen in early-stage cancers.[90,160] This OP cell expansion displaces HSCs and strengthens their interactions with GMPs, and consequently bone marrow GMPs are steered towards granulocytic differentiation. Remarkably, these tumor-induced bone marrow alterations persist even after removal of primary tumors, highlighting their long-lasting impact. Counteracting these changes with genetic or pharmacological interventions targeting OPs or MMP13 may mitigate systemic immunosuppression and enhance antitumor immunity.[174] In supporting our findings, Yohan Gerber-Ferder and colleagues also reported the link between increased osteogenic differentiation of MSCs and altered myelopoiesis in tumor-bearing mice.[175] Intriguingly, they further found that the myeloid-primed state conferred by remote tumors is retained in hematopoietic progenitor cells after multiple rounds of bone marrow transfers to the irradiated, tumor-free hosts.[175] These observations indicate a durable imprint of the primary tumor on bone marrow microenvironment, potentially due to a self-reinforcing loop between OP expansion and myelopoiesis in the bone.

6. Immunotherapies in treating bone-related tumors

Clinical treatment of bone-related tumors often ends up with poor prognosis. In addition to standard chemotherapies, patients with bone tumors are often treated with adjuvant bisphosphonates (BPs) and anti-RANKL monoclonal antibodies like Denosumab to prevent skeletal-related events. However, these treatments typically fail to eliminate all tumor cells, leaving behind residual cells in the bone marrow that can cause disease relapse. Moreover, these approaches often do not reverse suppressed anti-tumor immunity and may, in many cases, further dampen the immune response.[169] Not surprisingly, conventional therapies profoundly impact not only tumor cells but also the bone and immune cells. For example, traditional chemotherapeutic agents, such as alkylating agents and anthracyclines, are known for their myelosuppressive effects and hematopoietic toxicity. Bone marrow cells are particularly vulnerable to chemotherapy-induced genotoxic damage.[176] The bone marrow niches stressed by high-dose chemotherapy fail to support normal hematopoiesis and inadvertently protect residue tumor cells.[177–180] On the other hand, transplantation of bone marrow MSCs has been reported to mitigate the adverse effects of chemo/radiotherapies by enhancing the recovery of the hematopoietic system.[181,182] Recently, the immunostimulatory effects of specific chemotherapeutic regimens have gained attention. For instance, docetaxel-based adjuvant therapy significantly boosts lymphocyte infiltration in prostate cancer models by activating the cCAS-STING and IFN pathways.[183] Docetaxel can also directly stimulate T cells to release cytotoxic vesicles and thereby enhance their anti-tumor effects *in vivo*.[184] Similarly, in multiple myeloma, low-dose cyclophosphamide primarily exerts its tumor-controlling effects by selectively depleting Tregs, shifting the balance towards anti-tumor immunity.[185] Additionally, low-dose cyclophosphamide affects other immune subsets, such as $CD8^+$ dendritic cells, tumor-associated macrophages, and B cells, in peripheral lymph organs or triple-negative breast cancer tumors.[186,187] The impacts of these chemotherapies on the immune compartment of bone tumors likely mirrors the above-mentioned observations. They influence hematopoiesis and the overall immune response, which should be taken into consideration in bone tumor treatment.

Immunotherapies have revolutionized the clinical management of cancers in recent years. Currently, approved and experimental strategies include targeting T cells, MDSCs, macrophages, neutrophils, NK cells, and more (Fig. 4). Among these, immune checkpoint inhibitors (ICIs) targeting T cells are particularly promising, especially for cancers with high mutation burdens.[188] However, their effectiveness in treating osteosarcomas and bone metastases remains limited. Retrospective analyses indicate that bone metastatic TNBC patients show less favorable responses to PD-L1 inhibitors like atezolizumab combined with nab-paclitaxel, despite significant overall response rates in TNBC patients.[189] Similarly, only about 5% and 16% of patients with locally advanced or metastatic sarcomas respond to PD1 inhibitor nivolumab alone or in combination with CTLA-4 inhibitor ipilimumab, respectively.[190] In addition to their limited efficacy in bone-related tumors, ICI treatments may also cause bone loss and approximately double the risk of bone fractures,[191] likely due to the pro-osteoclastogenesis effects of activated T cells. Improving efficacy while minimizing immune-related adverse effects represents the new frontier of immunotherapies in bone tumor treatment.

To overcome ICI resistance in bone-related tumors, recent strategies focus on amplifying the anti-tumor immune response by simultaneously targeting inhibitory checkpoint molecules and co-stimulatory molecules. TGF-β is a promising target, given its pivotal role in both bone remodeling and immune regulation. In castration-resistant prostate bone metastasis, bone matrix degradation releases active TGF-β, prompting a shift of helper T cells towards the Th17 lineage over the Th1 lineage, and consequently diminishing ICI responsiveness.[100] Combining TGF-β neutralizing antibodies with ICIs enhances the infil-

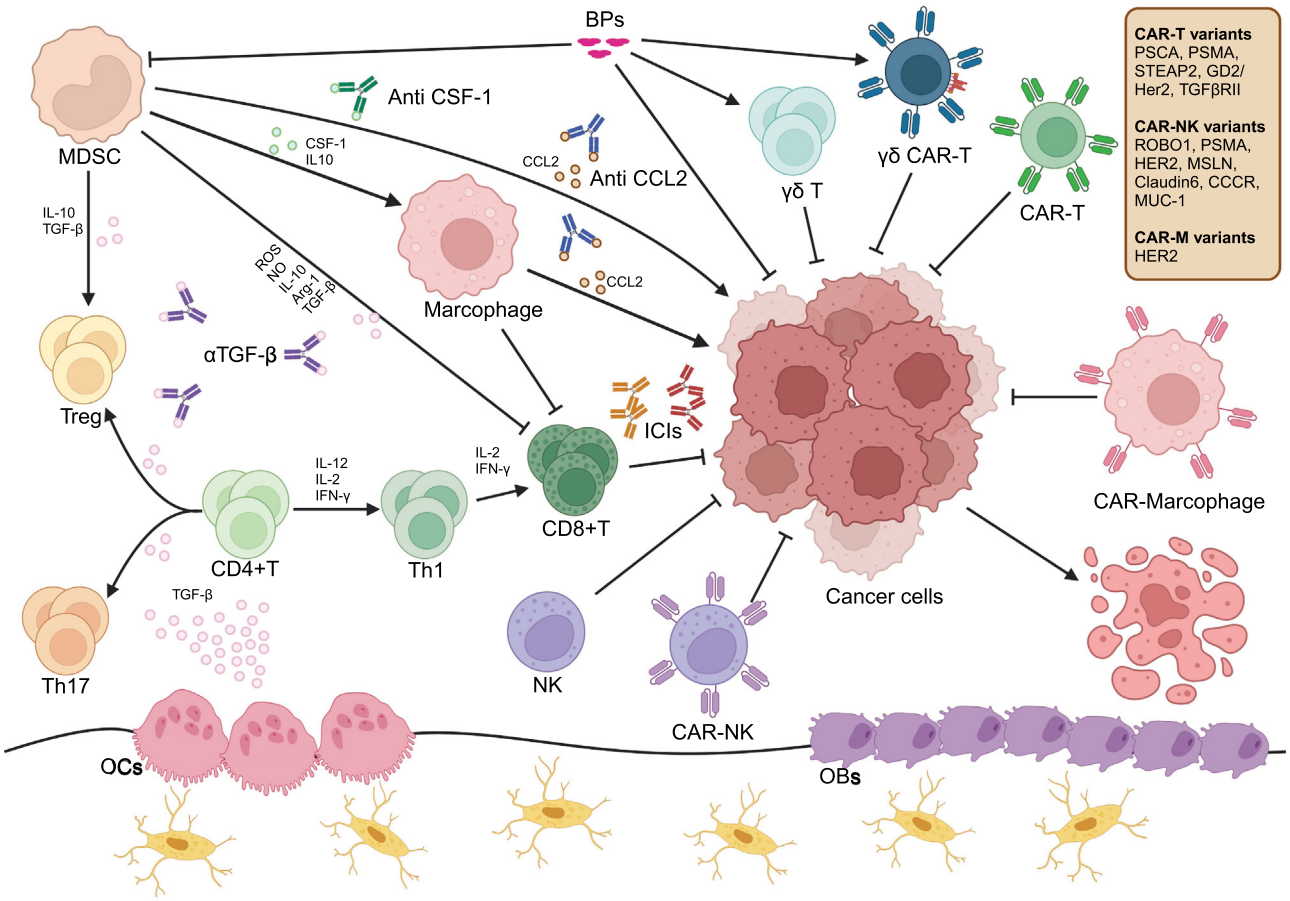

Fig. 4. Immunotherapies for bone-related malignancies. Immunotherapies in the bone tumor microenvironment target T cells, MDSCs, macrophages, and NK cells, aiming to boost anti-tumor immunity through ICIs and co-stimulatory molecules. Blocking TGF-β shifts CD4$^+$ *T* cell differentiation from Th17 and Treg cells to the Th1 lineage, enhancing CD8$^+$ *T* and NK cell activation and ICI efficacy. Additionally, TGF-β blockade reduces MDSC-mediated suppression of CD8$^+$ *T* cells and limits Treg proliferation. Targeting CSF-1 and CCL2 disrupts macrophage differentiation and hinders immunosuppressive cell chemotaxis and migration, thereby counteracting immunosuppressive environments within bone tumors. BPs complement ICIs by multiple ways, reducing MDSC immunosuppression and enhancing $\gamma\delta$ T and $\gamma\delta$ CAR-T cell cytotoxicity. CAR-T cells target diverse bone tumors expressing specific cancer-associated antigens. CAT-T cells engineered to express dominant negative TGFβRII counteracts TGF-β-mediated immunosuppression, significantly decreasing bone metastasis. BPs, bisphosphonates; CAR, chimeric antigen receptor; ICIs, immune checkpoint inhibitors; MDSCs, myeloid-derived suppressor cells; OB, osteoblast; OC, osteoclast.

tration of Th1 cells and effector T cell clones in tumors and thereby significantly delays the progression of bone metastasis.[100] Following this rationale, osteoclast-inhibiting reagents like BPs or RANKL neutralizing antibodies, which prevent bone degradation and consequent TGF-β release, may work synergistically with ICIs in bone tumor treatment. However, a preliminary study found that combining bone-targeted therapies with ICIs shows discrete efficacy in bone metastasis patients across different cancers,[192] potentially due to the pleiotropic effects of bone-targeted reagents. BPs, for example, not only inhibit osteoclast activity but also possess direct antitumor properties and can modulate the immune system in a context-dependent manner.[164,193] Therefore, the outcome of combining ICIs with bone-targeted therapies may hinge on the genetic and immunological characteristics of the bone tumors. In addition to TGF-β, approaches targeting myeloid cells to mitigate MDSC-mediated immunosuppression and boost ICI efficacy are under investigation (discussed above). Yet, translating these strategies into clinical practice poses substantial challenges. Further research into the interactions between ICIs and adjunctive therapies is necessary to identify patients who could benefit from these combinational therapies and improve therapeutic outcomes.

Cell-based immunotherapies are being actively evaluated for treating bone tumors. Chimeric antigen receptor-engineered T (CAR-T) therapy has shown a substantial successful rate in treating hematological malignancies like leukemia, multiple myeloma, and non-Hodgkin B-cell lymphoma.[194,195] However, CAR-T therapies face challenges in solid tumors due to factors such as the lack of universal antigens, limited infiltration, rapid exhaustion, and severe adverse effects.[196] Yet, promising results have emerged from targeting specific antigens with CAR-T cells in bone-related tumors, including PSCA, PSMA, and STEAP2 for prostate cancer, as well as GD2 and Her2 for osteosarcomas.[197–201] To overcome the immunosuppressive microenvironment and enhance T cell activation, engineered T cells can be further modified to adapt specifically for bone tumor treatment. A notable modification involves equipping CAR-T cells with a dominant-negative TGF-β type II receptor (TGFβRII), which has been demonstrated to effectively counteract TGF-β-mediated immunosuppression and significantly reduce CRPC bone metastasis in both research and clinical settings.[199,201]

$\gamma\delta$ T cells have been shown to exert direct anti-tumor effects in osteosarcomas and other metastatic diseases.[202,203] Bone-targeting BPs potently expand $\gamma\delta$ T cells and therefore enhance their cytotoxicity. Jeremy S. Frieling and colleagues recently developed a $\gamma\delta$ T cell-based CAR-T therapy targeting PSCA for metastatic CRPCs.[197] They found that pre-treatment with the BP zoledronate enhances the efficacy of $\gamma\delta$-enriched CAR-T cell, resulting in notable regression of established bone metastases and reduction of tumor-induced bone damage in mouse models.[197] This suggests that combining $\gamma\delta$ T cell therapies with BPs could offer a promising approach for treating bone-related tumors.

CAR-NK therapies are emerging as attractive alternatives to CAR-T therapies, especially for solid tumors.[204–206] These NK cell-based therapies possess inherent advantages for cancer treatment, including non-MHC restricted recognition and reduced alloreactivity. Multiple clinical trials are currently underway to evaluate the efficacy of CAR-NK cells targeting various tumor antigens. For example, there trials include CAR-NKs targeting ROBO1 in broad-spectrum solid tumors (NCT03940820), PSMA for prostate cancer (NCT03692663), ERBB2 for advanced or metastatic $HER2^+$ solid tumors (NCT04319757, NCT03383978), MSLN for ovarian cancer (NCT03692637), Claudin-6 for gynecologic and genitourinary cancers (NCT05410717), CCCR for non-small cell lung cancer (NCT03656705), and MUC-1 for recurrent or treatment-resistant solid tumors (NCT02839954). Notably, MUC1-specific CAR-NK cells have shown safety and preliminary effectiveness in preventing tumor recurrence and graft-versus-host disease.[207] Furthermore, a phase II clinical trial (NCT02100891) reported that early infusion of donor NK cells showed promising preliminary results in improving overall survival with manageable side effects in patients with high-risk bone tumors, including Ewing's sarcoma, rhabdomyosarcoma, and osteosarcomas.[208]

However, CAR-T and CAR-NK therapies encounter several common challenges, such as the immunosuppressive tumor milieu that hampers their infiltration and cytotoxicity. In contrast, macrophages are more abundant in many tumors compared to T and NK cells. As a result, CAR macrophage (CAR-M) therapy, which integrates CAR structures with macrophage functions, offers a complementary approach for tumor treatment. These engineered macrophages can infiltrate the tumor microenvironment more effectively and, when polarized towards a M1-like phenotype, exhibit enhanced anti-tumor efficacy.[209,210] For example, M1-poloarized CAR-Ms, induced by LPS/IFN-γ treatment or by adding the TIR domain of the TLR4 protein, have demonstrated superior phagocytic and cytotoxic abilities against tumor cells both *in vitro* and *in vivo*. A phase I trial is currently assessing CAR-M therapy targeting HER2-overexpressing solid tumors (NCT04660929).[211] Although CAR-M therapies show potential advantages over traditional CAR-T therapies, they are still in the early stages of development. Further validation is needed to fully establish their clinical efficacy in treating bone tumors.

In addition to the previously discussed immunotherapies, cancer-associated vaccines represent another promising approach for cancer treatment. These vaccines train T cells to recognize and attack cancer cells more effectively through activating antigen presenting cells (APCs), primarily DCs. Provenge® (Sipuleucel-T), the first FDA-approved cancer vaccine, activates the immune system in prostate cancer patients through the autologous transfer of APCs exposed to a fusion protein of prostatic acid phosphatase (PAP) and GM-CSF.[212] Various forms of antigens, such as DNA, RNA, synthetic peptides, and recombinant proteins, have been developed to activate APCs using different delivery methods.[213] Provenge® has shown durable effects in controlling bone metastases in some CRPC patients.[214,215] More cancer vaccines are currently under development for the treatment of bone metastasis or primary bone tumors.[216] The bone marrow-homing properties of DCs make them ideal for delivering tumor-associated antigens into the bone marrow.[21] However, DC-based vaccines often fail to induce durable immunity against tumors in most patients.[215] Studies have indicated that only a small cohort of bone tumor patients achieve a satisfactory immune response when receiving DC-based vaccines.[217,218] This may be due to the inefficiency of infused DCs in triggering sustained activation of T lymphocytes within the disordered microenvironment of bone tumors, a phenomenon that remains poorly understood. Investigating the interplay between these cancer-associated vaccines and other components of the bone microenvironment may provide clues on this perspective.

Significant challenges remain in the clinical application of immunotherapies for bone-related malignancies. Immunosuppressive cytokines are enriched in bone, and the dense bone matrix along with associated stromal cells create additional barriers for effective immune responses. Therefore, bone-specific immunotherapies may be needed to further improve the outcomes and quality of life in patients with bone tumors.

7. Conclusion and perspectives

The interactions between bone and immune cells are concentrated within the bone marrow and create a symbiotic system and function much like a symphony orchestra. The bone microenvironment is vital for supporting immune cell maturation and maintaining the balance of bone remodeling. Disruptions in these processes can lead to bone-related diseases. This review focuses on one of the most significant pathological conditions affecting this system: bone tumors, including both primary and metastatic forms. Besides its cellular components, bone is also unique with many physiochemical properties, including a rigid and dense mineral matrix, specific microstructural organization, and compartmentalized oxygen tensions within the bone marrow cavity. While accumulating evidence has linked these physicochemical features to bone development and pathologies,[219] less is known about their influence on immune cells within bone tumors. Understanding these influences and the underlying mechanisms will aid the development of new therapeutic approaches to combat bone-related cancers.

Tumor cells exploit and reconfigure bone-immune communications to facilitate their development. Consequently, the emergence of bone tumors disturbs the equilibrium between bone destruction and formation, resulting in osteoblastic, osteolytic, or mixed lesions, depending on the type of cancer. For instance, prostate bone metastases and osteosarcomas usually cause osteoblastic lesions, whereas bone metastases from breast cancer and multiple myeloma tend to be osteolytic. The determinants of these distinct bone phenotypes are not fully understood but likely involve both the genetic trait of the cancer cells and the host's biological environment. For example, wild-type MDA-MB-231 breast cancer cells typically form osteolytic bone metastases; however, when engineered to overexpress PDGF-BB, they produce mixed bone lesions.[220] Likewise, ER^+ MCF-7 breast cancer cells can generate osteoblastic metastases in the absence of estrogen supplementation,[220] which is a stark contrast to their well-known osteolytic behavior when estrogen is present, demonstrating how internal and external factors influence the nature of bone lesions.

The discrepancy in bone tumor histology prompts questions into how diverse bone phenotypes correlate with specific immune landscapes, and the contribution of the immune system in the shift of these bone phenotypes. Studies have demonstrated that specific cancer cell phenotypes can selectively attract neutrophils or macrophages, forming distinct immune compartments that influence treatment outcomes.[221] This suggests that the genetic trait of tumor cells may dictate a specific immune ecotype. Moreover, the host's genetic background plays a role in bone tumor progression via the immune system. For example, a recent study has shown that a specific GRM4 polymorphism drives the development of osteosarcomas predominantly through myeloid cells, rather than directly through tumor cells.[207] These insights support a co-evolutionary model of cancer cells and their surrounding microenvironments in tumor progression. Unlocking the spatial-temporal dynamics of bone tumors and harnessing the underlying mechanisms for better therapies necessitates the use of advanced technologies, such as single-cell omics, alongside clinically relevant models.

The clinical treatment of bone-related cancers often yields disappointing results, partly due to the bone marrow's susceptibility to conventional anti-tumor therapies. Exploiting immunoregulatory mechanisms within standard treatment modalities could expand the therapeutic landscape in these patients. The unique and evolving bone microenvironment necessitates tailored approaches that can effectively leverage the immune system to manage bone tumors. Preclinical models have demonstrated the synergistic effects of combining immunotherapies with specific bone-targeted treatments. For example, immune checkpoint blockade has shown favorable results in some bone cancer patients when used alongside with Denosumab.[178] Additionally, novel ap-

proaches targeting the bone stromal compartment, rather than immune cells, are being actively pursued for other bone-related diseases.[222] Investigating the impact of these approaches on bone tumor immune cells will likely inform the prediction of patient responses, identifying those who may benefit from combination therapies and ultimately refining the clinical management.

In summary, understanding the intricate interactions between tumor cells, immune cells, and bone cells enables the development of more effective treatments that not only inhibit tumor growth but also prevent metastasis and recurrence. Insights into the immunosuppressive mechanisms within the bone marrow will guide the creation of novel immunotherapies designed to overcome bone-specific barriers, thereby improving overall response and patient survival.

Declaration of competing interest

The authors 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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Author contributions

W.Z. conceived, reviewed and edited the manuscript. C.S., T.T., B.D. and Y.Z. wrote the original draft and revised the article with comments of M.Z. and E.C.

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