Immunotherapy targeting PD-1/PD-L1: A potential approach for the treatment of cancer bone metastases (Review)

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Abstract. Immune checkpoint molecules, such as programmed cell death 1 (PD-1) and programmed cell death ligand 1 (PD-L1), have a critical role in regulating immune responses, including in tumor tissues. Monoclonal antibodies against these molecules, known as immune checkpoint inhibitors (ICIs), have been shown to be effective against a variety of cancers; however, significant patient populations are resistant to such treatment. Clinical studies to date have shown that ICIs are less effective in cancer patients with bone metastases. The effect of anti-PD-1/PD-L1 antibodies on bone metastases, as assessed by the bone metastasis-specific response classification criteria, was relatively low. In addition, the presence of bone metastases showed a trend toward worse progression-free survival and overall survival in cancer patients treated with ICIs. To improve the efficacy of ICIs in bone metastases, several combination therapies are under investigation and certain studies have reported better responses. The present review summarizes the current understanding of the effects of anti-PD-1/PD-L1 antibodies on bone metastases based on the reported clinical and preclinical studies.

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Abbreviations: BMA, bone-modifying agent; BP, bisphosphonate; CCL2, chemokine C-C motif ligand 2; Dmab, Denosumab; FDA, Food and Drug Administration; ICI, immune checkpoint inhibitor; NSCLC, non-small cell lung cancer; OS, overall survival; PD-1, programmed cell death 1; PD-L1, programmed cell death ligand 1; PFS, progression-free survival; RANKL, receptor activator of NF-κB ligand; RCC, renal cell cancer; TGFβ, transforming growth factor β; Treg, regulatory T; ZA, zoledronic acid

Key words: immunotherapy, immune checkpoint inhibitor, programmed cell death 1, programmed cell death ligand 1, bone metastasis

Contents

1. Introduction

Metastasis is the primary cause of death in up to 90% of cancer patients (1). Bone is one of the most common sites of metastasis for several types of cancer, particularly breast, prostate, lung, kidney and thyroid cancer (2). Bone metastases are frequently associated with skeletal-related events, including bone pain, pathologic fractures, spinal compression and hypercalcemia, which severely impact quality of life. In addition, the 5-year survival rate of patients with bone metastases is 1-13% and their median survival ranges from 12 to 33 months (3). Despite recent advances in clinical interventions, such as bone-targeted therapies and radiopharmaceuticals, bone metastases remain incurable and the development of new treatment strategies is eagerly anticipated.

Immune checkpoint molecules are crucial for maintaining self-tolerance and modulating immune responses (4). However, certain cancers protect themselves from anti-tumor immunity by utilizing this system. Immune checkpoint molecules are mainly expressed on the surface of T cells and co-inhibit T-cell activation by binding to their ligands on antigen-presenting cells and/or cancer cells at the time of recognition of cancer antigens (4). Recently, cancer immunotherapy utilizing immune checkpoint inhibitors (ICIs) has demonstrated the potential of the immune system to eradicate cancer cells, thereby impressively improving the survival of cancer patients (5,6). Several kinds of ICIs have been approved for the treatment of a wide range of tumor types. Among oncology therapeutic products

approved by the US Food and Drug Administration (FDA) in this century, ICIs are the second most approved drugs despite having been on the market since 2011 (7). The successful development of ICIs suggests that ICIs may be a potentially powerful strategy for the treatment of bone metastases.

Among the currently available ICIs, monoclonal antibodies that block programmed cell death 1 (PD-1) and programmed cell death ligand 1 (PD-L1) are the mainstay of cancer immunotherapy in the clinic. In the tumor microenvironment, PD-L1 expressed on tumor cells interacts with the PD-1 receptor on activated T cells, leading to suppression of the immune response through T-cell exhaustion, anergy and apoptosis, and facilitating tumor immune evasion and tumor growth (8). Antibodies against PD-1 and PD-L1 block this pathway, increase immune cell proliferation, enhance anti-tumor activity and thereby suppress tumors. However, clinical studies have shown that the efficacy of anti-PD-1/PD-L1 antibodies against bone metastases remains lower than expected. To overcome this limitation, it is necessary to learn more about the immune microenvironment of bone metastases and to find ways to better utilize the antibodies. Therefore, the present review focuses on the effects of anti-PD-1/PD-L1 antibodies on bone metastases; it i) summarizes the current understanding of the immune microenvironment of bone metastases, ii) provides a comprehensive analysis of clinical reports to date, iii) presents possible candidates for combination therapies and discusses the shortcomings of the existing literature to guide future studies.

2. Effects of immune checkpoint molecules on bone metabolism

In addition to their effects on immune cells, immune checkpoint molecules have been shown to have certain effects on bone metabolism, particularly bone resorption. Several papers reported that osteoclast differentiation and bone resorption were suppressed in PD-1-deficient mice and in mice treated with anti-PD-1 antibody, whereas little effect was found on bone formation indices (9,10). Wang *et al* (11) showed similar results only in tumor-bearing bone but not in naive bone. These preclinical data are partially supported by a clinical study showing that ICIs increased plasma levels of C-terminal telopeptide of type I collagen, a bone resorption marker, in cancer patients without bone metastases, while plasma levels of N-terminal propeptide of type I procollagen, a bone formation marker, showed a trend toward a decrease but was not statistically significant (12). Although the precise mechanisms of the suppressive effects of PD-1 inhibition on osteoclastogenesis are still unclear, Wang *et al* (11) proposed that, in the bone metastasis microenvironment, binding of soluble PD-L1 produced by tumor cells to PD-1 in preosteoclasts causes the release of chemokine C-C motif ligand 2 (CCL2), thereby promoting osteoclast differentiation. Therefore, anti-PD-1 treatment prevents osteoclast differentiation by inhibiting CCL2 production (Fig. 1). Given that osteoclastic bone destruction has critical roles in the progression of bone metastases, these effects of PD-1 blockade on osteoclasts are expected to act to reduce bone metastases. In support of this notion, Zuo and Wan (13) reported that anti-PD-L1 antibody treatment reduced bone metastases of breast cancer and melanoma by simultaneously suppressing osteoclast differentiation and enhancing immunity. By contrast, Wang *et al* (11) showed that PD-1 blockade inhibited osteoclastogenesis but did not affect tumor burden in bone.

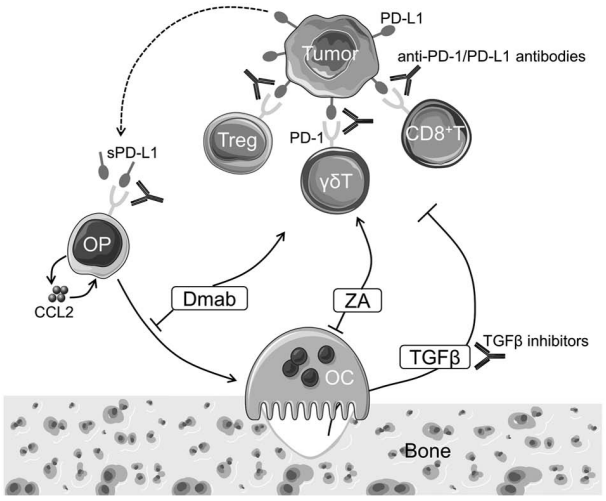

Figure 1. Association of tumor, immune and bone cells and targeted therapeutic agents in cancer bone metastasis. The interaction of PD-L1 expressed on tumor cells with PD-1 expressed on immune cells suppresses the anti-tumor effects of $CD8^+$ cytotoxic T cells and γδ T cells and the pro-tumor effects of Treg cells, which is blocked by anti-PD-1/PD-L1 antibodies. Anti-PD-1/PD-L1 antibodies have also been shown to prevent osteoclast differentiation, likely by inhibiting CCL2 production by osteoclast precursor cells. Several combination therapies are being developed to improve the efficacy of anti-PD-1/PD-L1 antibodies. The bisphosphonate ZA not only inhibits bone resorption but also has effects on expanding γδ T cells. The other bone resorption inhibitor, Dmab, has been suggested to have immunomodulatory effects by regulating signals mediated through receptor activator of NF-κB expressed on immune cells. TGFβ, released from the bone matrix by bone resorption, has potent immunosuppressive effects, which can be blocked by TGFβ inhibitors. PD-1, programmed cell death 1; sPD-L1, soluble programmed cell death ligand 1; OP, osteoclast precursor cells; OC, osteoclasts; Dmab, Denosumab; Treg, regulatory T cell; CCL2, chemokine C-C motif ligand 2; ZA, zoledronic acid.

3. Bone marrow immune systems and bone metastasis

Bone marrow provides a niche that supports hematopoietic stem cells and has a central role in hematopoiesis (14). In addition, bone marrow contains various immune cells and acts as a site of primary or memory immune response, indicating that bone marrow functions not only as a primary lymphoid organ but also as a secondary lymphoid organ (15). Thus, immune cells residing in bone marrow most likely have an important role in the development of bone metastases.

Various studies have indicated that immune checkpoint molecules regulate not only T cells but also a variety of immune cells, including B cells, natural killer cells and myeloid cells (4-6). However, currently available ICIs largely target $CD8^+$ cytotoxic T cells against tumor cells. High $CD8^+$ cytotoxic T-cell infiltration has been reported to be associated with response to ICI treatment (16). When $CD8^+$ T cells were PD-1-deficient in murine colon cancer models, anti-PD-1 antibody was ineffective (17).

Because most preclinical studies of bone metastasis have been conducted using immunodeficient mice, the current knowledge of the role of immunity in bone metastasis is limited. However, some recent work has shown that $CD8^+$ cytotoxic T

Table I. PD-L1 expression in primary tumors and metastatic lesions.

		PD-L1-positive rate, %								
Author, year	Cancer type	Primary	Bone	LN	Lung	Liver	Brain	Evaluated cells	Threshold	(Refs.)
Rozenblit, 2020	Breast	63.7	16.7	51.1	68.8	17.4	55.6	Immune cells	≥1%	(39)
Chao, 2023	Breast	26.0	6.0	-	-	-	-	Immune cells	≥1%	(26)
Fankhauser, 2018	Prostate	6.2	0	0	0	-	0	Tumor cells	≥1%	(40)
Zhang, 2019	RCC	32.5	14.6	47.9	45.8	-	36.8	Tumor cells	≥1+[a]	(41)

[a]Evaluated by staining intensity. LN, lymph node; -, not determined; RCC, renal cell carcinoma; PD-L1, programmed cell death ligand 1.

lymphocytes have a key role in antitumor immune responses also in the formation of bone metastases of melanoma (18,19), as well as breast (20,21) and prostate (22) cancers. A recent study by our group using syngeneic immunocompetent mouse models revealed that primary tumor-primed immune cells inhibit the development of bone metastases of breast cancer, which is predominantly mediated by $CD8^+$ cytotoxic T lymphocytes (23). In addition, a study using clinical specimens showed that the proportion of $CD8^+$ memory T cells was increased in the bone marrow of patients with breast cancer compared with that of healthy donors (24), and adoptive transfer of bone marrow T cells from patients with breast cancer into immunodeficient mice induced regression of xenografted autologous tumors (25). These results suggest that adequate control of the function of $CD8^+$ cytotoxic T lymphocytes allows us to eliminate bone metastases. On the other hand, a study using clinical specimens of bone metastases of breast cancer showed that the number of $CD8^+$ tumor-infiltrating lymphocytes was significantly lower than those in primary breast tumors (26), suggesting the immunologically cold microenvironment of bone metastases.

In contrast to $CD8^+$ T cells, clinical and preclinical studies have reported that regulatory T (Treg) cells, which suppress effective tumor immunity, are increased in bone metastases of breast and prostate cancer (27,28). PD-1 expression is also found on Treg cells and blocking PD-1 activates PD-1-expressing Treg cells. Kumagai *et al* (17) proposed that the balance of PD-1 expression between $CD8^+$ T and Treg cells determines the efficacy of PD-1 immunotherapy. Therefore, the $CD8^+$ T-low and Treg-high microenvironment of bone metastases may be hostile to immune checkpoint blockade therapies.

4. PD-L1 as a predictive biomarker for response to anti-PD-1/PD-L1 antibodies

It is crucial to define the biomarkers to predict the therapeutic effects of ICIs. Among several proposed biomarkers, PD-L1 expression quantified by immunohistochemistry is currently the most widely validated, used and accepted biomarker for selecting patients to receive anti-PD-1 or anti-PD-L1 antibody treatment (29). However, PD-L1 expression is not always associated with the prognosis of cancer patients. Several studies have indicated a positive association across cancers (30,31), while certain others have reported no association (32,33). One critical reason is that there are some technical issues with this screening method. Each pharmaceutical company developed its own diagnostic antibody and corresponding protocol for PD-L1 staining and interpretation; however, the use of different antibodies has been shown to affect PD-L1 positivity in the same tumors (34,35). PD-L1 expression is assessed on tumor cells and/or tumor-infiltrating immune cells, including macrophages, dendritic cells, neutrophils, myeloid-derived suppressor cells, and T cells and/or B cells (29). However, it has not been standardized which cells should be evaluated and what formulas should be used for scoring the threshold. Furthermore, the concordance between pathologists' scores was particularly poor for immune cells compared to tumor cells (36). These issues of inter-assay heterogeneity and inter-observer reproducibility are major challenges in the development of PD-L1 expression as a universal predictive biomarker. In addition to PD-L1, the FDA approved two other markers, tumor mutational burden and deficient mismatch repair/microsatellite instability-high, both of which still require refinement to improve validity across cancers (37).

As mentioned above, PD-L1 is a widely used but imperfect predictive biomarker, particularly for selecting patients to receive anti-PD-1/PD-L1 antibodies. Recently, the discordant expression of PD-L1 between primary tumors and paired metastases has been increasingly demonstrated. According to a meta-analysis by Zou *et al* (38), the percentage of PD-L1 that changed from positive to negative was 41% and that from negative to positive was 16%, although the conversion trend differed among cancer types and metastatic sites. Regarding bone metastases, certain papers reported the differential expression of PD-L1 in primary tumors and metastatic lesions in bone and other organs of breast, prostate and renal cell cancer (RCC) (Table I) (26,39-41). When compared with primary tumors, the PD-L1-positive rate was generally lower in bone metastases, regardless of the cancer type and cells examined. Furthermore, of note, Zhu *et al* (42) reported that the PD-L1 expression level in primary non-small cell lung cancer (NSCLC) was significantly lower in patients with bone metastases than in those without. These characteristics may contribute to the poor efficacy of ICIs in bone metastases.

5. Effects of anti-PD-1/PD-L1 antibodies on bone metastases

Preclinical studies have shown that treatment with anti-PD-1/PD-L1 antibodies reduced bone metastases of

Table II. Therapeutic effects of anti-PD-1/PD-L1 antibodies on bone metastases of non-small cell lung cancer.

		MDA criteria: N (%)					
Author, year	Anti-PD-1/PD-L1	CR	PR	SD	PD	N (%) for each bone-modifying agent	(Refs.)
Asano, 2022	A	0 (0)	0 (0)	2 (100)	0 (0)	20 (71.4) with Dmab; 1 (3.6) with ZA	(46)
	Niv	0 (0)	0 (0)	5 (50)	5 (50)		
	P	2 (12.5)	5 (31.2)	7 (43.8)	2 (12.5)		
Asano, 2023	A	5 (50)[a]		5 (50)[a]		29 (52.7) with Dmab; 6 (10.9) with ZA	(47)
	D	2 (66.7)[a]		1 (33.3)[a]			
	Niv	1 (6.7)[a]		14 (93.3)[a]			
	P	9 (33.3)[a]		18 (66.7)[a]			
Bongiovanni, 2021	A, Niv or P	0 (0)	12 (34.3)	11 (31.4)	12 (34.3)	23 (65.7) with Dmab or ZA	(48)
De Giglio, 2023	A, Niv or P	1 (1.6)	7 (11.4)	17 (27.8)	36 (59.0)	12 (19.75) with ZA	(49)
Nakata, 2020	Niv	1 (6.7)	5 (33.3)	8 (53.3)	1 (6.7)	2 (13.3) with Dmab; 10 (66.7) with ZA	(50)

[a]CR and PR or SD and PD, respectively, were combined. CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; N, number of patients included; PD-1, programmed cell death 1; PD-L1, programmed cell death ligand 1; Dmab, Denosumab; ZA, zoledronic acid; MDA, University of Texas MD Anderson Cancer Center; A, Atezolizumab; D, Durvalumab; Niv, Nivolumab; P, Pembrolizumab.

breast cancer and melanoma in mice (13,43,44). The increase in infiltrating $CD8^+$ T cells in metastatic bone lesions was found in these antibody-treated mice. Consistent with the findings described in Section 2, studies by Zuo and Wan (13) and Arellano *et al* (21) showed that the activation of T cells by immunotherapy suppressed osteoclast formation, which may also contribute to the inhibition of bone metastases. These results suggest the potential of anti-PD-1/PD-L1 antibodies to inhibit bone metastases. Clinical studies have reported the effects of anti-PD-1 antibodies (nivolumab and pembrolizumab) and anti-PD-L1 antibodies (atezolizumab and durvalumab) on bone metastases evaluated using the bone metastasis-specific response classification criteria developed by the University of Texas MD Anderson Cancer Center (MDA criteria) (45), all of which are data from patients with NSCLC (Table II) (46-50). Although responses varied among studies and agents used, the overall response rates were unsatisfactory compared with the preclinical studies. This is consistent with the findings that PD-L1 expression was low in bone metastases in cancer patients (Table I). It should be noted, however, that the number of studies and their sample sizes are limited to date.

6. Bone metastasis as a predictor of poor efficacy of anti-PD-1/PD-L1 antibodies

Numerous clinical studies have been conducted on the effects of ICIs on cancer patients with bone metastases. The impact of bone metastases on the prognosis of cancer patients treated with anti-PD-1 antibodies (camrelizumab, nivolumab, pembrolizumab, sintilimab, tislelizumab and toripalimab) and anti-PD-L1 antibodies (atezolizumab, avelumab and durvalumab) is summarized in Table III (42,49,51-85). Although these studies did not show the effects on bone metastases themselves and the results were not always consistent across the studies, the overall trend suggests that the presence of bone metastases is a potential predictor of poor progression-free survival (PFS) and overall survival (OS). The contribution of bone metastases to the survival rates appears to be similar to that of liver metastases and more severe than that of other metastatic sites. These data again suggest a lower efficacy of ICIs in bone metastases, although it should be noted that most of the studies are retrospective analyses with limited sample sizes. Most of these data were reported in patients with NSCLC. Numerous clinical trials have been completed or are ongoing (86,87); however, the number of trials conducted on cancers that frequently metastasize to bone, such as breast and prostate cancer, is limited.

7. Combination of anti-PD-1/PD-L1 antibodies with bone-targeted therapy

Due to the limited efficacy of ICIs in the treatment of cancer, there is a growing interest in the development of therapeutic combinations with ICIs. With respect to bone metastases, bone-modifying agents (BMAs), including the anti-receptor activator of NF-κB ligand (RANKL) antibody Denosumab (Dmab) and bisphosphonates (BPs), are often combined with ICIs to reduce skeletal-related events and cancer progression in patients with bone metastases. In the studies listed in Table II, a significant proportion of patients were treated with ICIs in combination with BMAs, such as Dmab or the BP zoledronic acid (ZA), and the combination therapy showed a trend toward improved response in bone metastases (46-50). Several studies also evaluated the prognostic impact of the combination of ICIs and BMAs in patients with bone metastases (Table IV)(42,46,48,60,64,75,88-90). A substantial proportion of studies have shown that the combination of

Table III. Effects of distant metastases on PFS and OS of cancer patients treated with ICIs.

Author, year	Cancer type	ICIs	Line of treatment: N (%)	Metastatic sites										(Refs.)
				Bone		LN		Lung		Liver		Brain		
				PFS	OS	PFS	OS	PFS	OS	PFS	OS	PFS	OS	
Botticelli, 2019	NSCLC	Niv	2nd: 102 (100)	NS	NS	NS	NS	↓	↓	↓[a]	↓[a]	-	-	(51)
Cortellini, 2020	NSCLC	P	1st: 1026 (100)	↓[a]	↓[a]	-	-	-	-	↓[a]	↓[a]	↓	↓	(52)
De Giglio, 2023	NSCLC	A, I, Niv, P	1st: 61 (27.5) 2nd: 124 (55.9) ≥3rd: 37 (16.7)	↓	↓[a]	-	-	-	-	↓[a]	↓[a]	NS	↓	(49)
Debieuvre, 2021	NSCLC	Niv	2nd: 1771 (68.9) ≥3rd: 799 (31.1)	-	↓	-	-	-	-	-	↓	-	NS	(53)
Deng, 2022	NSCLC	P	1st line: 40 (100)	↓[a]	NS	-	-	-	-	↓[a]	↓[a]	NS	NS	(54)
Du, 2023	NSCLC	A, C, Niv, P, S, Tor	1st & 2nd: 97 (53.9) ≥3rd: 83 (46.1)	↓[a]	NS	NS	NS	NS	NS	↓	NS	NS	↓[a]	(55)
Garde-Noguera, 2018	NSCLC	Niv	2nd: 65 (37.1) 3rd: 66 (37.7) ≥4th: 44 (25.1)	NS	NS	-	-	NS	NS	-	-	NS	NS	(56)
Hosoya, 2021	NSCLC	P	1st: 88 (100)	↓	-	-	-	-	-	NS	-	NS	-	(57)
Kawachi, 2020	NSCLC	P	1st: 213 (100)	NS	-	NS	-	NS	-	NS	-	NS	-	(58)
Landi, 2019	NSCLC (non-squamous)	Niv	≥2nd: 1588 (100)	↓[a]	↓[a]	-	-	-	-	-	↓[a]	-	↓	(59)
	NSCLC (squamous)		≥2nd: 371 (100)	↓[a]	↓[a]	-	-	-	-	-	↓[a]	-	NS	
Li, 2020	NSCLC	Not specified	1st: 13 (12.6) ≥2nd: 90 (87.4)	↓	↓[a]	-	-	-	-	-	-	-	-	(60)
Ma, 2021	NSCLC	A	2nd: 569 (100)	-	↓	-	-	-	-	-	-	-	NS	(61)
Mouritzen, 2022	NSCLC	Niv, P	2nd: 536 (63.8) 3rd: 205 (24.4) 4th: 68 (8.1) ≥5th: 31 (3.7)	NS	↓[a]	NS	NS	-	-	↓[a]	↓[a]	NS	NS	(62)
Petrova, 2020	NSCLC	P	2nd: 119 (100)	-	↓[a]	-	-	-	-	-	-	-	-	(63)
Qin, 2021	NSCLC	A, Niv, P, other	1st: 91 (27.6) 2nd: 160 (48.5) ≥3rd: 79 (23.9)	-	↓	-	-	-	-	-	-	-	-	(64)
Rounis, 2021	NSCLC	Not specified	2nd: 66 (100)	↓[a]	↓[a]	-	-	-	-	↓[a]	NS	NS	NS	(65)
Shi, 2022	NSCLC	A, C, D, Niv, P, S, T	1st: 14 (4.3) 2nd: 99 (30.6) ≥3rd: 241 (65.1)	↓[a]	-	-	-	NS	-	↓[a]	-	NS	-	(66)
Tamiya, 2018	NSCLC	Niv	≥2nd: 201 (100)	NS	-	NS	-	↓[a]	-	↓[a]	-	NS	-	(67)
Yang, 2023	NSCLC	Not specified	1st: 85 (59.4) ≥2nd: 58 (40.6)	↓	-	-	-	-	-	NS	-	-	-	(68)
Yao, 2020	NSCLC	C, Niv, P, S, Tor	2nd: 21 (36.8) ≥3rd: 36 (63.2)	NS	-	-	-	-	-	↓[a]	-	-	-	(69)

Table III. Continued.

Author, year	Cancer type	ICIs	Line of treatment: N (%)	Metastatic sites										(Refs.)
				Bone		LN		Lung		Liver		Brain		
				PFS	OS	PFS	OS	PFS	OS	PFS	OS	PFS	OS	
Yoneda, 2022	NSCLC	A, Niv, P	1st: 96 (22.1) ≥2nd: 339 (77.9)	↓[a]	↓[a]	NS	NS	↓	↓	↓	↓	NS	NS	(70)
Zeng, 2021	NSCLC	C, D, Niv, P, Pen, S, T	1st: 57 (66.3) ≥2nd: 29 (33.7)	↓[a]	-	-	-	NS	-	NS	-	NS	-	(71)
Zhu, 2022	NSCLC	C, Niv, P, S, T, Tor	1st: 57 (39.6) 2nd: 42 (29.2) ≥3rd: 45 (31.3)	↓[a]	↓[a]	-	-	-	-	-	-	-	-	(42)
Lee, 2022	SCLC	A	1st: 1026 (100)	↓[a]	↓	-	-	-	-	-	-	↓[a]	NS	(72)
Zhou, 2023	NSCLC, SCLC	Not specified	1st: 84 (44.2) 2nd: 52 (27.4) ≥3rd: 54 (28.4)	↓	-	-	-	NS	-	NS	-	NS	-	(73)
Tanaka, 2022	Gastric	Niv	≥3rd: 70 (100)	-	↓	-	NS	-	NS	-	↓	-	-	(74)
Gambale, 2023	RCC	I, Niv	Not specified	-	NS	-	-	-	-	-	-	-	-	(75)
Rebuzzi, 2021	RCC	Niv	2nd: 394 (69.0) 3rd: 120 (21.0) ≥4th: 57 (10.0)	-	↓[a]	-	NS	-	-	-	-	-	-	(76)
Velev, 2023	RCC	Niv	2nd: 354 (50.0) 3rd: 193 (27.3) 4th: 96 (13.6) ≥5th: 65 (9.2)	↓[a]	↓	-	-	-	-	-	-	-	-	(77)
Shimizu, 2022	Urothelial	P	2nd: 113 (83.1) ≥3rd: 23 (16.9)	-	↓	-	-	-	-	-	↓	-	-	(78)
Makrakis, 2022	Urothelial	Not specified	1st: 504 (55.0) ≥2nd: 413 (45.0)	↓ ↓	↓ ↓	↓ ↓	↓ ↓	↓ ↓	↓ NS	↓ ↓	↓ NS	-	-	(79)
Raggi, 2022	Urothelial	P, others	1st: 26 (12.5) 2nd: 134 (64.4) 3rd: 46 (22.1) 2nd/3rd: 2 (1.0)	↓[a]	↓[a]	-	-	NS	NS	NS	↓[a]	-	-	(80)
Hoshi, 2023	Head & neck	Niv	1st: 12 (29.3) ≥2nd: 29 (70.7)	-	NS	-	NS	-	NS	-	↓[a]	-	-	(81)
Bilen, 2019	Melanoma, gastrointestinal lung, head & neck, breast, gynecological, genitourinary, others	Not specified	1st or 2nd: 28 (31.1) ≥3rd: 62 (68.9)	NS	NS	NS	NS	NS	NS	↓	↓[a]	NS	NS	(82)
Botticelli, 2020	NSCLC, melanoma, RCC, others	A, Ave, Niv, P	1st: 94 (32.5) 2nd: 147 (50.9) 3rd: 33 (11.4) 4th: 11 (3.8) ≥5th: 4 (1.4)	↓	↓	NS	NS	-	-	NS	↓[a]	↓	↓[a]	(83)
Qin, 2022	NSCLC, melanoma, urothelial, HCC, head & neck, RCC	A, I, Niv, P, D/Trem, Niv/I	1st: 137 (46.1) ≥2nd: 160 (53.9)	↓[a]	↓	NS	NS	NS	NS	↓	↓	NS	NS	(84)

Table III. Continued.

Author, year	Cancer type	ICIs	Line of treatment: N (%)	Metastatic sites										(Refs.)
				Bone		LN		Lung		Liver		Brain		
				PFS	OS	PFS	OS	PFS	OS	PFS	OS	PFS	OS	
Topalian, 2019	Melanoma, RCC, NSCLC	Niv	≥2nd: 270 (100)	-	↓[a]	-	NS	-	NS	-	↓[a]	-	NS	(85)

[a]Multivariate analysis. N, number of patients included; LN, lymph node; HCC, hepatocellular carcinoma; RCC, renal cell carcinoma; NSCLC, non-small cell lung cancer; A, Atezolizumab; Ave, Avelumab; C, Camrelizumab; D, Durvalumab; I, Ipilimumab; Niv, Nivolumab; P, Pembrolizumab; S, Sintilimab; T, Tislelizumab; Tor, Toripalimab; Trem, Tremelimumab; ↓, significantly decreased; NS, not significant; -, not determined; PFS, progression-free survival; OS, overall survival; ICI, immune checkpoint inhibitor.

Table IV. Prognostic effects of ICIs in combination with BMAs.

Author, year	Cancer type	ICIs	Line of treatment: N (%)	BMAs	PFS	OS	(Refs.)
Asano, 2022	NSCLC	A, Niv, P	1st line: 8 (27.6) 2nd line: 13 (44.8) ≥3rd line: 8 (27.6)	Dmab	-	↑	(46)
Bongiovanni, 2021	NSCLC	A, Niv, P	1st line: 10 (21.7) 2nd line: 26 (56.5) ≥3rd line: 10 (21.7)	ZA/Dmab	↑	↑	(48)
Li, 2020	NSCLC	Not specified	1st line: 69 (33.8) ≥2nd line: 135 (66.2)	BP	-	NS	(60)
Li, 2022	NSCLC	Not specified	1st line: 171 (76.6) 2nd line: 29 (17.0) 3rd line: 9 (5.3) 4th line: 2 (1.2)	Dmab	↑[a]	-	(88)
Qiang, 2022	NSCLC	P	1st line: 58 (52.7) ≥2nd line: 52 (47.3)	PAM/ZA	↑[a]	NS	(89)
Qin, 2021	NSCLC	A, Niv, P, Other	1st line: 91 (27.6) 2nd line: 160 (48.5) ≥3rd line: 79 (23.9)	BP/Dmab	-	NS	(64)
Zheng, 2022	NSCLC	C, Niv, P, S	1st line: 20 (38.5) ≥2nd line: 32 (61.5)	ZA	↑[a]	NS	(90)
Zhu, 2022	NSCLC	C, Niv, P, S, T, Tor	1st line: 57 (39.6) 2nd line: 42 (29.2) ≥3rd line: 45 (31.3)	BP	↑	↑	(42)
Gambale, 2023	RCC	Niv, I	1st line: 1 (2.3) ≥2nd line: 42 (97.7)	BP/Dmab	-	↓	(75)

[a]Multivariate analysis. N, number of patients included; A, Atezolizumab; C, Camrelizumab; D, Durvalumab; I, Ipilimumab; Niv, Nivolumab; P, Pembrolizumab; T, Tislelizumab; Tor, Toripalimab; BP, bisphosphonate (not specified); PAM, pamidronate; ↑, significantly increased; ↓, significantly decreased; NS, not significant; -, not determined; PFS, progression-free survival; OS, overall survival; ICI, immune checkpoint inhibitor; RCC, renal cell carcinoma; NSCLC, non-small cell lung cancer; ZA, zoledronic acid; BMA, bone-modifying agent; Dmab, Denosumab.

BMAs confers a survival benefit. The mechanisms of action of individual BMAs are discussed below. In addition to BMAs, inhibitors of transforming growth factor β (TGFβ) and angiogenesis are also potential candidates for combination therapy.

BPs. Among several kinds of BPs, ZA is currently the most widely used for the treatment of bone metastases (91). As a ZA-specific effect, ZA has been shown to expand γδ T lymphocytes and induce their effector phenotype in cancer patients (92). Iwasaki *et al* (93) reported that, although PD-1

expression was rapidly induced on γδ T cells after antigenic stimulation, the attenuated effector function was reversed by anti-PD-L1 monoclonal antibody. ZA also has the effect of sensitizing tumor cells to γδ T cells (93). These effects of ZA may potentiate the effects of ICIs (Fig. 1). Li *et al* (44) showed in a mouse model of breast cancer bone metastasis that combined treatment with ZA and anti-PD-1 antibody suppressed bone tumor growth more effectively than either treatment alone with no apparent toxicity. The combined treatment increased the infiltration of $CD8^+$ T cells and decreased that of myeloid-derived suppressor cells in bone tumors, although the mechanisms remain to be determined.

Anti-RANKL antibody. RANKL is currently best known for its role in osteoclast lineage cells; however, RANKL was originally identified as a dendritic cell-specific survival factor, which was upregulated in activated T cells (94). In addition to dendritic cells, myeloid cells, such as macrophages and myeloid-derived suppressor cells, also express RANK in the tumor microenvironment and RANKL-RANK signaling provides immunomodulatory signals (95). However, in several mouse models, RANKL inhibition alone exhibited minimal efficacy in controlling subcutaneous tumor growth and experimental lung metastases (95).

Ahern *et al* (96) demonstrated that the combination of anti-RANKL and anti-PD-1 antibodies significantly suppressed subcutaneous growth of colon and prostate cancer compared with treatment with each antibody alone. The combination therapy of anti-RANKL and anti-PD-L1 antibodies also exhibited additive inhibitory effects on experimental lung metastases of melanoma and prostate cancer (96). These effects were attributed to either natural killer or $CD8^+$ T cells, which may be due to favorable immunological alterations in the tumor microenvironment caused by the combined treatment (Fig. 1). However, mechanistic studies of the combined effects in preclinical models have not been conducted.

TGFβ inhibitors. TGFβ is known to have both tumor-promoting and tumor-suppressive functions (97). In this context, TGFβ signaling has been shown to suppress the function of adaptive and innate immune cells, which is associated with cancer immune evasion (98). TGFβ is one of the most abundant growth factors stored in the bone matrix, which is released into the bone microenvironment as a result of osteoclastic bone resorption (99). Bone-derived TGFβ induces tumor production of osteolytic factors, such as interleukin 11, prostaglandin E2 and parathyroid hormone-related peptide, which cause further osteolysis, thereby supporting tumor growth and progression in the bone microenvironment. At the same time, it is also highly likely that bone-derived TGFβ suppresses immune cell functions in bone, thereby promoting the development of bone metastases. Preclinical studies demonstrated that targeting TGFβ with an antibody (22), a small molecule (100) and oncolytic adenoviruses (101) inhibited bone metastases and enhanced the efficacy of antibodies against PD-L1 and cytotoxic T-lymphocyte-associated protein 4 (Fig. 1). TGFβ is likely to be a promising target for combination with immune checkpoint molecules.

Anti-angiogenic agents. The abnormal vascular network in cancer tissue disrupts both the trafficking of immune effectors and the delivery of ICIs (102). Angiogenic factors, including vascular endothelial growth factor, angiopoietin, hepatocyte growth factor and platelet-derived growth factor, also directly affect immune cell function and impair optimal anti-tumor immunity. Therefore, it is expected that anti-angiogenic agents may be able to restore both cellular and molecular pathways in favor of improved efficacy of ICIs. In support of this notion, several clinical trials have demonstrated beneficial effects of these combinations (103). A study by Xie *et al* (104) showed that combined treatment with ICIs and anti-angiogenic agents significantly prolonged median bone PFS compared with ICIs alone, whereas median PFS and OS were not changed in patients with NSCLC with bone metastases.

Each of these agents not only has its own immunologic effects, but also affects the microenvironment of bone metastases, both of which are likely to contribute to the improved efficacy of ICIs when used in combination. Extensive studies are still needed to elucidate the precise mechanisms of action of these combinations. Further understanding the effects of ICIs on bone metastases may also lead us to find better candidates for combination therapy, including not only bone-targeted therapy but also chemotherapy, radiotherapy and other immunotherapeutic agents.

8. Limitations of the research to date

As described above, a number of studies have been conducted at the clinical and preclinical levels to investigate the effects of ICIs on bone metastases. However, these reports have several limitations that may affect the interpretation of the results.

Clinical studies. The most obvious limitation is that most of the available studies, listed in Tables II-IV, are observational and retrospective. As a result, patient status, anti-PD-1/PD-L1 antibodies used, lines of treatment and combination therapies are variable and at times unclear. Future prospective studies are needed to confirm these findings. Regarding the effects of anti-PD-1/PD-L1 antibodies on bone metastases, the number of studies and their sample sizes are limited. Furthermore, although the MDA criteria have been widely used to assess the therapeutic response of bone metastases (45), it remains to be determined whether this is the best evaluation system (105).

Preclinical studies. The most critical issue is that our understanding of the bone immune microenvironment and the roles of immune cells in bone metastases is immature. As presented in Table III, the response to anti-PD-1/PD-L1 antibody treatment is different among metastatic sites, suggesting that the immune response varies among organs. Preclinical studies focusing on bone-specific immunology are definitely needed to properly interpret the effects of immunotherapies on bone metastases. For instance, Yin *et al* (106) proposed that the transcription factor basic helix-loop-helix family member e22 is upregulated in bone metastatic prostate cancer and drives an immunosuppressive bone microenvironment. Since the majority of clinical studies have been conducted in patients with NSCLC, the study using animal models of NSCLC may

aid in the interpretation of clinical data. A significant number of patients are known to be resistant to ICI treatment (107). Some of these patients are primarily resistant and others acquire resistance after an initial response. To investigate the mechanisms of resistance, animal models of bone metastases that mimic not only primary but also acquired resistance need to be developed.

9. Conclusion and future perspectives

Immunotherapies targeting immune checkpoint molecules have been shown to be effective in several types of cancer and have led to a paradigm shift in cancer treatment. However, therapies with ICIs have several challenges that need to be addressed to broaden their application, including bone metastases (6). Current ICIs are not effective against cancers with low inherent immunogenicity. In addition, a considerable proportion of cancer patients exhibit resistance to ICIs (107). Both tumor-cell-intrinsic and tumor-cell-extrinsic mechanisms have been suggested to contribute to the resistance, but the precise mechanisms are still unclear. To overcome these issues and maximize the clinical efficacy of ICIs, numerous clinical trials of ICIs in combination with other therapeutic modalities, including chemotherapy, radiotherapy and other immunotherapeutic agents, are ongoing (108). With respect to bone metastases, although an immunologically cold microenvironment is thought to be one of the possible reasons for the limited efficacy of ICIs, there is still a lack of studies that comprehensively investigate the immune microenvironment and the regulation of immune checkpoint molecules of bone metastases. Clinical data on the effects of ICIs on bone metastases of cancers that frequently metastasize to bone, such as breast and prostate cancer, are also limited. Future translational and reverse translational approaches, combined with knowledge from other cancer fields, are expected to facilitate our understanding of the immunology of bone metastases. The study of the bone immune microenvironment and the roles of immune cells in bone metastases is still in its infancy. It is esteemed that the accumulated knowledge will lead to improved efficacy of ICIs on bone metastases.

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Authors' contributions

TH drafted the manuscript, collected and assembled the data, and read and approved the final manuscript. Data authentication is not applicable.

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Competing interests

The author declares that he has no competing interests.

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