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Review Article

Immune checkpoint inhibitors in bone metastasis: Clinical challenges, toxicities, and mechanisms

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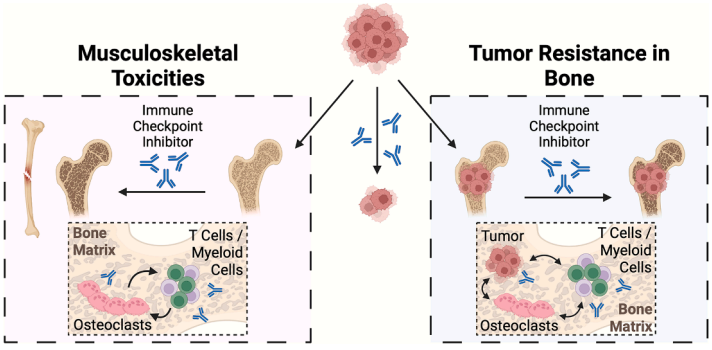
HIGHLIGHTS

- Patients with bone metastases have comparably poorer survival in response to immune checkpoint inhibitors (ICIs) compared to patients without bone metastases.
- Co-administration of ICIs with bone-targeted agents improve outcomes in patients with bone metastasis.
- Fracture risk is elevated in patients treated with ICIs, regardless of whether they have bone metastatic disease.
- There are limited pre-clinical studies investigating osteoimmunology in the tumor-bone microenvironment, but myeloid cells and $CD4^+$ and especially $CD8^+$ T cells are likely to play a role in bone metastatic progression.

GRAPHICAL ABSTRACT



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ABSTRACT

Immune checkpoint inhibitors (ICIs) have revolutionized the field of anti-cancer therapy over the last decade; they provide durable clinical responses against tumors by inhibiting immune checkpoint proteins that canonically regulate the T cell-mediated immune response. Despite their success in many primary tumors and soft tissue metastases, ICIs function poorly in patients with bone metastases, and these patients do not have the same survival benefit as patients with the same primary tumor type (e.g., non-small cell lung cancer [NSCLC], urothelial, renal cell carcinoma [RCC], etc.) that has not metastasized to the bone. Additionally, immune-related adverse events including rheumatologic and musculoskeletal toxicities, bone loss, and increased fracture risk develop after treatment with ICIs. There are few preclinical studies that investigate the interplay of the immune system in bone metastases; however, the current literature suggests a role for $CD8^+$ T cells and myeloid cell subsets in bone homeostasis. As such, this review focuses on findings from the clinical and pre-clinical studies that have investigated immune checkpoint blockade in the bone metastatic setting and highlights the need for more comprehensive investigations into the relationship between immune cell subsets, ICIs, and the bone-tumor microenvironment.

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1. Introduction

The development of immunotherapy has revolutionized cancer treatment over the past decade, appropriately being named Science magazine's "breakthrough of the year" in 2013. Immune checkpoint inhibitor (ICI) therapy (e.g., α-PD-1, α-PD-L1, and α-CTLA-4) has become a standard of care for the treatment of cancer patients. ICIs work by blocking immune checkpoint proteins that regulate the immune response, thereby allowing for expansion of active immune cell subsets. Since first being approved by the FDA in 2011, ICIs have become increasingly popular, with a 600% increase in clinical trials implementing PD-1 and PD-L1 inhibitors between 2015 and 2017 alone [1]. The market for ICIs was over $34 billion in 2021 and is projected to reach over $155 billion by 2031, further solidifying their relevance as an anti-cancer therapy.

Bone is a common site of metastasis, with many cancer types (e.g., myeloma, renal, melanoma, breast, lung, prostate, etc.) preferentially metastasizing to the bone and correlating with a worse prognosis [2]. Upwards of 50% of all cancer patients develop bone metastases [3], including more than 88% of metastatic prostate cancer patients [3,4] and 70% of metastatic breast cancer patients [4]. Despite the widespread success of ICIs as an anti-cancer treatment, they are comparably less effective at treating bone metastases. Poor clinical outcomes in ICI-treated patients with bone metastases suggest a potential link to resistance to this anti-cancer therapy which is otherwise successful in the primary tumor; as such, it would be clinically significant to investigate the phenomenon of why ICI therapy is ineffective in the bone microenvironment.

1.1. Immune response activation and regulation

ICIs have achieved success through blocking the activation of immune checkpoint proteins on the surface of T cells or tumor cells, thereby preventing tumor-mediated T cell inactivation. In order to appreciate how ICIs function, it is important to first understand the main steps needed for T cell activation. In the first stage of T cell activation, the T cell receptor (TCR) on a naïve T cell recognizes an antigen specific peptide presented by a major histocompatibility complex (MHC) on an antigen presenting cell (APC) [5,6]; this creates an intracellular signaling cascade resulting in activation of transcription factors including activator protein 1 (AP-1), nuclear factor of activated T cells (NFAT), and nuclear factor-kappa B (NF-κB), which regulate transcription of target genes correlating with cell survival and proliferation [7]. In the second stage of T cell activation, a costimulatory signal is created by CD28, a T cell surface protein, recognizing and binding to its respective ligands (e.g., B7-1, B7-2) on the APC; this secondary signal amplifies the signaling cascade created from TCR activation, enhancing T cell survival and proliferation [8] (Fig. 1A). The last part of T cell activation involves cell differentiation and expansion, which is induced by cytokines secreted from other immune cells in the environment, particularly APCs [9]. Once a T cell response is mounted, the immune system has mechanisms in place to inactivate or eliminate the active T cells which, if otherwise left unchecked, could result in the formation of uncontrolled inflammatory responses leading to tissue damage and autoimmune disease.

An effective method of T cell inhibition involves immune checkpoint proteins expressed on the surface of activated T cells. Upon engagement with the appropriate ligand, these proteins initiate T cell apoptosis and disrupt the TCR signaling cascade that is critical for activation of key transcription factors [10,11] (Fig. 1B). Three of the more commonly studied checkpoint proteins are cytotoxic T-lymphocyte associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1), and programmed death ligand 1 (PD-L1). Upon T cell activation, CTLA-4 is translocated to the T cell surface and outcompetes CD28 for binding to B7 ligands, resulting in T cell inactivation [12,13]. PD-1 expressed by T cells interacts with its ligands (i.e., PD-L1 and programmed death ligand 2 [PD-L2]) on APCs to promote T cell inactivation and apoptosis, but tumor cells also express PD-L1 as a means to evade T cell-mediated destruction, resulting in an impaired anti-tumor response [14,15]. This ability of cancer cells to induce immunosuppression and evade immune attack provides much of the basis for ICI therapy as an anti-cancer immunotherapy. As such, antibodies have been developed against CTLA-4, PD-1, and PD-L1 to prevent inhibition of T cell signaling in the tumor microenvironment.

1.2. Tumor metastasis to bone

Bone metastases are common for many cancer types and are often

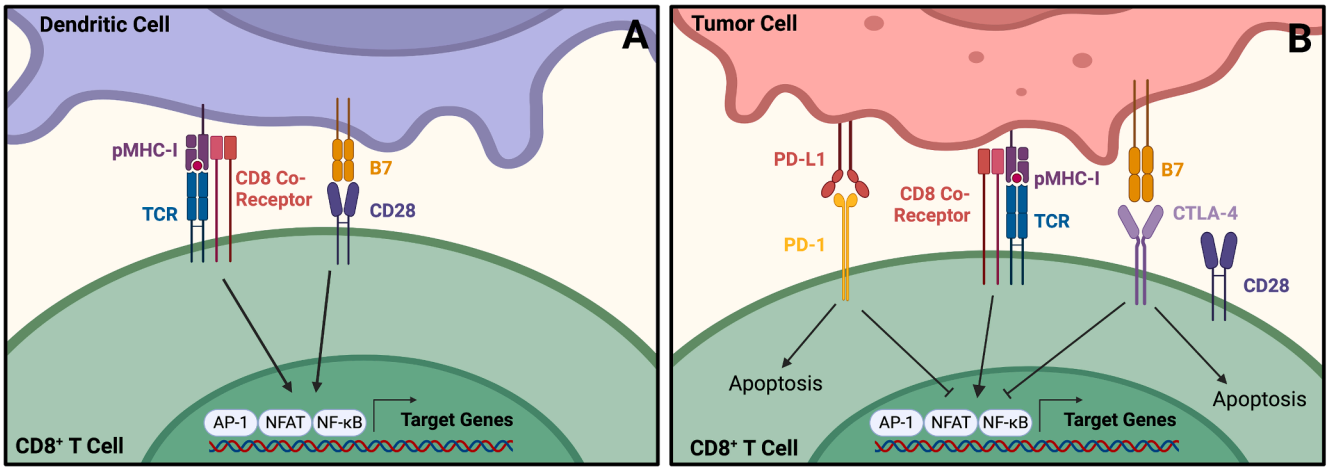

Fig. 1. Protein interactions between T cells and antigen-presenting cells (APCs) with (A) and without (B) involvement of tumor cells and immune checkpoint proteins. In tumor naïve settings (A), dendritic cells (purple) present antigen to the T cell (green) via MHCs to be recognized by the TCR on the T cell, creating a primary signal. A B7 ligand presented on the dendritic cell binds to CD28 on the T cell, providing a costimulatory signal. The primary and costimulatory signals trigger activation and nuclear localization of transcription factors important for target gene transcription. In tumor settings (B), these processes are disrupted by PD-L1 on tumor cells binding to PD-1 on T cells, disrupting T cell signaling and causing T cell apoptosis. CTLA-4 on the T cell competes with CD28 for binding to B7 on the tumor cell, eliminating the costimulatory signal required for T cell activation and causing T cell apoptosis. TCR = T cell receptor, pMHC-I = peptide-presenting major histocompatibility complex I, AP-1 = activator protein 1, NFAT = nuclear factor of activated T cells, NF-κB = nuclear factor-kappa B, CTLA-4 = cytotoxic T-lymphocyte associated protein 4, PD-1 = programmed cell death protein 1, PD-L1 = programmed death ligand 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

associated with poor clinical prognosis. To successfully metastasize to bone, disseminated tumor cells must go through the traditional steps of metastasis in which they travel from the primary site, circulate through the bloodstream, and ultimately reach a distant secondary site where they colonize the tissue [16]. Tumor cells home to the bone through surface expression of C-X-C motif chemokine receptor 4 (CXCR4) and C-X-C motif chemokine receptor 7 (CXCR7) [17,18], receptors which follow the C-X-C motif chemokine ligand 12 (CXCL12) gradient produced by endothelial cells and osteoblasts [19–21]. Once in the bone, tumor cells enhance their survival and colonization of the bone by establishing a vicious cycle of osteolysis that fuels tumor growth. In this cycle, tumor cells secrete parathyroid hormone-related protein (PTHrP) [22,23] which binds to its receptor (parathyroid hormone receptor 1 [PTHR1]) on osteoblasts [24]; this stimulates receptor activator of nuclear factor kappa-B ligand (RANKL) production and secretion by osteoblasts [25,26]. RANKL binds to its receptor (receptor activator of nuclear factor kappa-B [RANK]) on osteoclast precursors, resulting in the formation of mature osteoclasts capable of resorbing bone [27]. As the bone is resorbed, growth factors including transforming growth factor beta (TGF-β) [28–30] and insulin-like growth factor 1 (IGF-1) [31] are released from the bone matrix and interact with disseminated tumor cells, triggering tumor cell growth and proliferation. This cycle repeats, fueling tumor colonization and bone resorption that together contribute to tumor-induced bone disease (Fig. 2).

1.3. Clinical analysis of ICIs in bone metastatic patients

Treatment for bone metastases can be divided between treatment targeting the tumor and treatment targeting tumor-induced bone disease. Anti-cancer treatment traditionally includes chemotherapy, surgery, and radiation therapy [32], while treatments to mitigate tumor-induced bone disease are limited to anti-resorptive drugs [33]. The most commonly used anti-resorptive drugs are denosumab, a RANKL monoclonal antibody traditionally used to treat osteoporosis [34], and bisphosphonates (e.g. zoledronic acid), which slow bone degradation by inducing osteoclast apoptosis [35]. Although these drugs work to counteract bone loss, they do not actively target cancer cells and therefore must be used in combination with another anti-cancer drug to reduce tumor burden.

Many cancers with high rates of bone metastases (e.g., breast and prostate cancer) have low response rates to ICI therapy. Furthermore, tumor types found to be responsive to ICI therapy (e.g., melanoma, RCC, etc.) appear to have inferior outcomes when associated with bone metastases. While there is ample evidence to suggest that the tumor-bone microenvironment may limit ICI efficacy, it is important to note that most studies have been retrospective or post-hoc analyses restrained by

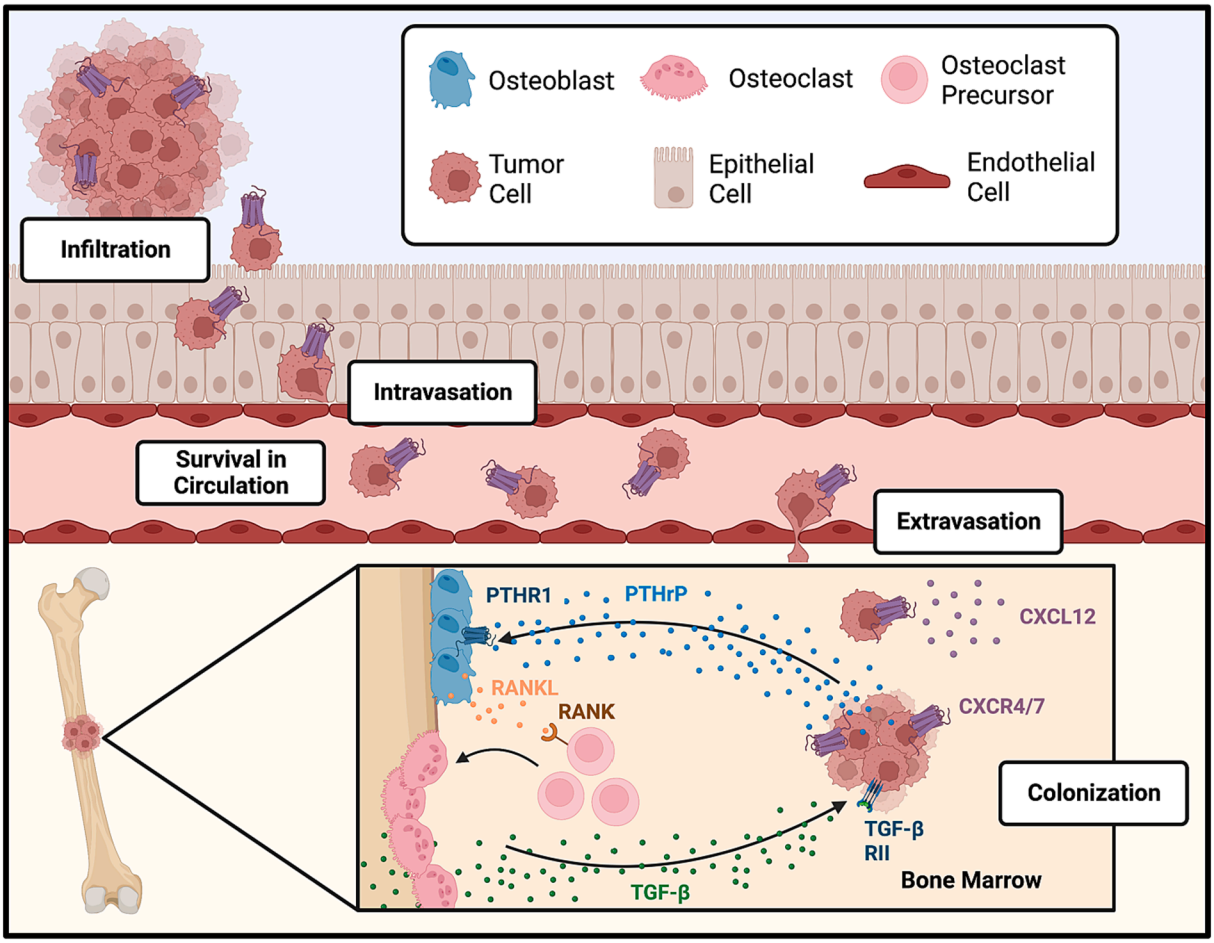

Fig. 2. Tumor metastasis to bone. To metastasize to bone, tumor cells move through the stages of metastasis including infiltration into surrounding tissue, intravasation into the bloodstream, survival in circulation, extravasation out of the bloodstream, and colonization of the bone marrow and matrix. In the bone, tumor cells contribute to the vicious cycle of bone metastasis by secreting PTHrP which triggers release of RANKL from osteoblasts. RANKL stimulates osteoclastogenesis and associated bone resorption, causing the release of growth factors including TGF-β and IGF-1 from the bone matrix which fuel tumor cell growth. PTHrP = parathyroid hormone-related protein, PTHR1 = parathyroid hormone receptor 1, RANK = receptor activator of nuclear factor kappa-B, RANKL = receptor activator of nuclear factor kappa-B ligand, TGF-β RII = transforming growth factor beta receptor II, TGF-β = transforming growth factor beta, IGF-1 = insulin-like growth factor 1, CXCR4 = C-X-C motif chemokine receptor 4, (CXCR7) = C-X-C motif chemokine receptor 7, CXCL12 = C-X-C motif chemokine ligand 12.

sample size, thus limiting definitive conclusions.

1.4. Therapies targeting PD-1

Nivolumab is one of the most commonly administered FDA-approved α-PD-1 drugs that binds to and blocks PD-1 commonly expressed on monocytes, B cells, natural killer (NK) cells, macrophages, and activated T cells [36–38]. The PD-1 cognate ligands, PD-L1 and PD-L2, are expressed on dendritic cells, macrophages, and tumor cells [39,40]. Circulating T cells can rapidly express PD-1 upon TCR-mediated activation [39,41] or exposure to various cytokines [42,43]; however, PD-1 is sustained on antigen-specific T cells [44], indicating that PD-1 expression can reflect the status of T cell activation. There are multiple mechanisms by which PD-1 suppresses immunity and promotes disease progression, but the main avenue is through inactivation of T cells by blocking signaling downstream of TCR activation [45,46]. One study examining non-small cell lung cancer (NSCLC) patients with non-squamous-cell histology treated with nivolumab found that the presence of bone metastases, compared to the absence, decreased overall survival (OS) from 15.3 months to 7.4 months [47]. Overall survival was also decreased in bone metastatic NSCLC patients with squamous-cell histology treated with nivolumab compared to patients without bone metastases (10.9 months vs 5.0 months, respectively) [47]. A retrospective study examining 144 NSCLC patients found that bone metastases were independently associated with inferior OS and progression free survival (PFS), and that bone metastatic tumors were significantly associated with an immunologically "cold" phenotype (i.e., low PD-L1 expression and reduced infiltration of $CD3^+$ T lymphocytes, $CD8^+$ cytotoxic T cells, $CD68^+$ tumor-associated macrophages (TAMs) and $Foxp3^+$ Tregs) [48]. These studies suggest that α-PD-1 treatment may not be effective at reducing tumor burden in patients with bone metastases, specifically in NSCLC patients (Table 1).

For multiple tumor types including lung cancer, ICIs are commonly used in combination with chemotherapy, but ICI use as a first-line treatment without concurrent chemotherapy is becoming more prevalent [49,50]. An example of this is in a study examining colorectal cancer patients receiving pembrolizumab (α-PD-1) followed by post-treatment surgical resection in which 79 % of patients reached pathologic complete response [51]. While ICI single agent therapy (e.g., α-PD-1, α-PD-L1, or α-CTLA-4 without chemotherapy) works well in many tumor types, patients with bone metastases do not typically achieve the same clinical response as patients without bone metastases. A retrospective study of stage IV NSCLC patients found that in 101 patients treated with ICI agents in combination with chemotherapy, there was no significant difference in PFS or OS between patients with bone metastases and those without bone metastases; however, in 103 patients who received ICI single agent therapy, patients with bone metastases had significantly lower PFS (4.2 vs 6.7 months) and OS (12.5 months vs 23.9 months) compared to patients without bone metastases [52]. These data suggest that in NSCLC, chemotherapy may alter the tumor microenvironment in such a way that benefits ICI efficacy. In a separate study, urothelial cancer patients with bone metastases treated with single agent ICI monotherapy, after failure of at least one line of previous chemotherapy, had a shorter median OS compared to patients without bone metastases (3.9 vs 7.8 months, respectively) [53] (Table 1). These data cannot be directly compared to the NSCLC study since the patients with urothelial cancer had previously failed chemotherapy, which may have resulted in more resistant, aggressive tumors. Nonetheless, these data suggest that patients treated with ICIs as a single agent have a poorer prognosis if they present with bone metastases.

1.5. Combination therapies targeting PD-1/CTLA-4

Combining PD-1 blockade (nivolumab) with CTLA-4 blockade (ipilimumab), termed ipi/nivo, is now standard of care in multiple different cancers and is associated with higher response rates compared with ICI monotherapy. CTLA-4 is expressed by many immune cell types including NK cells, T memory cells, T regulatory cells, and other T cell subsets [54]. Upon a prolonged TCR activation stimulus, CTLA-4 is transported to the immunological synapse [12] where it out-competes CD28 for binding to B7 ligands [55,56]; disruption of the CD28 costimulatory signal results in inhibition of T cell signaling [46] and a reduced immune response to tumor antigens. A recent retrospective study of renal cell carcinoma (RCC) bone metastatic patients treated with ipi/nivo identified a relatively low response rate (21 %) and median OS (25.6 months) [57], indicative of poor clinical outcome. Supporting these results, another study in advanced RCC found that patients with bone metastases receiving ipi/nivo had a lower 12-month OS rate (41.7 %) compared to patients without baseline bone metastases (82.7 %) treated with ipi/nivo [58]. Taken together, these studies suggest bone metastatic patients are less responsive to combination treatment with PD-1 and CTLA-4 inhibitors than non-bone metastatic patients.

While much of the clinical data has been reported in patients with NSCLC, use of ICIs as a monotherapy or combination therapy (e.g., with other ICIs or standard of care [e.g., chemotherapy, radiation therapy]) is common in cancers that form bone metastases at high frequencies (e.g., breast, prostate, etc.). In January 2022, almost 300 trials targeting PD-1/PD-L1/CTLA-4 in breast cancer were enrolling patients [59]; as of 2021, 6 ongoing trials targeting metastatic castrate resistant prostate cancer with ICI monotherapy or combination therapy had enrolled more

Table 1
Summary of clinical data reporting on patients presenting with bone metastases and treated with ICIs. Overall, the data indicate that ICIs are ineffective at treating bone metastases. NSCLC = non-small cell lung cancer, RCC = renal cell carcinoma, OS = median overall survival, ICI = immune checkpoint inhibitor.

Clinical Studies					
Cancer Type	**Treatment**	**Number of Patients**	**Outcome in patients with bone metastases *Compared to patients with no bone metastases**	**P-Value**	**Reference**
Non-squamous NSCLC	Nivolumab	626 BoM+ 962 BoM-	7.9 month decrease in OS*	$p < 0.0001$	[47] – Landi, et al, *J Immunother Cancer*, 2019
NSCLC	Nivolumab	59 BoM+ 78 BoM-	OS not achieved*	$p < 0.0001$	[48] – Zhu, et al, *Lung Cancer*, 2022
	Unspecified ICI	103 total	11.4 month decrease in OS*	$p = 0.0036$	[52] – Li, et al, *Thorac Cancer*, 2020
Squamous NSCLC	Nivolumab	120 BoM+ 251 BoM-	5.9 month decrease in OS*	$p < 0.0001$	[47] – Landi, et al, *J Immunother Cancer*, 2019
Urothelial	Unspecified ICI	86 BoM+ 122 BoM-	3.9 month decrease in OS*	$p = 0.005$	[53] – Raggi, et al, *Clin Genitourin Cancer*, 2022
RCC	Ipilimumab/ Nivolumab	80 BoM+	25.6 month OS (relatively low response rate)	N/A	[57] – Desai, *Target Oncol*, 2021
	Ipilimumab/ Nivolumab	9 BoM+ 27 BoM-	41% decrease in 12-month OS rate*	$p = 0.021$	[58] – Pham, et al, *Biomedicines*, 2022

than 1,600 patients, and 13 trials including more than 4,000 patients had been completed [60]. Despite the clinical relevance of using ICIs in these cancers, there is a lack of studies reporting on the relationship between bone metastatic patients and response to ICIs in these tumor types. For future studies, it will be important to account for PD-L1 expression levels both globally and in the bone marrow and to monitor bone mineral density (BMD), bone microarchitecture, or serum markers of bone turnover, when clinically available. Taken together, the current data suggest that ICIs alone or in combination are comparably less effective at treating and eliminating bone metastases (Table 1). However, some patients with ICI-responsive tumor types and bone metastases do experience durable responses. Additional clinical and preclinical follow-up studies are warranted to identify the mechanisms for ICI resistance or sensitivity of tumor cells in the bone marrow.

1.6. ICIs in combination with bone targeted therapies

Since current data suggest that bone metastases negatively impact prognosis in patients treated with ICIs, it follows that investigation would shift towards combination therapy of ICIs with anti-resorptives commonly used to treat tumor-induced bone disease (i.e., denosumab and bisphosphonates). A retrospective study on NSCLC patients presenting with bone metastases treated with an ICI plus denosumab showed that duration of concurrent treatment positively correlated with median OS; patients treated with combination therapy for less than 3 months had a median OS of 3.6 months whereas patients treated for more than 3 months had a median OS of 11.5 months [61]. While selection bias may play a role in this result, it could also reflect the importance of the relationship between T lymphocytes and mature osteoclasts / osteoclast precursors for bone remodeling [62–66], which may be a critical mechanism for the poor prognosis in bone metastatic patients treated with ICIs. Also in bone metastatic NSCLC patients, median OS was increased from 15.8 months to 21.8 months when patients were treated with an ICI alone versus with combination ICI therapy and a bone targeted therapy such as denosumab or zoledronate [67]. Similar results are shown in an advanced NSCLC study which found that patients treated with zoledronic acid in combination with an α-PD-1 had a 16.7 month median OS compared to a 12.8 month median OS in patients treated with the ICI alone [68], although this difference was not statistically significant. In contrast to these studies, PFS was significantly lower in patients treated with nivolumab who had bone metastases compared to patients without bone metastases, and combination treatment with nivolumab and denosumab across multiple tumor types did not improve progression free survival; however, this study spanned patients with renal cell carcinoma, melanoma, NSCLC, gastric cancer, and head and neck cancer, and the outcomes were not reported for individual tumor types [69]. Collectively, these data suggest that ICIs in combination with a bone targeted treatment may increase patient survival in NSCLC (Table 2); however, it is unclear whether this is through an effect on the tumor itself or on the bone. There is a distinct lack of clinical and pre-clinical studies looking at the effects of ICIs in combination with bone targeted treatments in other cancer types, but these studies will be critical in determining how to improve survival for the substantial number of oncology patients who are receiving ICI therapy and have bone metastatic disease.

Success of ICI therapy can be attributed partially to immune infiltration into the primary and metastatic tumors. NSCLC is a characteristically “hot” tumor microenvironment, in which there are tumor infiltrating lymphocytes (TILs) present in the tumor; however, immunofluorescence for 10 immune antibodies revealed NSCLC bone metastases as immunologically "cold," correlating with a lack of efficacy of immune checkpoint inhibitors in these patients [48]. It is important to note that the bone contains multiple niches and its mineralized nature makes immunohistochemistry (IHC) challenging; this has at times limited our understanding of tumor cell behavior in bone and our ability to interpret findings, since staining patterns in tumor cells residing along the endosteal bone surface can vary dramatically from tumor cells residing in the marrow or perivascular niche [70]. Other common bone metastatic cancers including breast and prostate cancer tend to be cold tumors lacking TILs. For example, only a minority of patients with triple negative breast cancer (TNBC) present with TIL expansion; however, patients with TNBC often have upregulation of PD-1 on T cells and PD-L1 on tumor cells [71]. This may partly explain the modest efficacy of ICIs in TNBC and supports further studies of ICI-based combination approaches. Given that the bone is a common site of metastasis in NSCLC and breast cancer patients, further investigation is warranted into the lack of function of ICIs in the bone microenvironment and if immunologically cold tumors with upregulated immune checkpoint protein expression can still be targeted with ICI therapy.

1.7. Low PD-L1 positivity in bone metastatic patients

PD-L1 positivity rate is commonly used as a predictive marker of success for administration of α-PD-1 treatment. Most of the clinical studies reporting on the effects of ICIs on patient survival in the bone metastatic setting stated “failure to examine PD-L1 expression” as a limitation, although one group reported low PD-L1 expression in NSCLC patients with bone metastases compared to patients without bone metastases [52]. This suggests that the lower median OS in patients treated with ICIs who have bone metastases compared to those without bone metastases may be due to low PD-L1 expression in bone-disseminated tumor cells.

Table 2
Summary of clinical and preclinical data reporting on treatment of bone metastases with co-administration of ICI therapy and a bone targeted therapy. The data indicate that combination treatment shows positive results in improving the prognosis of bone metastatic patients. OS = median overall survival, BTT = bone targeted therapy, NSCLC = Non-small cell lung cancer, mCRPC = metastatic castration resistant prostate cancer, ICI = immune checkpoint inhibitor.

Clinical Studies

Cancer Type	Combination Treatment	Number of Patients	Outcome in patients with bone metastases	P-Value	Reference
NSCLC	Denosumab + ICI	40 BTT + ICI	7.9 month increase in OS with >3 months treatment	p = 0.0005	[61] – Cao, et al, *J Thorac Dis*, 2021
	BTT (Denosumab or Zoledronic Acid) + ICI (Nivolumab, Pembrolizumab, or Atezolizumab)	16 ICI alone 30 BTT + ICI	6 month increase in OS compared to ICI alone	p < 0.001	[67] – Bongiovanni, et al, *Front Immunol*, 2021
	Zoledronic acid + α-PD-1 (Pembrolizumab, Sintilimab, Camrelizumab, or Nivolumab)	29 ICI alone 52 BTT + ICI	NS change in OS compared to ICI alone	p = 0.101	[68] – Zheng, et al, *Int Immunopharmacol*, 2022

Preclinical Studies

Cancer Type	Combination Treatment	Number of Samples	Outcome in mice with bone metastases	P-Value	Reference
Breast Cancer	α-PD-1 + Zoledronic acid	n = 10 mice/group	Lower bone tumor volume than α-PD-1 alone	p < 0.05	[96] – Li, et al, *BMC Cancer*, 2018
mCRPC	α-PD-1/α-CTLA-4/α-TGF-β	Data not provided	Higher percent survival than α-CTLA-4/α-PD-1	p < 0.0001	[97] – Jiao, et al, *Cell*, 2019

PD-L1 positivity rates in metastatic breast cancer have been investigated by quantifying the total number of PD-L1 positive tumor cells and immune cells. There is only 12% pooled PD-L1 positivity in bone metastases compared to the primary tumor and other common sites of metastasis, which ranged from 19% to 60% positivity [72]. Thus, bone-disseminated breast cancer cells have lower PD-L1 expression, but whether PD-L1 suppression is caused by the bone microenvironment or is due to PD-L1^{low} tumor cells having a greater propensity to disseminate to bone is an intriguing unanswered question. A separate study found an immune cell PD-L1 positivity rate of 16.7% in breast cancer bone metastases compared with other metastatic sites ranging from 17.4% in the liver to 68.8% in the lungs [73]. These data identify the bone as being a metastatic site with characteristically low PD-L1 expression in both tumor and immune cells, providing evidence for the lack of function of α-PD-1 therapy in bone metastatic patients, specifically, and suggest that low PD-L1 expression may be a product of the bone microenvironment.

2. Immune related adverse events associated with ICI therapy

2.1. Commonly reported ICI-induced irAEs

ICIs frequently induce primary tumor regression; however, they also correlate with immunologic toxicities stemming from inflammation and immune cell expansion. These immune-related adverse events (irAEs) are associated with development of a robust immune response that correlates with anti-tumor activity and better treatment efficacy [74,75]. The type and severity of irAEs vary according to the type of ICI therapy administered. Up to 60% of patients receiving ipilimumab develop irAEs, with 30% of patients developing irAEs of grade 3 or higher, and the severity of the irAE oftentimes positively correlates with the dose and duration of treatment [76]. Patients treated with α-PD-1 (e.g., nivolumab or pembrolizumab) are at a lower risk for irAEs compared to patients treated with α-CTLA-4, with only 10–15% of patients having irAEs of grade 3 or higher [76,77]. Ipi/Nivo combination therapy increases the prevalence and severity of irAEs over treatment with a single agent [76]; depending on the primary tumor type, the prevalence of severe irAEs in patients receiving combination therapy can range from 46% to 55% [78–80]. Common ICI-associated irAEs include cutaneous, gastrointestinal, pulmonary, and endocrine toxicities [76,81–83]. Despite this wide range of toxicities, many can be controlled for without stopping ICI therapy with close observation and symptomatic management [84].

2.2. Musculoskeletal ICI-induced irAEs

In addition to the commonly reported ICI-induced toxicities, patients also present with rheumatologic and musculoskeletal toxicities including arthralgias, which present in up to 43% of patients, and rheumatoid arthritis, which presents in 1–7% of patients [85–89]. There are also reports of bone loss and fractures following treatment with ICIs. These adverse events appear to be increasing in prevalence as patients treated with ICIs are living longer but suffering from the long-term effects of these irAEs. An analysis of the FDA adverse event reporting system found that patients with no underlying risk factors receiving ICIs had a significant increase in pathological fractures compared to age-matched peers, regardless of whether they had bone metastases; furthermore, almost 33% of patients treated with ICIs who reported a bone or joint injury died [90]. A recent study determined the risk of developing a fracture to be approximately 20% higher in the first year after starting on ICI therapy compared to the year prior to starting therapy. Furthermore, fractures accounted for more than 60% of injuries in α-PD-1-treated individuals [90–92]; these patients had a significant increase in spinal compression and femoral neck, vertebral, thoracic, pathological, and osteoporotic fractures [90]. In addition to fractures, clinical case reports have found that patients treated with α-PD-1 or α-PD-1/α-CTLA-4 can present with generalized bone loss and/or focal bone lesions not due to tumor-induced osteolysis [91], indicating an effect of PD-1 blockade on the bone remodeling process. These data suggest a possible negative impact of ICI therapy on the bone microarchitecture, potentially via disruption of bone remodeling, which could correlate with the lack of function of ICIs in bone metastases; however, there is a lack of knowledge concerning the mechanisms involved, and the role of the immune system in bone metastases remains understudied. One group analyzed changes in serum bone turnover markers in advanced NSCLC and RCC patients without bone metastases after 3 months of ICI treatment and found a trend towards a decrease in P1NP (N-terminal propeptide of type I procollagen) and significantly increased CTX-I (type I collagen C-terminal telopeptide) compared to serum bone turnover levels assessed prior to initiating ICI therapy [93]. Furthermore, the increase in serum CTX-I levels correlated with poor clinical prognosis [93]. These data suggest ICIs cause an increase in bone resorption possibly coupled with a decrease in bone formation, independent of bone metastases, which may contribute to the elevated fracture risk and lack of bone-metastatic tumor responses reported in patients treated with ICIs. A preclinical study that investigated the effects of PD-1 on the skeleton found that both PD-1 global genetic knockout and pharmacologic blockade (i.e. nivolumab) had no impact on bone volume in young male tumor naïve mice, but rescued the low bone volume phenotype induced after inoculation with Lewis Lung carcinoma cells, suggesting that PD-1 signaling has negative effects on bone microarchitecture in a bone-tumor environment [94]. Another preclinical study investigated the effects of PD-1 and PD-L1 blockade in an inflammatory arthritis model and observed that PD-1 and/or PD-L1 knockout in young male mice resulted in a significant reduction of bone volume and trabecular thickness and an increase in trabecular separation, overall suggesting a bone loss phenotype when immune checkpoint proteins are inhibited [95]. This study contradicts Wang *et al* [94] and suggests that PD-1 and PD-L1 are essential for maintaining bone homeostasis, at least in settings of chronic inflammation, and appears to more closely align with clinical reports of bone and joint injuries and increased fracture risk in patients receiving ICIs [85–91]. These contradictory data, combined with the overall lack of studies investigating the role of ICIs in altering bone microarchitecture, particularly in female pre-clinical models, highlight a need for a more comprehensive examination of this relationship.

2.3. Preclinical models investigating ICIs in combination with bone targeted therapies

In addition to clinical studies, the combination of ICIs and bone targeted therapies has also been investigated in pre-clinical models. In a murine model of bone metastatic breast cancer, α-PD-1 treatment in combination with zoledronic acid produced a better anti-tumor response compared to α-PD-1 treatment alone, evident by decreased tumor burden in bone as assessed by bioluminescence imaging [96]. Another preclinical study investigated combination therapy with α-PD-1/α-CTLA-4/α-TGF-β in metastatic castration resistant prostate cancer (mCRPC) and found decreased growth of bone metastases and a higher percent OS compared to the α-PD-1/α-CTLA-4 treated group [97]. TGF-β inhibitors increase bone mass and strength [98] by inducing expression of factors important in osteoblast differentiation (e.g., Runx2 and EphB4) and by reducing osteoclast differentiation and activity [99]. There is conflicting information concerning the benefits of TGF-β inhibition in patients with osteolysis and bone metastatic disease. It has been observed that the use of TGF-β inhibitors in myeloma bone disease protects the bone without eliminating tumor burden [100], but data in other solid tumor models suggest that inhibition of TGF-β, a key player in the vicious cycle of bone metastasis, has success in reducing bone metastatic tumor burden in combination with ICIs [101,102]. While capable of reducing tumor burden, TGF-β inhibition has also been linked to promotion of epithelial-mesenchymal transition and immune suppression, thereby enhancing the metastatic progression of tumor cells in

the bone [103]. The likelihood that TGF-β inhibitors will progress clinically is low, but taken together, these data suggest that combination treatment with ICI therapy and bone-targeted agents shows promising results for patient survival (Table 2).

3. Immune cells in bone metastasis and tumor-induced bone disease

3.1. Immune cells in bone remodeling

Although there is clinical evidence that ICIs are less effective in the bone metastatic setting, we have a limited understanding of how immune cells in the bone marrow influence tumor progression in bone. This is in part due to the difficulty associated with preclinical modeling of bone metastases, which has heavily relied on immune-compromised xenograft models since these provide the most robust studies in which to examine mechanisms of bone metastasis in human cells [104]. As murine carcinoma cell lines become more widely used for modeling bone metastases in immunocompetent mice, we will discover more about the complex interactions between immune cells and the bone microenvironment.

Immune cells have already been established to play an important role in bone remodeling [105]; however, studies investigating the mechanisms behind these interactions are limited. Bone remodeling and tumor-induced bone destruction are dependent on osteoclast maturation, which typically begins with myeloid lineage cells, notably monocytes, that mature into osteoclast precursors in the presence of macrophage colony stimulating factor (M-CSF); these precursors eventually fuse to create mature osteoclasts in the presence of RANKL. Aside from this differentiation pathway, there are alternative myeloid lineages that can contribute to osteoclast maturation. In addition to promoting inflammatory T cell responses, bone marrow derived dendritic cells cultured *ex vivo* can also contribute to the formation of CX_3CR1^+ inflammatory osteoclasts capable of expressing functional MHCII to activate T cells [106]. Whether dendritic cells act as osteoclast precursors and generate pro-inflammatory osteoclasts *in vivo* remains unclear. Bone marrow derived macrophages can be polarized into their pro-inflammatory M1 phenotype through *ex vivo* stimulation by sustained RANKL expression, after which they can differentiate into osteoclasts [107]. In conditions of chronic inflammation, macrophages can also release cytokines and molecules including tumor necrosis factor α (TNFα), interleukin 6 (IL-6), and reactive oxygen species (ROS) that contribute to osteoclastogenesis [108,109]. Myeloid derived suppressor cells (MDSCs), immature myeloid lineage cells capable of immunosuppression, have also been shown to differentiate into osteoclasts in the presence of RANKL and M-CSF [110–112]. Aside from myeloid lineage cells, T cells can be activated by IGF-1 released by bone matrix degradation [113], resulting in production of RANKL and TNFα by T cells which can trigger osteoclastogenesis [114–116]. Taken together, this suggests the importance of myeloid and T cells in osteoclastogenesis. Given the importance of osteoclasts in tumor-induced bone disease and progression of bone metastases, these cell populations likely contribute to tumor growth and proliferation by modifying the bone microenvironment in the context of ICIs (Fig. 3).

3.2. T Cells in bone metastasis

Various immune cell populations are demonstrated to play a role in

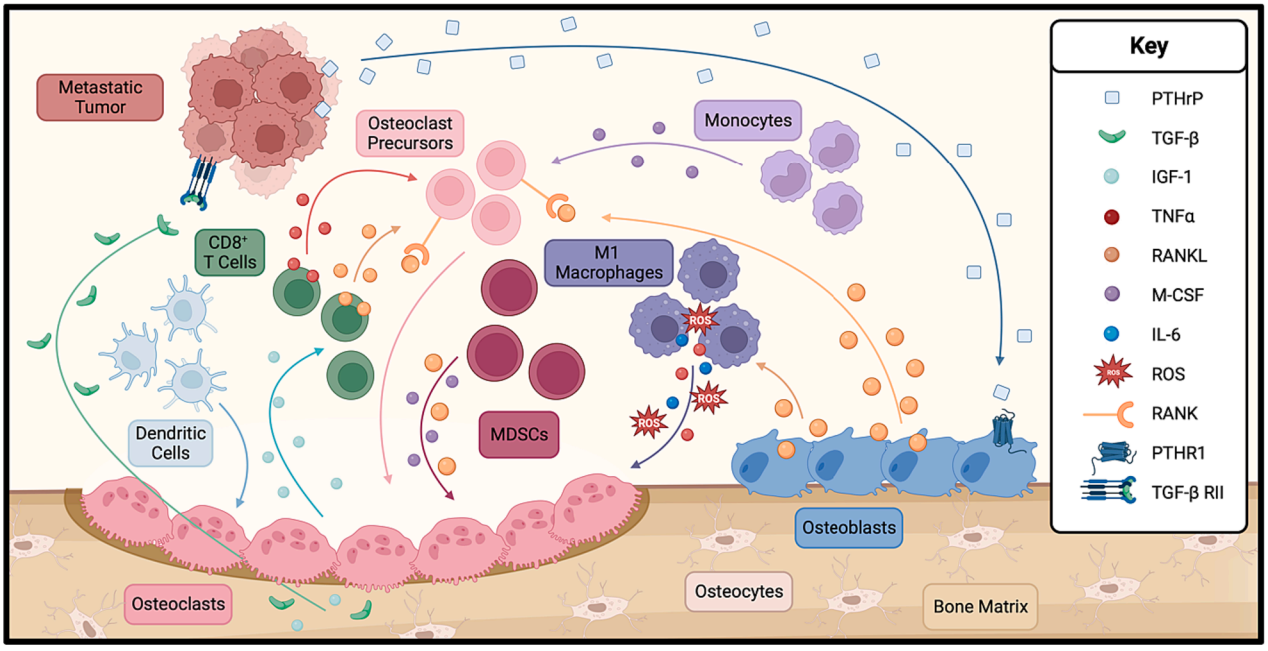

Fig. 3. Immune involvement in the vicious cycle of bone destruction. Metastatic tumors (red) release PTHrP (blue squares) which bind to PTHR1 (blue receptor) to stimulate the release of RANKL (orange circles) from osteoblasts (blue). RANKL binds to RANK (orange receptor) on osteoclast precursors (light pink), triggering the formation of mature osteoclasts (pink, multinucleated) and associated bone resorption. TGF-β (green) is released from the bone matrix and binds to its receptor, TGF-β RII, on tumor cells to stimulate growth and the further release of PTHrP from the tumor. Monocytes (light purple) differentiate into osteoclast precursors in the presence of M-CSF (purple circles). RANKL secreted from osteoblasts can stimulate M1 macrophages (dark purple) to differentiate directly into mature osteoclasts through the production of ROS, TNFα (red circles), and IL-6 (dark blue circles). MDSCs (magenta) can differentiate directly into mature osteoclasts in the presence of RANKL and M-CSF. IGF-1 (light blue circles) released from the bone matrix during bone resorption can stimulate $CD8^+$ T cells (green), which in turn can release TNFα and RANKL to trigger osteoclastogenesis. Dendritic cells (light blue) may directly differentiate into inflammatory osteoclasts. PTHrP = parathyroid hormone-related protein, PTHR1 = parathyroid hormone receptor 1, RANKL = receptor activator of nuclear factor kappa-B ligand, RANK = receptor activator of nuclear factor kappa-B, TGF-β = transforming growth factor beta, TGF-β RII = transforming growth factor beta receptor type II, M-CSF = macrophage colony-stimulating factor, TNFα = tumor necrosis factor alpha, ROS = reactive oxygen species, IL-6 = interleukin-6, MDSC = myeloid derived suppressor cell, IGF-1 = insulin-like growth factor 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

bone homeostasis, especially in osteoclastogenesis; however, only a handful of preclinical studies have been performed to investigate the effects of these populations in bone metastasis (Summarized in Fig. 4). $CD8^+$ T cells stand out as being a major player in osteoclast maturation, but there is conflicting evidence on whether they play a pro- or anti-osteoclastogenic role. In a 4T1 breast cancer model, $CD8^+$ T cells in the bone increase osteoclast formation and drive bone metastasis, but this is specific to non-activated T cells; active $CD8^+$ T cells actually inhibit osteoclast formation in this model [117]. Furthermore, tumor volume increases upon treatment with CD4 and CD8 neutralizing antibodies [117], further emphasizing the importance of T cells in the development of osteolytic bone metastases. This is relevant in the tumor microenvironment where T cell populations are expected to be in an active state; however, PD-L1-expressing tumor cells can activate PD-1 on T cells and suppress T cell activation [118], possibly leading to pro-osteoclastogenic effects that may help to fuel tumor-induced bone destruction. A separate study revealed that bone marrow derived $CD8^+$ T cells primed by non-metastatic primary breast tumor antigens can interfere with bone homeostasis by producing anti-osteoclastogenic cytokines and low levels of RANKL [119]. They observed that bone marrow derived $CD4^+$ T cells primed by antigens from metastatic primary breast tumors produce low levels of anti-osteoclastogenic cytokines and high levels of RANKL [119]. The authors also indicate that $CD8^+$ regulatory T cells are capable of suppressing $CD4^+$ pro-osteoclastogenic cells, suggesting opposing roles for $CD8^+$ and $CD4^+$ T cells in metastatic versus non-metastatic tumors, respectively, in the formation of bone metastases [119]. The non-metastatic specific $CD8^+$ T cells inhibit primary tumor growth and metastatic colonization, further emphasizing the complex interplay between $CD8^+$ and $CD4^+$ tumor-specific T cell populations. These findings are intriguing and introduce a potentially complicated model, in which tumors with different metastatic potential within the same patient might oppositely regulate bone metastases through different T cell subsets. It will therefore be important to assess whether this model persists in patients. In addition to having effects on the bone metastatic niche, there is also evidence that T cells can stimulate osteolytic bone disease prior to tumor colonization of the bone. Tumor-specific T cells, primed by antigen from 67NR and 4T1 breast cancer cell lines, produce RANKL [120]. Furthermore, inhibition of T cell-derived RANKL *in vivo* prevents bone loss and metastasis as evident by a decrease in metastatic clones in the iliac bone marrow [120], suggesting that RANKL produced by T cells may contribute to the pro-metastatic niche in the bone. Interestingly, contrasting evidence has shown that $CD8^+$ T cells regulate the development of bone metastases independent of osteoclastogenesis and osteoclast-mediated bone resorption in a B16 melanoma model [121]. When mice with dysfunctional osteoclasts and increased tumor burden are injected with antigen specific $CD8^+$ T cells, tumor growth is normalized; similarly, when mice with hyperactive osteoclasts and reduced tumor burden are depleted of $CD8^+$ T cells, tumor growth is normalized [121]. Taken together, these data suggest that T cells, specifically $CD8^+$ subsets, can have important effects on osteoclastogenesis and bone homeostasis and may prime the bone metastatic niche for metastasis.

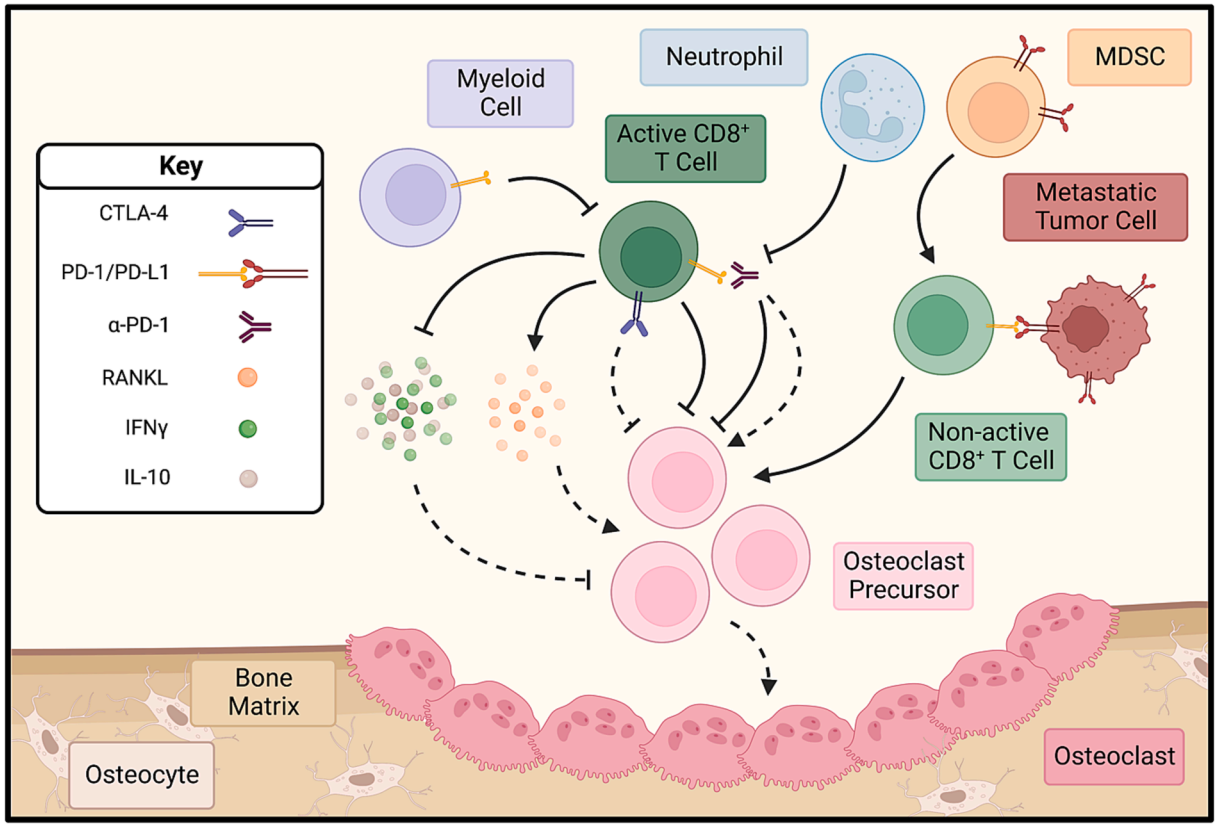

Fig. 4. Current understanding of immune involvement in tumor-induced bone disease and osteoclastogenesis. Active (dark green) and non-active (light green) $CD8^+$ T cells both appear to play a role in osteoclastogenesis and tumor induced bone disease. Non-active $CD8^+$ T cells, potentially developing from interactions with MDSCs (orange) and tumor cells (red), promote osteoclastogenesis. Conflicting information about $CD8^+$ T cells activated with tumor antigen suggests that these cells can both inhibit osteoclastogenesis and promote the production of pro-osteoclastogenic RANKL (orange circles) and inhibit the production of anti-osteoclastogenic IFNγ and IL-10 (green and grey circles). Neutrophils (blue) can inhibit the action of ICIs and prevent T cell activation, similar to PD-1 on myeloid (purple) cells. CTLA-4 on active T cells can inhibit osteoclastogenesis. PD-1 blockade can have differential effects on osteoclastogenesis depending on if administered in a tumor-bearing or tumor-naïve setting. MDSC = myeloid derived suppressor cell, RANKL = receptor activator of nuclear factor kappa-B ligand, IFNγ = interferon gamma. Solid lines = tumor dependent effects on osteoclastogenesis, dashed lines = tumor independent effects on osteoclastogenesis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. PD-1 and PD-L1 signaling in bone metastasis

In addition to immune cell lineages affecting osteoclastogenesis, immune checkpoint proteins themselves (e.g., PD-1, PD-L1) directly regulate bone homeostasis, which could impact tumor progression. PD-1 global knockout inhibits osteoclastogenesis and rescues bone loss in mice challenged with Lewis lung carcinoma (LLC) cells by intra-femoral injection without altering tumor burden [94], suggesting that PD-1 blockade may be beneficial for tumor-induced bone destruction, but ineffective at eliminating tumor burden in bone. Since PD-1 blockade also temporarily increases inflammation, it is unclear whether the effects of α-PD-1 therapy on the bone microenvironment are due to direct effects of disrupted PD-1/PD-L1 signaling or elevated inflammation. In an inflammatory rheumatoid arthritis model, PD-1 and PD-L1 global knockout mice have increased RANKL production and osteoclast formation, and reduced trabecular bone volume [95]; however, there was no difference in inflammation between control mice and PD-1 or PD-L1 knockout mice, suggesting that the effects of ICIs are signaling-specific, and not due to changes in inflammation. Notably, the authors compared their data to Wang *et al* who identified that PD-1 blockade decreased bone destruction in a tumor inoculation model [94]. The contradictory results suggest that PD-1 could have different roles depending on the source of inflammation (e.g., tumor-induced inflammation versus chronic joint inflammation). One limitation of these studies is the use of global PD-1 and PD-L1 knockout models as opposed to pharmacologic inhibitors, which appropriately model patients being treated with ICIs. Additionally, most studies have used global, rather than conditional, PD-1 or PD-L1 knockout models, which would identify the cell lineage(s) responsible for driving the pro-osteoclastogenic effects seen after immune checkpoint blockade. As such, further investigation needs to be performed to evaluate the intricacies with which immune checkpoint proteins, notably PD-1 and PD-L1, affect bone homeostasis.

3.4. CTLA-4 signaling in the bone microenvironment

The immune checkpoint protein CTLA-4 also has effects on the bone microenvironment, but there is a lack of data investigating the effects of CTLA-4 signaling in bone metastasis. Similar to the function of PD-1 described in LLC bone metastases, CTLA-4 inhibits RANKL and TNF-mediated osteoclastogenesis by blocking monocyte precursor differentiation into osteoclasts [122]. Furthermore, CTLA-4 interacts with costimulatory molecules (e.g., CD80/86) and acts as a negative regulatory signal for osteoclastogenesis (Fig. 4) [123]. Thus, blocking CTLA-4 could also have detrimental effects on bone remodeling and microarchitecture, but the impact of this in a bone metastatic setting is unknown.

3.5. ICI mechanisms of resistance

Aside from its canonical function in T cell suppression, PD-1 has also been shown to alter myeloid cell effector function. Notably, PD-1 inhibits signaling pathways in myeloid cells that are important for T cell activation, suggesting that myeloid specific PD-1 blockade may subvert myeloid cell-mediated immunosuppression [124] (Fig. 4). As a potential anti-tumor therapy, myeloid cell-specific PD-1 genetic deletion more effectively decreased tumor growth in a B16-F10 melanoma model compared to T cell-specific PD-1 deletion [124]. Despite this, accumulation of myeloid lineage neutrophils in the tumor microenvironment correlates with resistance to ICIs [125], likely due in part to the fact that PD-1 can enhance myeloid cell-mediated immunosuppression (Fig. 4). Given that neutrophils are highly prevalent in the bone marrow [126,127] and that tumor associated neutrophils play roles in tumorigenesis and metastasis, this could be a potential mechanism for the lack of efficacy of ICIs in the bone metastatic niche.

The primary and/or metastatic tumor microenvironment can also be influential in conferring resistance to ICI therapy. Hypoxia is an important characteristic of the tumor microenvironment due to its association with increased survival, proliferation, and metastatic properties of malignant cells [128]. Hypoxia also upregulates PD-L1 expression in tumor cells, dendritic cells, macrophages, and MDSCs [129,130]. PD-L1 is important for MDSC function in hypoxia; by binding to the hypoxia response element on the PD-L1 promoter, hypoxia inducible factor 1 subunit alpha (HIF-1α) increases PD-L1 expression to induce immunosuppression [129]. Given that both the bone and tumor microenvironments are hypoxic [128,131], this suggests a possible hypoxia-driven mechanism for ICI resistance in bone metastases; however, since ICIs have high efficacy in primary tumors, which are also typically hypoxic, there are likely other factors unique to the bone microenvironment that play into ICI resistance in the bone.

Taken together, preclinical data indicate that ICIs may contribute to bone loss in addition to being ineffective at mitigating tumor burden in the bone, suggesting that irAEs following ICI therapy may not be worth the risk–benefit ratio in patients with bone metastases. This highlights the urgent need for preclinical studies to investigate mechanisms of ICI resistance and combination therapy with bone-targeted agents in the bone metastatic niche.

4. Conclusion

Bone metastases are common for many cancer types, notably prostate, breast and lung cancer. The development of immune checkpoint inhibitors as a new anti-cancer treatment came with exciting success in the primary tumor and some sites of distant metastases; however, there is a striking lack of regression of bone metastases following ICI treatment. Clinical reports indicate that patients with bone metastases have a comparably poor survival benefit from ICIs compared to patients without bone metastases, suggesting the efficacy of ICIs is compromised in the bone microenvironment. Furthermore, patients treated with ICIs develop bone loss and have increased fracture risk, independent of the presence of bone metastases, suggesting a negative impact of ICI therapy on the skeleton. Pre-clinical studies suggest the importance of multiple immune cell subsets (e.g., MDSCs, $CD8^+$ T cells), PD-1/PD-L1 expression in the bone microenvironment, and the hypoxic bone and tumor microenvironments in tumor-induced bone disease. However, there remains a lack of preclinical studies comprehensively investigating the role of the immune system in bone metastases and the regulation of immune checkpoint protein expression in the bone microenvironment. As such, further investigation into the mechanism of action of ICIs in the bone microenvironment as well as the effect of ICIs on the skeleton is needed.

Declaration of Competing Interest

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