

The possible different roles of denosumab in prevention and cure breast cancer bone metastases: A ‘hypothesis-generator’ study from clinical practice

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Abstract. The most frequent site of recurrence in breast cancer (BC) is the bone, particularly in patients with ‘luminal-like’ disease. Denosumab has been shown to prevent aromatase inhibitors (AIs) induced bone resorption in postmenopausal early BC patients and reduce skeletal-related events (SREs) in bone metastatic breast cancer (BMBC). A ‘real life’ analysis of 90 BMBC patients treated with denosumab was performed. Eighty-six patients (95.6%) had ‘luminal-like’ disease, 72 (80%) had bone metastases at the time of first recurrence of disease. Among 50 patients with metachronous ‘luminal-like’ disease, 40 (80%) had first recurrence to the bone. Among these patients median time to skeletal recurrence (TSkR) was shorter for patients who were previously exposed to AIs compared to those who were not (53.0 vs. 102.0 months, respectively; $P=0.0300$) and longer for patients previously treated with tamoxifen compared to those who were not (102.0 vs. 59.0 months, respectively; $P=0.0466$). Both of them were not confirmed at multivariate analysis. In the overall population, 17 first SREs were observed (16 radiation therapy) and median time to first SRE was not reached. A statistically significant difference in the incidence of SREs was detected only between patients with exclusively osteolytic bone metastases vs. those

without ($P=0.013$). The presence of exclusively-osteolytic bone metastases was the only factor significantly associated with a shorter time to first SRE ($P=0.011$). The only G3 toxicity reported was hypocalcemia in one patient. No osteonecrosis of the jaw events (ONJ) occurred. This study demonstrated that a pro-active attitude enables the treatment of the majority of patients with denosumab without significant class-related toxicities. The majority of SREs were from radiation therapy, so pain still remains the clinical hallmark of bone metastases, particularly for osteolytic ones. The suggestion that estrogen deprivation with AIs can favor a ‘bone-related’ risk conditions for developing bone metastases must be considered with caution and surely needs further validations.

Introduction

Breast cancer (BC) is the most frequent tumor and the second leading cause of cancer death among women, with an estimate rate of about 250.000 new cases in 2016 (1). Despite the increase in chances of cure, approximately 20–45% of affected patients will develop metastases (2), particularly bone metastases in 60–80% of cases (3), bone-only metastases in 17–37% (4), and from 25 to 40% of patients have bone metastases at the diagnosis (3). Study data suggest that bone-only patients survive more than others (5). In a recent ‘real-life’ study of metastatic BC (MBC) patients, the median overall survival (OS) was 37.22 months (6), while some studies reported a median OS up to 72 months for bone-only patients (7).

Microarray analyses identified different gene expression profiles in BC, thus differentiating molecular subgroups with different clinical course; several studies evaluated the association between subgroups and metastatic spread, showing that bone is the most common metastatic site in the luminal A (66.6%), luminal B (71.4%) and luminal/HER2+ tumors (65%), while it is less frequently involved in basal-like tumors (39%) (8). So, the expression of hormone receptors in BC is associated with a higher risk of developing bone metastases, but their presence does not necessarily imply that the metastatic

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involvement remains confined exclusively to the bone (9,10). Indeed, experimental evidences prove that BC metastasis to the bone is mediated by a specific set of genes beyond those involved in the development of the primary tumor (11).

Osteoporosis, a process of bone mineral density (BMD) reduction, is accelerated by estrogen deficiency in postmenopausal women. Tamoxifen reduces BMD in premenopausal women, while promotes bone formation in postmenopausal patients. On the other hand, adjuvant aromatase inhibitors (AIs) therapy enhances the BMD decrease to about 2.5% per year, due to a long-lasting significant deprivation of circulatory and tissue estrogens (12). In the bone companion study of the MA.17 trial, patients treated with anastrozole reported a significant decreases in BMD (~4%), compared to those treated with tamoxifen (13). Due to osteoporosis, the bone microarchitecture is impaired and the microenvironment is modified. The 'seed and soil' hypothesis, proposed by Paget more than a hundred years ago (14), speculates that pro-metastatic tumor cells (the 'seed') take root in specific organ sites (the 'soil') where the microenvironment is favorable for metastasis. The primary tumor can promote metastasis by inducing the creation of a permissive microenvironment in a secondary organ site, termed the pre-metastatic niche (15,16). The alteration of bone health associated with osteoporosis may provide fertile soil for the activation of the metastatic cascade, from the seeding of tumor cells to the activation of indolent micrometastases and finally to the expansion of bone lesions (17).

Several large randomized trials of adjuvant bisphosphonates vs. placebo showed an improvement, in both disease-free survival (DFS) and OS, in women with early BC treated with endocrine therapy (18-21). The role of denosumab in the prevention of AI-induced bone resorption was demonstrated in the ABCSG-18 phase III trial, which evaluated the effects of adjuvant denosumab in postmenopausal patients with early BC receiving AIs (22).

It is well known that bone metastases negatively affect patients' quality of life. Bisphosphonates showed to improve it, by reducing pain and the consequent consumption of analgesics, but they did not demonstrate to prolong survival (23).

Bisphosphonates like zoledronic acid, limit the loss of bone density, by binding and blocking the enzyme farnesyl diphosphate synthase (FPPS) in the HMG-CoA reductase pathway, leading to inhibition of both osteoclastogenesis, cell survival, and cytoskeletal dynamics, which is vital for maintaining the 'ruffled border' that is required for contact between a resorbing osteoclast and a bone surface. Denosumab is a human monoclonal antibody which acts on 'pre-osteoclasts'. These precursors express on their cell surface receptors called RANK (receptor activator of nuclear factor-kappa B). RANK is activated by RANKL (the RANK-Ligand), which exists as cell surface molecules on osteoblasts and this binding promotes the maturation of pre-osteoclasts into osteoclasts. Denosumab proved to be more effective than zoledronic acid in terms of pain reduction, allowing a smaller percentage of patients makes use of strong opioids (24).

In the phase 3 study published by Stopeck *et al* (25), in 2010, 2049 patients with bone MBC (BMBC), were randomized to receive denosumab or zoledronic acid. The median time to first skeletal-related event (SRE) was 26.4 months in the group of patients treated with zoledronic acid, while it had not

yet been reached in the group treated with denosumab, with a reduction in terms of time to first SRE by 18% over zoledronic acid (25). The subsequent uploaded data showed a median time to first SRE in the denosumab arm of 32.4 months (26). Moreover, denosumab demonstrated to decrease the risk of occurrence of multiple SREs by 23% and to reduce the skeletal morbidity rate (ratio between the number of SREs per patient divided by the patient of the time at risk) by 22% vs. zoledronic acid. Overall survival and disease progression were similar in the two groups. As to adverse events, pyrexia, bone pain, arthralgia, renal failure and hypercalcemia were more frequent during treatment with zoledronic acid, while hypocalcemia and toothache during treatment with denosumab. The risk of osteonecrosis of the jaw (ONJ) was not higher with denosumab compared to zoledronic acid ($P=0.39$) (25).

Here we report a 'real life' multicenter retrospective analysis of BMBC patients treated with denosumab, focusing both to clinical outcomes commonly related to the treatment (safety and efficacy in reduce skeletal related events) and to the possible correlations between patients/diseases' features and clinical patterns of recurrence to the bone.

Patients and methods

Study design and statistical analysis. A retrospective analysis of BMBC patients treated with denosumab, at the medical oncology departments of St. Salvatore Hospital in L'Aquila and Campus Bio-medico University Hospital in Rome, was conducted. Data cut-off was August 2017. Comorbidities were classified according to the Cumulative Index Rating Scale (CIRS) (27). Estrogen and Progesterone Receptor expressions were evaluated by immunohistochemistry (IHC), using Dako monoclonal antibodies. HER2 analysis was performed by IHC on paraffin embedded tissue from the primary tumor and/or metastatic site (Hercept-Test[®], Genentech Inc. subject to licenses held by Dako Denmark A/S, Glostrup, Denmark and F. Hoffmann-La Roche Ltd., Basel, Switzerland). Fluorescence *in situ* hybridization (FISH) and silver *in situ* hybridization (SISH) were used for cases of doubtful interpretation. 'Luminal-like' disease was defined in any case of Estrogen and/or Progesterone Receptor expression. Toxicity was registered according to the National Cancer Institute Common Toxicity Criteria (v4.0). Clinical evaluation of bone metastases was performed by radiographic imaging (X-ray, computed tomography scan or magnetic resonance) every three months or as clinically indicated up to death or last contact.

Definition of SRE included pathological fractures (not due to major trauma), radiation therapy on a bone segment, bone surgery or spinal cord compression (28). Hypercalcemia of malignancy was not considered among SREs. Subsequent events which occurred within 30 days of each other were not counted as separate events but rather unique (for example, a surgery to repair a fracture or multiple doses of radiation therapy during a treatment cycle). Only SREs occurred during treatment with denosumab were included in the analysis. Time to first SRE was defined as the interval from the start of denosumab to the onset of the first SRE (but not within the first month of treatment). Median time to skeletal recurrence (TSkR) was defined as the length of time from the surgical radicalization of the primary tumor to the diagnosis of first

skeletal metastasis (it was calculated only for patients with metachronous recurrence to the bone). OS was defined as the interval from the start of denosumab to death or last contact.

Subgroup analyses were performed among patients according to the following variables: elderly status (< vs. \geq of 70 years) (29), ECOG-PS (Eastern Cooperative Oncology Group Performance Status) (0/1 vs. 2), CIRS stage (primary/intermediate vs. secondary), 'luminal-like' disease (yes vs. no), HER2 status (positive vs. negative), menopausal status, extension of disease (bone only vs. non-bone only), visceral involvement (excluding lymph nodes) (yes vs. no), previous bisphosphonates (yes vs. no), number of bone metastases (< 1 vs. \geq 1), involvement of axial bones (yes vs. no), exclusively-osteolytic type of metastases (yes vs. no).

Patient eligibility. Patients were eligible if they had histologically confirmed diagnosis of BC, radiological confirmation of at least one bone metastasis; age \geq 18 years; adequate haematological, renal and hepatic functions; albumin-adjusted serum calcium between 8.1 and 10.4 mg/dl. Previous intravenous bisphosphonate therapy was allowed. Exclusion criteria were recent (<3 months) surgery of the oral cavity or inflammatory untreated dental-periodontal or peri-implant disease. All patients performed an orthopantomography and a dental examination at baseline and twice a year thereafter. All patients provided written informed consent to the proposed treatment and to participate to this analysis. To guarantee the confidentiality of personal data for deceased patients, all the available procedures to ensure anonymity have been used. The procedures followed were in accordance with the precepts of Good Clinical Practice and the ethical standards of local responsible committee on human experimentation (Comitato Etico per le province di L'Aquila e Teramo).

Treatment. All patients received a subcutaneous injection of denosumab (XGEVA[®], Amgen Europe B.V. Breda, The Netherlands) 120 mg every 4 weeks. A daily calcium (\geq 500 mg) and vitamin D (\geq 1,000 U) oral supplement was recommended. All patients received concomitant specific antineoplastic treatment, chemotherapy or endocrine therapy (Table I).

Statistical analysis. Median TSKR, median time to first SRE and median OS were evaluated using the Kaplan-Meier method (30). Median period of follow-up was calculated according to the reverse Kaplan-Meier method (31). χ^2 and Fisher's exact test were used to compare the incidence of SREs among subgroups, using the appropriate test according to the sample size in contingency tables for each comparison (32,33). Log-rank (34) was used to compare TSKR among subgroups. Cox proportional hazards regression (35) was used for univariate and multivariate analyses of clinical outcomes among subgroups (time to first SRE and OS). For statistical analysis MedCalc Statistical Software, v18.6 (MedCalc Software bvba, Ostend, Belgium; <http://www.medcalc.org>; 2018) was used.

Results

Patients' features. From July 2012 to August 2017 90 consecutive BMBC patients were treated with denosumab. Clinical features of patients are summarized in Table I. One out of

Table I. Patients' features.

Clinical feature	No. of patients (%)
Total no. of patients	90 (100.0)
Sex	
Male	1 (1.1)
Female	89 (98.9)
Age	
Non elderly	69 (76.7)
Elderly	21 (23.3)
ECOG PS	
0-1	84 (93.3)
\geq 2	6 (6.7)
CIRS (Comorbidity)	
Primary/intermediate	73 (81.1)
Secondary	17 (18.9)
Luminal-like	86 (95.6)
HER2 positive	13 (14.4)
Triple negative	4 (4.4)
Type of disease	
Synchronous	36 (40.0)
Metachronous	54 (60.0)
Menopausal status	
Yes	74 (82.2)
No	16 (17.8)
Onset localization of metastases	
Bone	72 (80.0)
Visceral	18 (20.0)
>1 bone metastases	80 (88.9)
Bone-only disease	35 (38.9)
Visceral disease	38 (42.2)
Axial bone metastases	81 (90.0)
Type of bone metastases	
Osteolytic exclusively	48 (53.3)
Others	42 (46.7)
Concomitant treatments	
Chemotherapy	43 (47.8)
Hormonal therapy	70 (77.8)
Aromatase inhibitors	52 (57.8)
Anti-HER2 therapy	13 (14.4)
Everolimus	29 (32.2)
Bevacizumab	14 (15.6)
CDK inhibitors	5 (5.6)
Previous bisphosphonates	27 (30.0)

The median age of patients was 58 years (age range, 26-91). ECOG-PS, eastern cooperative oncology group-performance status; CIRS, cumulative illness rating scale; CDK, cyclin-dependent kinase; HER2, human epidermal growth factor 2.

90 patients was male, median age was 61 years (range 26-91). Twenty-one patients (23.3%) were elderly, 6 (6.7%) had ECOG-PS \geq 2, 17 (18.9%) had a secondary CIRS stage and

74 (82.2%) were in menopausal status. Eighty-six patients (95.6%) had 'luminal-like' disease, 72 (80%) had bone metastases at the time of diagnosis of metastatic disease, 18 (20%) had the first recurrence of disease to visceral organs. Disease-oriented systemic treatments were concomitantly administered to all patients; 27 (30%) patients received previous bisphosphonate therapy. Fifty-four patients (60%) had a metachronous disease; clinical features of these patients are listed in Table II. Among 50 patients with metachronous 'luminal-like' disease, 40 (80%) had first recurrence to the bone, 10 (20%) to the visceral organs. Nineteen (38%) were previously treated with tamoxifen, 26 patients (52%) with AIs adjuvant therapy (including 10 to both tamoxifen and AIs).

Clinical patterns of disease recurrence to the bone. Among 40 patients with metachronous 'luminal-like' disease and first recurrence to the bone, fisher exact test did not show any statistically significant difference between previously exposed to AIs vs. not ($P=0.4896$) and to tamoxifen vs. not ($P=1.0000$). Among these patients median TSKR was 70.0 months (95% CI: 50.0-95.0). At univariate analysis with log-rank test, median TSKR was significantly shorter for patients who were previously exposed to AIs compared to those who were not (53.0 vs 102.0 months, respectively; $P=0.0300$). Median TSKR was also significantly longer for patients previously treated with tamoxifen compared to those who were not (102.0 vs. 59.0 months, respectively; $P=0.0466$; Fig. 1). At multivariate analysis, neither previous exposure to AIs (HR=1.66, 95% CI: 0.48-5.72) nor treatment with tamoxifen (HR = 0.78, 95% CI: 0.22-2.75) were confirmed as independent predictors for TSKR.

Clinical outcomes. All 90 patients were evaluable for clinical outcomes, as summarized in Table III. A median number of 18.5 cycles of denosumab was administered. In the overall population, 17 first SREs were observed, represented by 16 radiation therapy on a bone segment and 1 pathological fracture, with an incidence of 18.8% (95% CI: 11.4-28.5). Among patients who developed a SRE, 5 (29.4%) developed a subsequent SRE (5 radiation therapy on a bone segment). As shown in Fig. 2, a statistically significant difference in the incidence of SREs was detected only between patients with exclusively-osteolytic bone metastases vs. not ($P=0.013$). Specifically, among the first group (48 patients with exclusively-osteolytic metastases) 14 (29.2%) SREs were observed (95% CI: 15.9-48.9) compared to 3 SREs (7.1%) among the second group (95% CI: 1.4-20.8) (42 patients with other types of bone metastases). After a median follow up of 33 months, median time to first SRE was not reached (Fig. 3) and median OS was 40.0 months (95% CI: 34.0-48.0). At univariate analysis, only type of bone metastases was significantly correlated to time to first SRE in favor of other than exclusively-osteolytic ones ($P=0.011$) (Table IV). As to OS, at univariate analysis ECOG-PS 0/1 ($P=0.027$), bone-only disease ($P=0.004$) and non-visceral disease ($P=0.023$) were significantly correlated to a better OS, while multivariate analysis confirmed just ECOG-PS ($P=0.006$) and bone-only disease ($P=0.035$) as independent predictors for OS (Table V).

Safety. In the present analysis, we reported only class-specific adverse events which could be related to denosumab and

Table II. Clinical features of patients with metachronous disease.

Clinical feature	No. of patients (%) (n=54)
Triple negative	4 (7.4)
Luminal-like	50 (92.6)
Adjuvant hormonal therapy	
Aromatase inhibitors	26 (52)
Tamoxifen	19 (38)
Onset localization of metastases	
Bone	40 (80)
Visceral	10 (20)

Table III. Efficacy data.

Variables	Value
Evaluable patients, no. of patients (%)	90 (100)
Incidence of SRE, (%)	18.8 ^a
Radiation therapy, no. of patients (%)	16 (17.8)
Pathological fracture, no. of patients (%)	1 (1.1)
Median time to first SRE, months (range)	Not reached (1-61)
Median OS time, months	40.0 (1-61) ^b
No. of mortalities	38

^a95%CI, 11.4-28.5; ^b95%CI, 35.0-48.0. SRE, skeletal-related events; OS, overall survival; CI, confidence interval.

not to disease-oriented treatments concomitantly administered (Table VI). All patients were evaluable for toxicity. Four patients (4.4%) discontinued denosumab due to adverse events. The only G3 toxicity reported was hypocalcemia in a patient, with a history of total thyroidectomy, completely recovered by strengthening of calcium and vitamin D support. Decrease in serum calcium levels was in most cases mild, recovered in a short time (within 2 weeks) with an increased in oral calcium and vitamin D support and was not accompanied by clinical complications. No ONJ events occurred. Patients experiencing toothache (10.0%) did not develop ONJ, while two patients (3.1%) reported dental infections in absence of radiographic signs of bone remodeling.

Discussion

Our 'real life' safety data confirmed the good tolerability of denosumab. We reported 7.8% of flu-like symptoms and no case of renal injury. We did not report cases of ONJ, even in patients who experiencing toothache (12.5%). As previously mentioned, all patients underwent orthopantomography and subsequent maxillofacial visit before starting denosumab, to identify possible risk factors such as recent dental alveolar surgery (<3 months), dental-periodontal or peri-implant inflammatory disease, incongruous removable dentures or poor oral hygiene, which could be responsible for an increased risk of

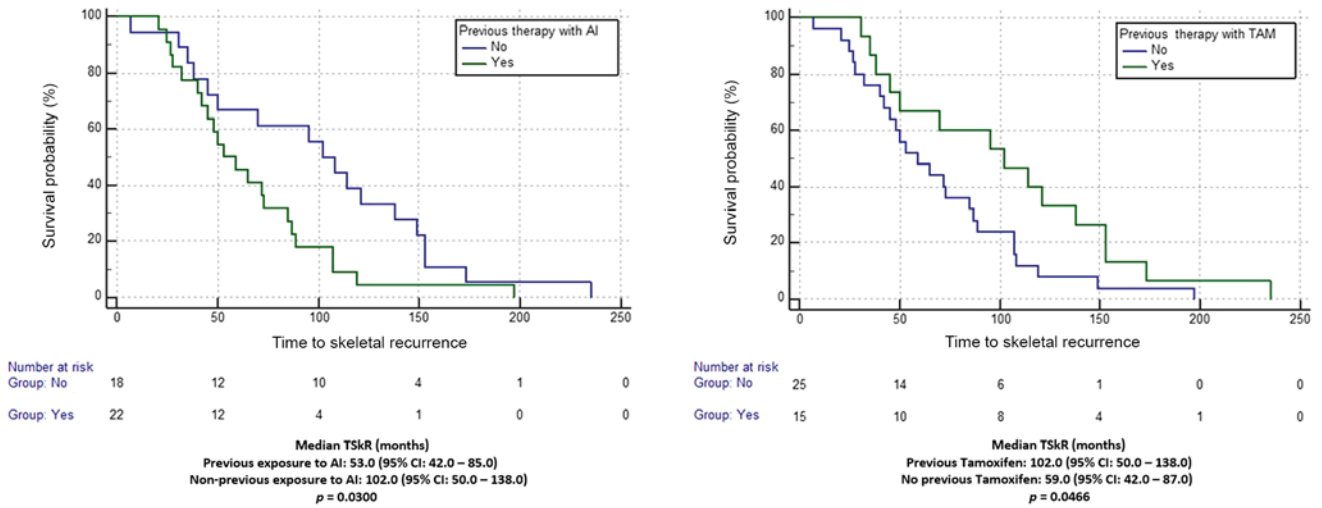


Figure 1. Log-rank test for median TSKR, measured in months, for previous exposure to AI during adjuvant therapy vs. not, and previous tamoxifen adjuvant therapy vs. not. TSKR, time to skeletal recurrence; AI, aromatase inhibitors.

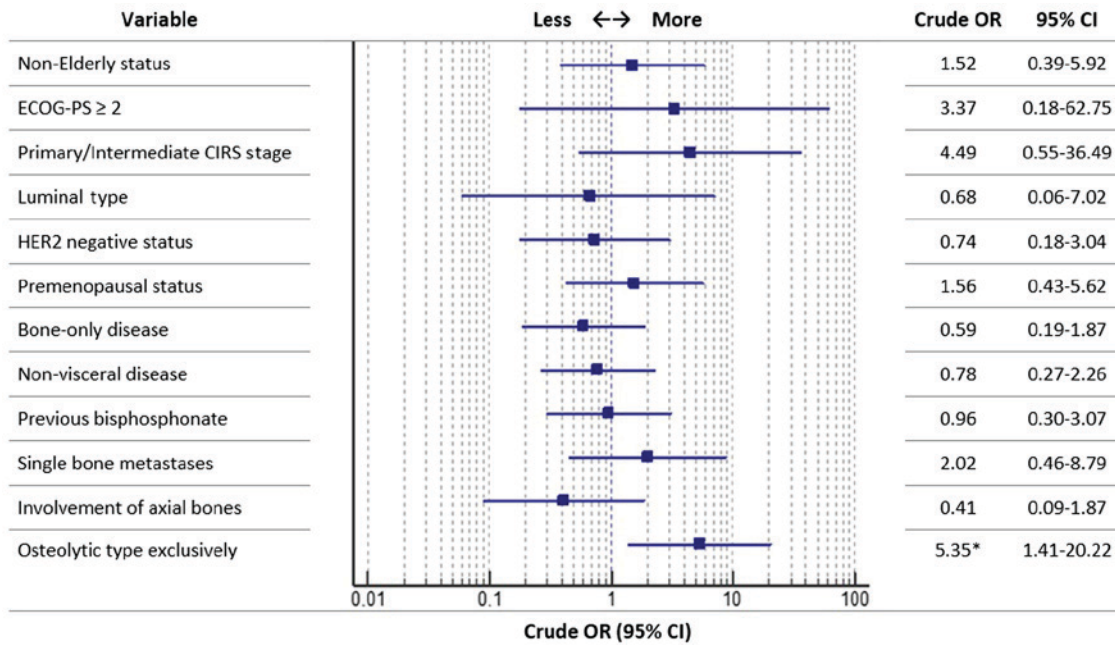


Figure 2. Forest plot graph for incidence of SRE. SRE, skeletal related events; OR, Odds Ratio; *statistically significant.

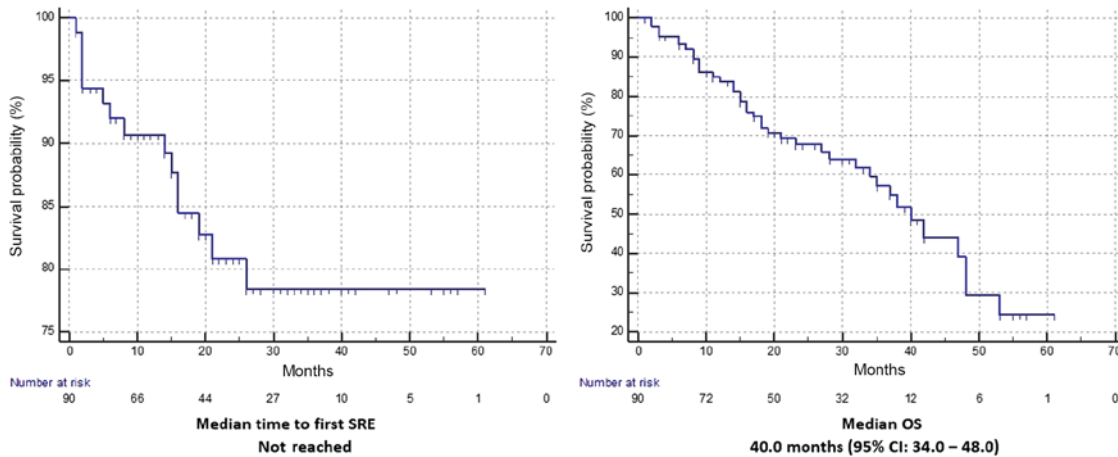


Figure 3. Kaplan-Meier survival estimate for time to first SRE and OS in the overall population. SRE, skeletal related event; OS, overall survival.

Table IV. Univariate analysis for time to first SRE.

Clinical features	No. of patients	Univariate analysis for time to first SRE	
		HR (95% CI)	P-value
Age			
Non elderly vs. elderly	69 vs. 21	0.87 (0.25-3.06)	0.836
ECOG-PS			
0-1 vs. \geq 2	84 vs. 6	Not computable	0.953
CIRS stage			
Primary/intermediate vs. secondary	73 vs. 17	0.29 (0.03-2.23)	0.237
Luminal-like disease			
Yes vs. no	86 vs. 4	1.34 (0.17-10.23)	0.777
HER2 status			
Positive vs. negative	13 vs. 77	1.13 (0.32-3.96)	0.839
Menopausal status			
Yes vs. no	74 vs. 16	0.76 (0.28-2.08)	0.605
Bone-only disease			
Yes vs. no	35 vs. 55	1.76 (0.62-5.03)	0.284
Visceral disease			
Yes vs. no	38 vs. 52	1.26 (0.48-3.27)	0.632
>1 bone metastases			
Yes vs. no	10 vs. 80	0.52 (0.14-1.83)	0.311
Axial bone metastases			
Yes vs. no	81 vs. 9	0.40 (0.11-1.43)	0.161
Previous bisphosphonates			
Yes vs. no	27 vs. 63	1.27 (0.44-3.62)	0.655
Type of bone metastases			
Osteolytic exclusively vs. others	48 vs. 42	0.19 (0.05-0.69)	0.011 ^a

^aStatistically significant (P<0.05). ECOG-PS, eastern cooperative oncology group-performance status; CIRS, cumulative illness rating scale; SRE, skeletal-related events; HR, hazard ratio; CI, confidence interval.

develop ONJ. In our study, G2 hypocalcemia occurred in 3.1% and G3 hypocalcemia in 1.6% of patients. Almost all events of hypocalcemia occurred in the first 6 months of treatment, not related to clinical complications and a more adequate support of calcium and vitamin D solved them within a week.

We observed 17 first SREs; exclusively-osteolytic type of metastases is the only factor significantly related to the incidence of SREs and to median time to first SRE. As in most cases (16 out of 17) the SRE was represented by radiotherapy, performed with a palliative aim for bone pain, thus we could hypothesize that exclusively-osteolytic bone metastases are associated with a greater incidence of pain. Cancer-induced bone pain is a complex syndrome, which involve inflammatory, neuropathic, ischemic and cancer-specific mechanisms, and it is related to tumor growth, release of pain mediators by the cancer cells and damage to the sensory nerves caused by infiltration and/or compression by the tumor cells (36). A different impact on bone pain between osteolytic and osteoblastic lesions has never been clearly demonstrated. Osteolytic metastases are caused by proliferation and hypertrophy of osteoclasts. While inducing bone remodeling, osteoclasts release various acidic

and lytic enzymes, causing bone degradation and a decrease in the pH of the tumor microenvironment; also local acidosis could probably be involved in the nociceptive stimulation of the primary afferents in the bone (37).

The 95.6% of our patients had 'luminal-like' disease. In line with that, a recent prospective study, aimed at identifying patterns of BC relapse according to the biological subtype, Luminal A and Luminal B tumors demonstrated a predominant rate of distant metastases to the bone, compared to HER2-enriched and triple-negative tumors (38).

Multivariate survival analysis showed that just ECOG-PS and bone-only disease result independent predictors for a longer OS. Several other studies reported an improved prognosis for bone-only MBC patients compared to patients with visceral or central nervous system metastases (39-41). In a recent retrospective analyses from the MD Anderson Cancer Center, median OS of bone-only MBC patients was 4.9 years (42). However bone-only disease and ECOG-PS were already been widely related with a longer OS (4,43).

Interestingly, regarding patients with metachronous 'luminal-like' disease, at univariate analysis we found that

Table V. Univariate and multivariate analyses for OS.

Clinical features	No. of patients	Univariate analysis for OS		Multivariate analysis for OS	
		HR (95% CI)	P-value	HR (95% CI)	P-value
Age					
Non elderly vs. elderly	69 vs. 21	1.45 (0.70-2.99)	0.313	-	-
ECOG-PS					
0-1 vs. ≥ 2	84 vs. 6	3.30 (1.14-9.55)	0.027 ^a	4.90 (1.57-15.30)	0.006 ^a
CIRS stage					
Primary/intermediate vs. secondary	73 vs. 17	1.75 (0.82-3.72)	0.144	-	-
Luminal-like disease					
Yes vs. no	86 vs. 4	1.54 (0.46-5.08)	0.476	-	-
HER2 status					
Positive vs. negative	13 vs. 77	1.02 (0.44-2.34)	0.952	-	-
Menopausal status					
Yes vs. no	74 vs. 16	0.93 (0.55-1.55)	0.792	-	-
Bone-only disease					
Yes vs. no	35 vs. 55	3.09 (1.41-6.76)	0.004 ^a	2.86 (1.06-7.69)	0.035 ^a
Visceral disease					
Yes vs. no	38 vs. 52	2.11 (1.10-4.02)	0.023 ^a	1.29 (0.55-3.02)	0.543
>1 bone metastases					
Yes vs. no	10 vs. 80	0.64 (0.22-1.86)	0.420	-	-
Axial bone metastases					
Yes vs. no	81 vs. 9	1.06 (0.32-3.47)	0.916	-	-
Previous bisphosphonates					
Yes vs. no	27 vs. 63	0.81 (0.42-1.55)	0.525	-	-
Type of bone metastases					
Osteolytic exclusively vs. others	48 vs. 42	1.22 (0.64-2.33)	0.527	-	-

^aStatistically significant (P<0.05). ECOG-PS, eastern cooperative oncology group-performance status; CIRS, cumulative illness rating scale; HR, hazard ratio; CI, confidence interval; OS, overall survival.

median TSKR was significantly shorter for patients who were previously exposed to AIs compared to those who were not (53.0 vs 102.0 months, respectively; P=0.0300), even if the significance was not confirmed at multivariate analysis, where only a trend was maintained. These findings are aligned with the abovementioned 'seed and soil' hypothesis (36), suggesting that AI-induced osteoporosis could increase the risk of developing bone metastases. Surely these data are not conclusive; we must take into account the strong limitations of our analysis: the small sample size (40 patients), the retrospective nature and the selection biases. A certain fact is that tumor cells interfere with the bone homeostasis by secreting growth factors, which stimulate bone resorption; bone resorption, in turn, leads to release of factors promoting tumor growth in a 'vicious cycle' of tumor expansion and bone destruction (44). Bisphosphonates and denosumab can block this cycle and prevent bone loss. In order to clarify if AIs could have a certain role in developing bone metastases, we have already planned a multicenter retrospective confirmatory study, which will evaluate clinical pattern of disease progression in early 'luminal-like' BC patients who underwent adjuvant hormonal treatments.

Table VI. Class-related toxicity data.

Grade	No. of patients (n=90) (%)		
	Any grade	G3	G4
Acute phase reactions	2 (2.2)	-	-
Hypocalcemia	17 (18.9)	1 (1.1)	-
Hypercalcemia	1 (1.1)	-	-
Fever	12 (13.3)	-	-
Bone pain/arthralgia	24 (26.7)	-	-
Toothache	9 (10.0)	-	-
Dental infections	2 (2.2)	-	-

Denosumab have demonstrated to improve osteoporosis progression in early BC postmenopausal patients receiving AIs. Assuming that AIs adjuvant treatments could create a 'bone-related' risk conditions for developing bone metastases, could denosumab have a role in prevention of this risk conditions?

The present study showed that denosumab in our hands have a good safety profile, and a pro-active attitude let us to treat in a 'real life' setting the majority of patients without significant class-related toxicities and no ONJ events. The majority of SRE were radiation therapy, so pain still remain the clinical hallmark of bone metastases, particularly of osteolytic ones. The suggestion that estrogen deprivation with AIs can favour a 'bone-related' risk conditions for developing bone metastases must be considered with caution and surely needs further validations; our next multicenter confirmatory study will try to shed light on this topic.

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Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

AC, KC, VC, DS, AI, GP, PB and PF and CF conceived and designed the study. AP, FP, CDO, LV, OV, LZ, TS, and PLB collected the data. AC, PB, PF and VC wrote the paper. KC, DS, CF and GP reviewed and edited the manuscript. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

All patients provided written informed consent to treatment. The procedures followed were in accordance with the ethical standards of the Local Responsible Committees on Human Experimentation (Comitato etico per le province di L'Aquila e Teramo).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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