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Therapeutic Effectiveness and Immune-Associated Toxicities of Pembrolizumab Combined With Bone-Modifying Agents in Women with Breast Cancer

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Abstract

Recent evidence suggests that certain bone-targeted therapies, including denosumab (Dmab), may enhance antitumor immunity by increasing the infiltration and diversity of T cells within tumors. In breast cancer, women frequently receive immune checkpoint inhibitors (ICIs) together with bone-modifying agents (BMAs), yet the influence of BMAs on immune-related adverse events (irAEs) and treatment efficacy remains largely undefined. We retrospectively analyzed female breast cancer patients treated with pembrolizumab between 2017 and 2024, stratifying them by BMA exposure: zoledronic acid (ZA), Dmab, or none. BMA use was considered relevant if administered within 12 months (ZA) or 6 months (Dmab) prior to ICI, during ICI therapy, or within one month after the last ICI dose.

Among 425 patients, 55 (12.9 percent) received Dmab, 31 (7.3 percent) received ZA, and 339 (80.0 percent) received no BMA. Early-stage breast cancer accounted for 255 patients (60%), while 170 (40 percent) had metastatic disease. After a median follow-up of 19.4 months (95 percent CI, 17.5–20.8), severe irAEs were more common in the Dmab group compared to patients without BMAs (21.8 percent vs 11.5 percent, P = .04). Objective response rates were higher in patients receiving Dmab (48.0%) than in those receiving ZA (31.6%) or no BMA (35.0%), though the difference was not statistically significant (P = .3). These findings suggest that Dmab may increase the risk of severe irAEs and potentially enhance the antitumor efficacy of ICIs in women with breast cancer.

Keywords: Denosumab, Pembrolizumab, Immunotherapy, Breast cancer, Immune-related adverse events

Introduction

Bone-modifying agents (BMAs), such as bisphosphonates and denosumab (Dmab), are widely incorporated into breast cancer management. In early-stage disease, BMAs are administered to prevent osteoporosis, and adjuvant bisphosphonates have been shown to reduce recurrence risk in postmenopausal women [1]. In metastatic breast cancer with bone involvement, BMAs help limit skeletal-related complications [2].

Preclinical studies indicate that Dmab, a monoclonal antibody targeting RANKL, can augment the immune system when combined with ICIs [3–7]. Mouse studies demonstrate that co-administration of anti-RANKL therapy and ICIs increases recruitment and activation of tumor-infiltrating CD4+ and CD8+ T cells, improving antitumor outcomes [3, 4]. Trials are ongoing to evaluate this combination in melanoma and non-small cell lung cancer [8–11].

RANK/RANKL signaling also plays a key role in central immune tolerance. Within medullary thymic epithelial cells (mTECs), RANKL drives expression of the autoimmune regulator (Aire), promoting deletion of

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autoreactive T cells and development of regulatory T cells (Tregs) [12–14]. Inhibiting RANK/RANKL with Dmab may impair this process, potentially allowing autoreactive T cells to persist and altering Treg function [12–14].

The combined effect of BMAs and ICIs on irAEs and clinical outcomes in female breast cancer patients remains largely unknown. This study aimed to assess irAE incidence and treatment response in women receiving concurrent ICI and BMA therapy.

Materials and Methods

We conducted a retrospective cohort study of female breast cancer patients treated with pembrolizumab from March 2017 to January 2024. Patients were grouped according to BMA exposure: ZA, Dmab, or none. Eligible BMAs had to be administered within 12 months (ZA) or 6 months (Dmab) before ICI, during ICI treatment, or within one month after the final ICI dose. Patients receiving both ZA and Dmab were excluded.

Demographic, clinical, and treatment-related data were extracted from electronic medical records. TNBC was defined as ≤10% nuclear staining for ER and PR and HER2 negativity following the 2018 ASCO–CAP guideline [15]. Severe irAEs (grade ≥3) were identified per CTCAE v5.0 and NCCN v1.2024 criteria, based on oncology provider assessment of ICI-related toxicity.

For metastatic patients, best responses were categorized as CR, PR, SD, or PD, with objective response defined as CR or PR. Patients with insufficient follow-up or imaging were classified as unknown. Early-stage patients receiving BMAs were not included in response analysis due to limited numbers. PFS was measured from the first ICI dose to progression or death, and OS from first ICI dose to last follow-up or death. For patients treated with pembrolizumab multiple times, PFS and best response were evaluated based on the initial course and BMA timing.

Comparisons across ZA, Dmab, and no BMA groups used Chi-square or Kruskal-Wallis tests. Logistic regression analyses were performed to identify factors associated with response in metastatic disease, including age, menopausal status, number of ICI cycles, BMA type, concurrent chemotherapy, and occurrence of severe irAEs.

Results and Discussion

A total of 425 female patients were included: 55 (12.9 percent) received Dmab, 31 (7.3 percent) received ZA, and 339 (80.0 percent) received no BMA **(Table 1)**. Early-stage disease was present in 255 (60%) patients, while 170 (40 percent) had metastatic breast cancer. TNBC accounted for 82.1% of cases. Patients received a median of 9 pembrolizumab cycles (IQR 4–15) over a median treatment duration of 6.9 months (IQR 2.8–12.1), with 98.1% receiving only one type of ICI.

Table 1. Baseline demographic and clinical characteristics of patients receiving pembrolizumab, stratified by bone-modifying agent use

Characteristic	Denosumab (n = 55)	Zoledronic Acid (n = 31)	No BMA (n = 339)	All Patients (n = 425)	P-value
Median age at initiation of ICI therapy, years (IQR)	57.9 (48.0–67.5)	56.1 (45.5–60.9)	55.4 (45.3–64.9)	55.9 (45.9–65.2)	.1
Race					.001
American Indian or Alaska Native	2 (3.6%)	3 (9.7%)	1 (0.3%)	6 (1.4%)	
Asian or Pacific Islander	15 (27.3%)	5 (16.1%)	63 (18.6%)	83 (19.5%)	
Black or African American	5 (9.1%)	0 (0.0%)	37 (10.9%)	42 (9.9%)	
White	30 (54.5%)	23 (74.2%)	213 (62.8%)	266 (62.6%)	
Other or Unknown	3 (5.5%)	0 (0.0%)	25 (7.4%)	28 (6.6%)	
Ethnicity					.9
Hispanic or Latino	13 (23.6%)	10 (32.3%)	99 (29.2%)	122 (28.7%)	
Not Hispanic or Latino	40 (72.7%)	20 (64.5%)	228 (67.3%)	288 (67.8%)	
Unknown	2 (3.6%)	1 (3.2%)	12 (3.5%)	15 (3.5%)	
Menopausal status					0.07
Premenopausal	9 (16.4%)	7 (22.6%)	113 (33.3%)	129 (30.4%)	

Postmenopausal	44 (80.0%)	23 (74.2%)	216 (63.7%)	283 (66.6%)	
Unknown	2 (3.6%)	1 (3.2%)	10 (2.9%)	13 (3.0%)	
Clinical stage at ICI initiation					<.0001
I	0 (0.0%)	1 (3.2%)	9 (2.7%)	10 (2.4%)	
II	1 (1.8%)	4 (12.9%)	119 (35.1%)	124 (29.2%)	
III	0 (0.0%)	3 (9.7%)	118 (34.8%)	121 (28.5%)	
IV	54 (98.2%)	23 (74.2%)	93 (27.4%)	170 (40.0%)	
Breast cancer subtype					<0.0001
HR+/HER2-	22 (40.0%)	12 (38.7%)	38 (11.2%)	72 (16.9%)	
HER2+	0 (0.0%)	1 (3.2%)	3 (0.9%)	4 (0.9%)	
TNBC	33 (60.0%)	18 (58.1%)	298 (87.9%)	349 (82.1%)	
Median number of pembrolizumab cycles (IQR)	5 (3–15)	8 (4–14)	9 (5–15)	9 (4–15)	0.08
Types of ICI received					0.2
Pembrolizumab only (1 ICI)	54 (98.2%)	30 (96.8%)	333 (98.2%)	417 (98.1%)	
≥2 ICIs	1 (1.8%)	1 (3.2%)	6 (1.8%)	8 (1.9%)	
Second ICI type					0.02
None	54 (98.2%)	30 (96.8%)	333 (98.2%)	417 (98.1%)	
Atezolizumab	1 (1.8%)	1 (3.2%)	4 (1.2%)	6 (1.4%)	
Nivolumab	0 (0.0%)	0 (0.0%)	2 (0.6%)	2 (0.5%)	
Median duration of pembrolizumab therapy, months (IQR)	4.1 (1.7–13.4)	7.2 (2.1–11.5)	7.1 (3.0–12.2)	6.9 (2.8–12.1)	0.3
Dose/regimen of concurrent bone-modifying therapy					<0.0001
None	0 (0.0%)	0 (0.0%)	339 (100%)	339 (79.8%)	
Denosumab 120 mg (oncology dose)	53 (96.4%)	0 (0.0%)	0 (0.0%)	53 (12.5%)	
Denosumab 60 mg (osteoporosis dose)	2 (3.6%)	0 (0.0%)	0 (0.0%)	2 (0.5%)	
Zoledronic acid 3–4 mg (oncology dose)	0 (0.0%)	31 (100%)	0 (0.0%)	31 (7.3%)	
Zoledronic acid 5 mg (osteoporosis dose)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Concurrent chemotherapy with ICI					<0.0001
No	28 (50.9%)	14 (45.2%)	43 (12.7%)	85 (20.0%)	
Yes	27 (49.1%)	17 (54.8%)	296 (87.3%)	340 (80.0%)	
Bone metastases at ICI start (among Stage IV patients, n=170)					<0.0001
No	3 (5.6%)	8 (34.8%)	79 (85.0%)	90 (52.9%)	
Yes	51 (94.4%)	15 (65.2%)	14 (15.0%)	80 (47.1%)	
Bone-only metastases at ICI start (among Stage IV patients, n=170)	7 (13.0%)	4 (17.4%)	4 (4.3%)	15 (8.8%)	0.4

Abbreviations: BMA, bone-modifying agent; ICI, immune checkpoint inhibitor; HR, hormone receptor; HER2, human epidermal growth factor receptor 2; TNBC, triple-negative breast cancer.

Over a median follow-up of 19.4 months (95 percent CI: 17.5–20.8), severe immune-related adverse events (irAEs) were documented in 12.7% of the total cohort (54 of 425 patients) **(Table 2)**. The occurrence of severe irAEs was notably higher among patients who received denosumab (Dmab) compared with those who did not receive any bone-modifying agent (21.8 percent vs 11.5 percent, P = .04). In the early-stage disease group, only a

single patient received Dmab, and this individual developed a severe irAE. Among the eight patients receiving zoledronic acid (ZA), one (12.5%) experienced a severe irAE, whereas 30 of 246 patients (12.2%) without BMA exposure developed severe irAEs. Although the number of early-stage patients treated with Dmab was limited, the proportion of severe irAEs was similar across Dmab, ZA, and no-BMA groups (P = .2). In the metastatic or recurrent disease subset, severe irAEs were observed in 11 of 54 patients (20.4%) receiving Dmab, compared with 2 of 23 patients (8.7%) receiving ZA and 9 of 93 patients (9.7%) without BMAs; however, these differences did not reach statistical significance (P = .2).

Table 2. Immune-related adverse events and clinical outcomes in patients treated with pembrolizumab, stratified by bone-modifying agent received

Characteristic	No BMA (n = 339)	Denosumab (n = 55)	Zoledronic Acid (n = 31)	All Patients (n = 425)	P-value
Grade 3–5 immune-related adverse events (irAE)					.09
No	300 (88.5%)	43 (78.2%)	28 (90.3%)	371 (87.3%)	
Yes	39 (11.5%)	12 (21.8%)	3 (9.7%)	54 (12.7%)	
Median time from pembrolizumab start to irAE, months (IQR)	3.7 (1.1–6.9)	1.8 (1.2–5.9)	2.3 (0.3–7.3)	2.3 (1.1–6.9)	.8
Objective response rate in metastatic disease, % (95% CI)	35.0 (24.3–45.7)	48.0 (33.7–62.3)	31.6 (8.6–54.6)	38.9 (31.0–46.8)	.3
Disease control rate in metastatic disease,** % (95% CI)	53.8 (42.6–64.9)	60.0 (41.7–70.3)	63.2 (39.3–87.0)	55.7 (47.6–63.8)	.8
Median progression-free survival, months (95% CI)	6.1 (4.1–10.2)	4.4 (3.5–13.9)	5.8 (2.8–10.3)	5.9 (4.4–9.0)	.5
Median follow-up, months (95% CI)	18.0 (15.9–19.4)	36.4 (20.9–55.7)	29.4 (21.2–42.9)	19.4 (17.5–20.8)	<.0001
Median overall survival (OS), months (95% CI)					
Early stage (I–III)	NR (36.4–NR)	NR (NR–NR)	NR (NR–NR)	NR (36.4–NR)	.6
Recurrent/metastatic (IV)	23.0 (19.0–54.5)	22.2 (13.6–43.1)	36.3 (10.9–50.9)	23.0 (19.2–36.6)	1.0
24-month OS rate, % (95% CI)					
Early stage (I–III)	93.2 (87.4–96.4)	100 (100–100)	100 (100–100)	93.6 (88.0–96.6)	.6#
Recurrent/metastatic (IV)	46.2 (33.9–57.6)	49.6 (34.4–63.1)	50.0 (26.7–69.6)	47.9 (49.1–56.2)	1.0#
Median OS in early-stage disease by severe irAE,* months (95% CI)					
Grade 3+ irAE	36.4 (NR–NR)	NR (NR–NR)	NR (NR–NR)	36.4 (NR–NR)	1.0
No Grade 3+ irAE	NR (34.6–NR)	—	NR (NR–NR)	NR (NR–NR)	.4
Median OS in recurrent/metastatic disease by severe irAE,* months (95% CI)					
Grade 3+ irAE	NR (2.7–NR)	19.1 (3.4–NR)	NR (23.6–NR)	23.6 (19.1–NR)	.6
No Grade 3+ irAE	20.6 (18.2–31.4)	36.3 (13.6–43.1)	36.3 (10.9–50.9)	22.0 (18.2–36.3)	.9

Abbreviations: BMA, bone-modifying agent; irAE, immune-related adverse event; NR, not reached; OS, overall survival. *Comparisons of patients with versus without grade 3+ irAE within each group yielded log-rank P-values ≥ 0.2. **Disease control rate defined as complete response + partial response + stable disease. #Log-rank test comparing survival curves.

The median duration from pembrolizumab initiation to the onset of an immune-related adverse event was 2.3 months (IQR 1.1–6.9), with no significant variation observed across different BMAs (P = 0.8) **(Table 2)**. In

cases of severe irAE, 87.0% (47 out of 54) of patients received systemic corticosteroid therapy, including two individuals who required treatment for two separate severe events. Hospitalization due to irAE occurred in 57.4% (31 of 54) of patients, with no patient admitted more than once. The use of corticosteroids and hospitalization rates did not differ significantly by BMA type (P = 0.5 and P = 0.7, respectively). **Figure 1** presents the number and categories of severe irAEs according to the type of BMA administered.

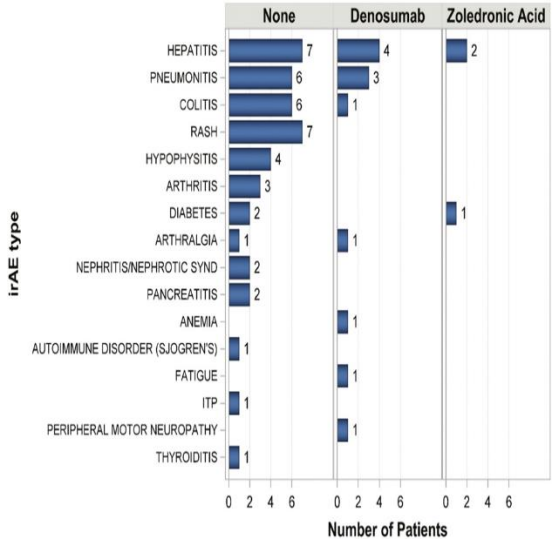

Figure 1. Type of severe immune-related adverse events according to bone-modifying agent received. Abbreviations: irAE= immune-related adverse event; ITP= immune thrombocytopenia.

Among patients with metastatic disease receiving pembrolizumab, those treated with denosumab (Dmab) demonstrated higher objective response rates (48.0% [95% CI, 33.7–62.3]) compared with patients receiving zoledronic acid (ZA) (31.6 percent [95 percent CI, 8.6–54.6]) or no BMA (35.0 percent [95 percent CI, 24.3–45.7]), although these differences were not statistically significant (P = 0.3) **(Table 2)**. Disease control rates were comparable across the three groups (Dmab: 60.0 percent [95 percent CI, 41.7–70.3]; ZA: 63.2 percent [95 percent CI, 39.3–87.0]; no BMA: 53.8 percent [95 percent CI, 42.6–64.9]; P = 0.8). Median progression-free survival also did not differ substantially between groups (Dmab: 4.4 months [95 percent CI, 3.5–13.9]; ZA: 5.8 months [95 percent CI, 2.8–10.3]; no BMA: 6.1 months [95 percent CI, 4.1–10.2]; P = 0.5). Kaplan-Meier curves depicting PFS for metastatic patients by BMA type are presented in **Figure 2**.

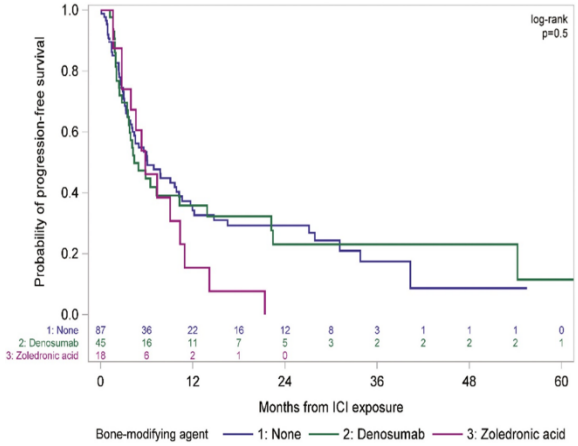

Figure 2. Kaplan-Meier curves for progression-free survival (PFS) among patients with metastatic cancer who received pembrolizumab, grouped by the type of bone-modifying agent (BMA) used. Abbreviation: ICI= immune checkpoint inhibitor.

Overall survival (OS) did not differ significantly depending on the BMA administered, whether in early-stage (P = 0.6) or metastatic (P = 1.0) disease **(Figures 3 and 4; Table 2)**. Median OS was not reached for early-stage patients (95% CI, 36.4 months to not reached). Across all metastatic patients, median OS was 23.0 months (95 percent CI, 19.2–36.6). Within the metastatic cohort, median OS was 22.2 months (95 percent CI, 13.6–43.1) for denosumab recipients, 36.3 months (95 percent CI, 10.9–50.9) for zoledronic acid recipients, and 23.0 months (95 percent CI, 19.0–54.5) for patients who received no BMA.

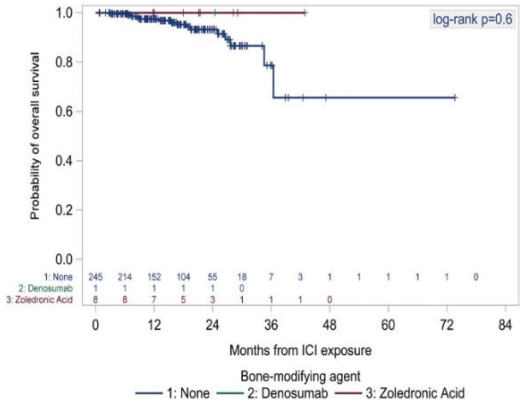

Figure 3. Kaplan-Meier curve for overall survival in patients with early-stage disease receiving pembrolizumab, categorized by the type of bone-modifying agent (BMA) administered. Abbreviation: ICI= immune checkpoint inhibitor.

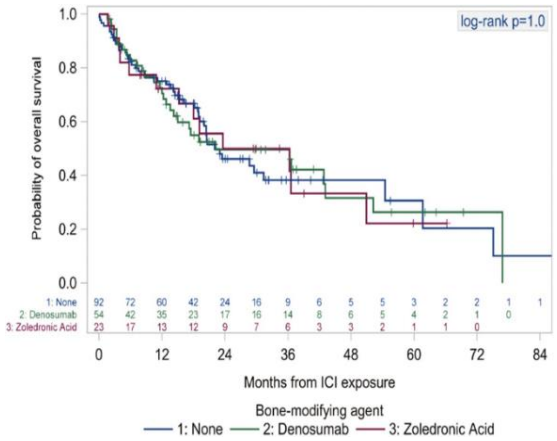

Figure 4. Kaplan-Meier curve for overall survival (OS) in patients with metastatic disease receiving pembrolizumab, stratified by the type of bone-modifying agent (BMA) used. Abbreviation: ICI= immune checkpoint inhibitor.

The 24-month OS estimates were 93.6% (95% CI, 88.0–96.6) for early-stage disease and 47.9 percent (95 percent CI, 49.1–56.2) for metastatic disease, with no significant differences across the three BMA groups (P = 0.6 and P = 1.0, respectively) **(Table 2)**. Among metastatic patients, the 24-month OS was 49.6 percent (95 percent CI, 34.4–63.1) for denosumab (Dmab) recipients, 50.0% (95% CI, 26.7–69.6) for zoledronic acid (ZA) recipients, and 46.2 percent (95 percent CI, 33.9–57.6) for those receiving no BMA (P = 1.0) **(Table 2)**.

Univariate and multivariate logistic regression analyses were conducted to determine predictors of response to immune checkpoint inhibitors (ICI) in metastatic disease. Univariate results showed that a greater number of ICI cycles was associated with better response (OR 1.13, 95 percent CI, 1.07–1.20, P < 0.0001). In the multivariate model, both a higher number of ICI cycles (OR 1.15, 95 percent CI, 1.08–1.22, P < 0.0001) and the occurrence of severe immune-related adverse events (irAE) (OR 5.18, 95 percent CI, 1.77–15.2, P = 0.0027) independently predicted response.

In women with breast cancer receiving pembrolizumab, those treated with denosumab exhibited higher rates of severe irAE than patients who received no BMA. Although not reaching statistical significance, clinical response rates appeared improved with denosumab compared to zoledronic acid or no BMA.

Elevated response rates and irAE incidence have been observed in other malignancies when combining ICI with denosumab [5-9]. For instance, a retrospective analysis of 86 patients with non-small cell lung cancer demonstrated that adding denosumab to ICI significantly improved response rate (40.4 percent vs. 20.5 percent, P = 0.01), disease control rate (67.3 percent vs. 38.7 percent, P = 0.02), real-world progression-free survival (7.4 vs. 3.6 months, P < 0.01), and overall survival (14.2 vs. 8.6 months, P = 0.02) relative to ICI alone [6]. IrAE rates also trended higher by 16.9 percent (29.7 percent vs. 12.8 percent, P = 0.07), though not significantly [6]. Given the common use of BMAs in breast cancer and ongoing trials exploring ICI plus denosumab in other tumor types, confirming these differences in response and irAE among breast cancer patients is clinically relevant.

Preclinical and clinical evidence supports synergy between denosumab and ICI [3-7]. In murine models, anti-RANKL therapy combined with ICI enhanced antitumor activity by boosting recruitment and activation of tumor-infiltrating CD4+ and CD8+ T cells [3, 4]. A prospective trial in stage IV melanoma comparing dual ICI plus denosumab versus dual ICI alone revealed elevated CXCL-13 levels—a chemokine involved in immune cell recruitment—and increased CD8+ T cells in the combination arm [8].

Beyond tumor microenvironment benefits, anti-RANKL agents influence systemic autoimmunity via effects on thymic development and T-cell homeostasis. RANKL blockade depletes Aire-expressing medullary thymic epithelial cells, impairing negative selection and broadening the peripheral T-cell repertoire [12]. This may heighten autoimmunity, as evidenced by increased inflammatory infiltrates and autoantibodies in tissues following transplantation of RANK-deficient thymic stroma in immunodeficient models [12].

Despite higher response rates with denosumab plus pembrolizumab versus other BMA groups, progression-free survival remained comparable across groups. This could reflect the unselected nature of the cohort, including more advanced cases and patients receiving pembrolizumab in any treatment line. The overall median PFS (5.9 months) was shorter than the 9.7 months reported in KEYNOTE-355 for first-line pembrolizumab plus chemotherapy in PD-L1-positive (CPS ≥10) advanced triple-negative breast cancer [16]. PFS assessment may also have been influenced by censoring at systemic therapy changes, often due to chemotherapy toxicity while continuing pembrolizumab.

Median OS in metastatic disease (23.0 months) aligned with the CPS ≥10 subgroup in KEYNOTE-355 [17]. The

relatively favorable OS despite more advanced disease in this cohort may stem from subsequent therapies, such as antibody-drug conjugates, which improve survival. In early-stage patients, the 24-month OS of 93.6 percent resembled the 36-month estimate of 89.7% from KEYNOTE-522 [18].

Severe irAE onset was linked to better objective responses in metastatic disease, consistent with reports across various cancers treated with ICI, suggesting irAE as a potential predictor of ICI efficacy [19].

Limitations include the sample size (425 patients), median follow-up (19 months), retrospective design, and non-primary focus on denosumab effects. The trend toward better responses with denosumab requires validation in larger prospective randomized trials. Additionally, inclusion of bone-only metastatic disease (more prevalent in the denosumab group) may have inflated response rates due to imaging challenges, and variability in treatment lines, regimens, and chemotherapy partners could have influenced irAE and response rates.

Conclusion

This represents the first report, to our knowledge, indicating potentially higher severe irAE rates and a possible synergistic antitumor benefit from combining denosumab with ICI in women with breast cancer. Combined with emerging data from melanoma and lung cancer [5-7], these findings support further clinical trial evaluation of denosumab's immunomodulatory potential.

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