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Research Article

Differential effect of zoledronic acid on normal trabecular and cortical bone density in oncologic patients with bone metastases

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ABSTRACT

Purpose: To evaluate bone density changes at the level of normal bone and bone metastases after zoledronic acid (ZA) treatment in oncologic patients.**Materials and methods:** We retrospectively evaluated 72 consecutive adult patients with histologically confirmed solid tumors with at least 1 newly diagnosed bone metastatic lesion. Bone metastases were diagnosed by bone scans and confirmed with computed tomography (CT). Patients received intravenous ZA, 4 mg, by 15-min infusion every 28 day through a peripheral or a central venous access and were monitored for at least 3 months and a maximum of 24 months. Bone density was determined at the level of bone metastases and at the level of normal trabecular and cortical bone using a ROI-based approach.**Results:** A significant increase was demonstrated at the level of normal trabecular bone of the calvarium and the femoral neck. No significant increase of density was observed at the level of the normal cortical bone. Bone metastases showed a significant increase in CT density as compared to baseline up to 24 months after zoledronic acid.**Conclusion:** We have found that long term treatment with ZA increases trabecular bone density in oncologic patients whereas normal cortical bone changes are not detectable.© 2012 Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Patients with advanced breast, prostate, lung and colo-rectal cancer frequently develop bone metastases (BMs). These lesions may be asymptomatic or may cause pain, pathologic fractures, malignant hypercalcemia, epidural spinal cord compression and/or shorten survival [1]. The underlying pathophysiology of BMs involves reciprocal interactions between tumor cells and the bone micro-environment that lead to the disruption of the balanced physiological activity between osteoblasts and osteoclasts. Loss of this critical balance results in a spectrum of osteolytic to osteoblastic bone lesions [2].

Computed tomography (CT) provides accurate morphological images of bone, allowing visualization of cortical and trabecular bone, tumor margins and dimensions [2]. Anatomical extension of lesions is depicted on CT studies as well as the presence of

sclerosis in the context of lytic lesions [3]. Whole-body CT scans are diffusely used to detect osteolytic, mixed or osteoblastic bone lesions in the staging, follow-up and re-staging studies of oncological patients. CT scans are also considered crucial in the assessment of the bone response to therapy [2,4,5]. In osteolytic bone metastases, indeed, an increase of density is considered an indicator of response to therapy [2].

As potent inhibitors of osteoclast function, bisphosphonates are being incorporated into the management of metastatic bone disease [6], with beneficial effects on skeletal complications [7,9], bone pain [9], quality of life [7–9], particularly in advanced breast cancer [7,10], multiple myeloma [8,11], and more recently in lung, prostate and kidney cancer [12,13]. Zoledronic acid (ZA) is a potent third generation nitrogen-containing bisphosphonate, which has been widely used in the treatment of Paget's disease of bone [14], hypercalcemia [15], multiple myeloma [16], breast cancer BMs [16], prostate cancer BMs [17], lung cancer BMs [18] and osteolytic BMs [19,20].

In patients with BMs, decrease of bone density is the consequence of several factors, not only of pathologic mechanisms at

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bone metastatic sites, but also of the normal ageing process, of concurrent postmenopausal or drug-related osteoporosis, or of androgen-deprivation therapy in men with prostate cancer [21].

In multicenter randomized controlled trials, once yearly injection of 5 mg of ZA has been demonstrated to significantly reduce the risk of vertebral and hip fractures in postmenopausal women [22] and the risk of recurrence of clinical fractures in men and women with a recent hip fracture [23].

Sclerosis of bone metastases has been documented by CT imaging after ZA treatment in studies [24–26] conducted on patients at an advanced stage of cancer. However, the CT changes of the normal bone after ZA treatment in oncological patients has not yet been established.

The primary aim of this study was to determine CT density changes of the normal trabecular and cortical bone tissue in oncological patients undergoing routine whole body CT examinations; as reference, in the same group of patients, the rate of sclerosis of bone metastases up to 24 months after the initiation of ZA treatment was evaluated.

2. Materials and methods

2.1. Patients

We conducted a retrospective analysis of adult patients with histologically confirmed solid tumors with at least 1 newly diagnosed bone metastatic lesion. Bone metastases were diagnosed by bone scans and confirmed on CT images. The study was carried out according to the principles of the Declaration of Helsinki. Our institutional review board approved the study and all patients provided a written informed consent to receive ZA administration and to undergo CT examinations.

The patients underwent therapy with ZA (Zometa[®], Novartis Pharma, USA) in the period between December 2004 and February 2010. We included patients with a total bilirubin level lower than 2 mg/dL, and serum creatinine level lower than 2 mg/dL, to avoid the effects of prolonged immobility and hepatic or renal osteodystrophy on bone metabolism. Patients with proven peptic ulcer, poor performance status unrelated to bone disease (WHO 3 ± 4), Paget's disease, primary hyperparathyroidism, administration of calcitonin and any prior treatment with bisphosphonates were excluded from the study.

Concurrent cytotoxic, hormonal or steroid therapy was permitted. Each patient was treated with chemotherapy and/or radiotherapy, using an individualized therapeutic approach according to the current international recommendations and the institutions' practice. Patients with metastatic breast cancer were allowed to receive hormonal therapy with tamoxifen or aromatase inhibitors in case of estrogen and/or progesterone receptor positive disease. Patients with castration-sensitive metastatic prostate cancer as well as patients with castration refractory disease were allowed to continue androgen deprivation therapy with luteinizing hormone-releasing hormone (LHRH) analogs and/or anti-androgens. Patients received intravenous ZA, 4 mg, by 15-min infusion every 28 day through a peripheral or a central venous access and were monitored for at least 3 months and a maximum of 24 months. According to standard procedures, supplementation with vitamin D (400 Units/die) and calcium (500 mg/die) was added.

All patients were monitored for skeletal related events (SREs) by physical examination and by diagnostic imaging techniques (X-rays, CT scan, or magnetic resonance imaging scan) at any symptom or sign indicating skeletal disease progression. The definition of SRE included pathologic fractures, surgery or radiotherapy to bone to treat or prevent an impending fracture, palliative radiotherapy to bone, spinal cord compression,

malignant hypercalcemia, and changes in antineoplastic therapy because of worsening bone pain.

2.2. CT scans

CT images were obtained using a 16 and a 64 slice CT scanner (Somatom Sensation, Siemens, Erlangen, Germany). Whole body CT scans were acquired at baseline (prior to treatment) and every 3 and/or 6 and/or 12 months, according to the staging and restaging needs of each patient, to the current international recommendations and to the institutions' practice. WBCT used for the analysis were performed up to 24 months after the initiation of treatment with ZA. Images were obtained using the whole body protocol (KV 120, 140 mAs, B30 kernel) and were reformatted at 5 mm section thickness, before and after bolus administration of non-ionic iodinated contrast agent, at the concentration of 350 mg/mL (Iobitridol, Xenetix[®], Guerbet, France), injected intravenously with a power injector (EnVision; Medrad Italia, PV, Italy) (total volume = 120 mL, flow = 3 mL/min).

Image evaluation was conducted on a separate workstation applying bone-specific Hounsfield Units (HU) windows (width: 2500 HU; window level: 480 HU). By using a two-reader consensus, C.C.Q. and P.D. drew regions of interest (ROIs) on images obtained in the contrast-enhanced scan (60–70 s after contrast injection). After opening the CT scans on the eFilm workstation (MERGE-Healthcare, NL), images were anonymized with no access to the date of the examination. ROIs were chosen at the level of the bone metastases on the basis of concordant CT and bone scans at baseline. Bone lesions previously treated by radiotherapy were not considered for analysis.

ROIs were drawn at the level of normal trabecular and cortical bone in the occipital calvarial bone chosen as a non-weight-bearing, and in the left femoral neck chosen as a weight-bearing skeletal segment. At the level of the normal trabecular bone of the left femoral neck, negative values related to the high content of fatty bone marrow were not considered for analysis and alternative ROIs were drawn.

At each site, three circle-sized ROIs (0.1 cm² for bone metastases and normal trabecular bone and 0.05 cm² for normal cortical bone) were drawn on an eFilm workstation (MERGE-Healthcare, NL) and the average value was used for analysis (Fig. 1). Since mixed bone metastases are frequent in patients with advanced cancer, two groups of predominantly lytic or predominantly sclerotic metastases were classified according to an attenuation threshold value of 300 HU, as previously reported [27]. Measurements were obtained at baseline CT and at the CT scans performed 12 and 24 months after the initiation of ZA treatment.

2.3. Statistical analysis

Patient demographic data, clinical data, primary tumor characteristics and CT density measurements were loaded on a database.

The absolute data were plotted and the relative increments to baseline were calculated.

Descriptive statistics (median, quartiles, ranges, average and standard deviation) was performed. The difference between groups was determined using the non-parametric Wilcoxon signed ranks test on the SPSS platform (SPSS, 14.0). The $p=0.05$ was considered as the threshold for a significant difference among groups.

3. Results

3.1. Patients

We included 72 consecutive patients (35 males and 37 females). The mean age at the diagnosis of bone metastases was 67 ± 12 (\pm standard deviation) years. Demographic details are

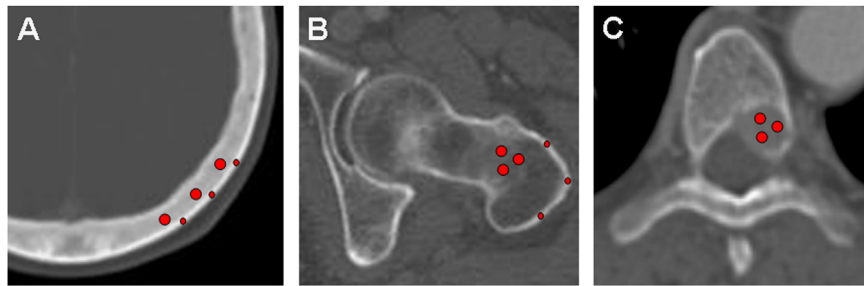


Fig. 1. Regions of interest (ROIs) (red spots) were drawn at the level of the trabecular and cortical occipital calvarium (A), trabecular and cortical left femoral neck (B) and in the context of bone metastatic lesions (C). Average of three values at each site was used for comparison between groups. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Patients demographics and characteristics.

	Total	Breast ca.	Prostate ca.	Colon ca.	Lung ca.	Other
Patients no.	72	29	14	10	6	13
M/F	35/37	1/28	14/0	8/2	5/1	7/6
Age (years)	67 ± 12	66 ± 12	73 ± 8	76 ± 9	68 ± 11	58 ± 13
Histotype	–	Ductal ca. 23 Infiltrative lobular ca. 6	Adenoca. 14	Adenoca. 9 Undifferentiated ca. 1	Adenoca. 2 Small cell ca. 2 Squamous cell ca. 2	
No. of patients with lytic/blastic metastases at baseline	46/26	21/8	6/8	7/3	4/2	8/5

provided in Table 1. According to the CT density threshold of 300 HUs, 64% (46/72) of the patients were included in the group of osteolytic metastases and 36% (26/72) of the patients were included in the group of osteoblastic metastases.

The largest group was represented by patients with a diagnosis of breast cancer and a high prevalence of osteolytic metastases (72%, 21/29) at baseline.

An acute reaction after the first administration of ZA was observed in 7% of the cases (5/72); episodes of asymptomatic hypocalcemia in 9% of the cases (6/72); a slight increase of creatinine serum levels (inferior to 1.5 mg/dL) in 11% of the cases (8/72), osteonecrosis of the mandible in 3% of the cases (2/72).

Skeletal related events were not observed in 50% of the cases (36/72). Among the group of patients that experienced a skeletal related event, 30 patients underwent radiotherapy, 4 patients experienced bone fractures and 2 patients received orthopedic surgery. No atypical fractures were reported.

3.2. CT density of normal bone

Among the 72 patients included in the study, density measurements were available at baseline in a group of 55 patients for the occipital calvarium and a group of 57 patients for the left femoral neck, because of the presence of metastatic lesions, suspicious focal areas of altered density, or the presence of prosthesis at these sites. Details are presented in Table 2.

After 12 months and 24 months of ZA treatment, we observed a significant increase of density at the level of normal trabecular bone of the occipital calvarium, as compared to baseline ($p < 0.05$) (Fig. 2). The average increment of bone density was higher at the level of the left femoral neck as compared to the occipital calvarium. However, the increase of density at the femoral neck reached the significance threshold only after 24 months since the initiation of ZA treatment ($p < 0.001$) (Fig. 2).

At the level of the normal cortical bone, we did not observe significant increases of density both in the occipital calvarium and in the left femoral neck (Table 2).

Table 2

CT density values and increments of normal bone at baseline, 12 months and 24 months after first administration of ZA.

	No. of observations	HUs median [25–75%]	Average of increment vs. baseline [± SEM]	p value [vs. baseline]
Calvarial cortical bone				
0	55	1536 [1327–1762]	–	–
12 months	55	1554 [1316–1906]	14% [± 8%]	0.05
24 months	37	1591 [1298–1954]	22 [± 15%]	0.11
Calvarial trabecular bone				
0	55	1056 [878–1234]	–	–
12 months	55	1138 [890–1338]	8 [± 3%]	0.01
24 months	37	1146 [961–1506]	11 [± 4%]	0.02
Femoral cortical bone				
0	57	726 [586–844]	–	–
12 months	57	715 [617–830]	12% [± 10%]	0.21
24 months	37	671 [587–846]	5% [± 4%]	0.43
Femoral trabecular bone				
0	57	120 [89–153]	–	–
12 months	57	137 [00–179]	23% [± 14%]	0.09
24 months	37	137 [100–173]	18% [± 6%]	0.005

3.3. CT density of bone metastases

CT density at the level of BMs increased during treatment (Table 3 and Fig. 3). Patients showed an average increment of 98% after 12 months of treatment. Density values were 1.75 times higher than baseline after 24 months of treatment. As shown in Table 2, the relative increment of density was higher for osteolytic metastases (1.34 times at 12 months and 2.56 times at 24 months) than for osteoblastic metastases (35% at 12 months and 31% at 24 months). The density of bone osteolytic metastases significantly increased at 24 months in comparison to the 12 months time point (Fig. 4).

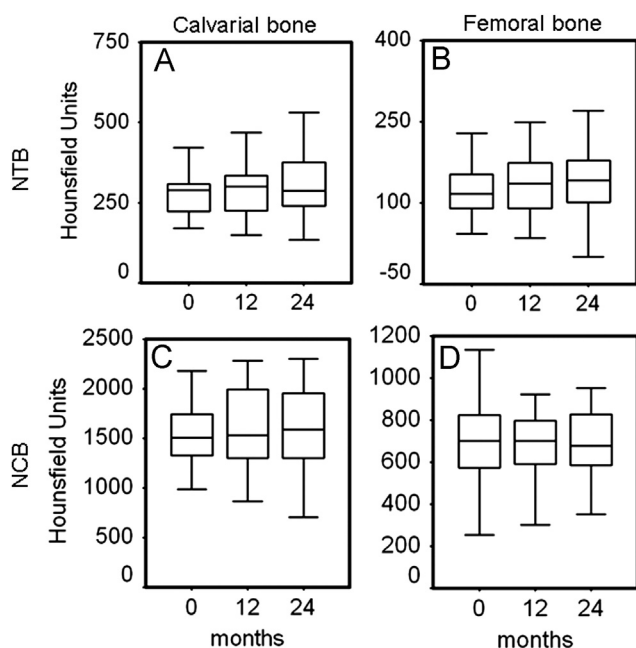


Fig. 2. Box plots represent median, quartiles, minimum and maximum values of absolute Hounsfield units of calvarium (A and C) and femoral (D and E) normal bone. NCB=normal cortical bone; NTB=normal trabecular bone.

Table 3

CT density values and increments of bone metastatic lesions at 12 months and 24 months after first administration of ZA. HU=Hounsfield units; SEM=standard error of the mean; BMs=bone metastases.

	No. of observations	HUs median [25–75%]	Average of increment vs. baseline [\pm SEM]	p value [vs. baseline]
Total patients				
0	72	260 [154–466]	–	–
12 months	54	525 [323–725]	98% [\pm 20%]	< 0.0001
24 months	35	555 [413–712]	X1.75 [\pm 37%]	< 0.0001
Patients with lytic BMs				
0	46	185 [103–251]	–	–
12 months	34	371 [222–541]	X1.34 [\pm 27%]	< 0.0001
24 months	23	554 [348–617]	X2.56 [\pm 40%]	< 0.0001
Patients with blastic BMs				
0	26	514 [463–779]	–	–
12 months	20	747 [611–1007]	35% [\pm 6%]	0.001
24 months	11	774 [588–1096]	31% [\pm 7%]	0.03

4. Discussion

We found that long-term treatment with ZA increases trabecular bone density in oncologic patients, whereas normal cortical bone changes are not detectable. These findings may have important implications in tumor treatment and in the management of osteoporotic patients who are treated with much lower doses of ZA.

Biphosphonates are known to reduce the incidence of skeletal complications in patients with BMs and delay the onset of SREs and the progression of skeletal disease. ZA is a third generation bisphosphonate that has been shown to be more effective than other biphosphonates [27] and significantly reduces skeletal related complications compared with placebo in patients with BMs [28].

This study shows increase of bone density at the level of the normal trabecular bone in oncologic patients. In non-oncologic

postmenopausal osteoporotic patients, once yearly injections of ZA (5 mg/year vs. much higher doses in oncology) significantly increase bone mineral density (BMD) of the trabecular compartment at the spine, femoral neck, trochanter and hip, when compared with placebo, as measured by both dual-energy X-ray absorptiometry (DXA) and quantitative computed analysis (QCT) [29].

In oncologic patients with bone metastases, bone resorption may be linked to several factors such as the normal ageing process, concurrent postmenopausal or drug-related osteoporosis, androgen-deprivation therapy in men with prostate cancer; in our group, even in the occurrence of these phenomena, we still observed an increase of bone density after 12 months (occipital calvarium) and 24 months (occipital calvarium and left femoral neck) of ZA treatment.

We observed a different response to ZA treatment over time: the results were different at the femoral neck as compared to the calvarium. Normal trabecular bone showed a significant increase after 12 months of ZA treatment at femoral neck but not at the calvarium. This could be explained either by a different effect of ZA in different skeletal segments or, alternatively, by concomitant higher bone turnover of the trabecular femoral neck.

At the 24 months time-point, the increase of density of the femoral neck showed a higher increase rate as compared to the calvarium: we interpreted these results possibly as a slow but progressive effect of weight-bearing at the femoral neck.

Density measures of the cortical bone did not yield, in respect to a trend toward a density increase, a significant change up to 24 months: still, these results may be explained by a different effect of ZA on the cortical bone in comparison to that on the trabecular bone or by HU measurements at a saturation level in the cortical compartment, so that slight increases could not be detected. These results, thus, confirm previous reports of a slight but not significant increase of cortical density after ZA treatment [29].

We, moreover, confirmed previous evidence of a net increase of CT density of bone metastases after ZA treatment and demonstrated that the changes persist at a 24 months follow-up. This study and previous reports [24–26] have demonstrated significant sclerotic changes of BMs, more evident in patients with osteolytic lesions. The current data show that changes persist after 24 months of treatment in both osteolytic and osteoblastic metastases. The CT density of osteolytic metastases shows a progressive increase at 24 months in comparison to the 12 months time-point, suggesting that the effect does not reach a plateau level, even after 24 months of treatment. These data support and confirm a dominant effect of ZA as an inhibitor of osteoclastic activity, even in the long-term treatment. Previous reports have shown the effect of concomitant ZA and radiation treatment on density of bone metastases [30]. Radiation therapy alone has been previously shown to increase density of bone metastases by means of quantitative computed tomography [31–33]; in our study, however, skeletal segments previously treated with radiation were not considered and excluded from analysis.

Since concurrent antitlastic and/or hormonal treatments were not discontinued, we cannot rule out an effect on density due to other drugs or to their interactions with ZA. However, bone density increase was seen both in extra-skeletal “responder” and “non-responder” patients, as previously shown [25]. In rat models, ZA alone produces dose-dependent increases in cancellous bone volume and connectivity, 100 times more effectively than pamidronate, and decreases bone resorption [34]. As such, due to the inclusion of patients with different primary tumors and different chemotherapeutic schedules, it is sufficiently safe to claim, on the basis of our and previous reported data, a direct and independent effect of ZA on the increase of density of bone

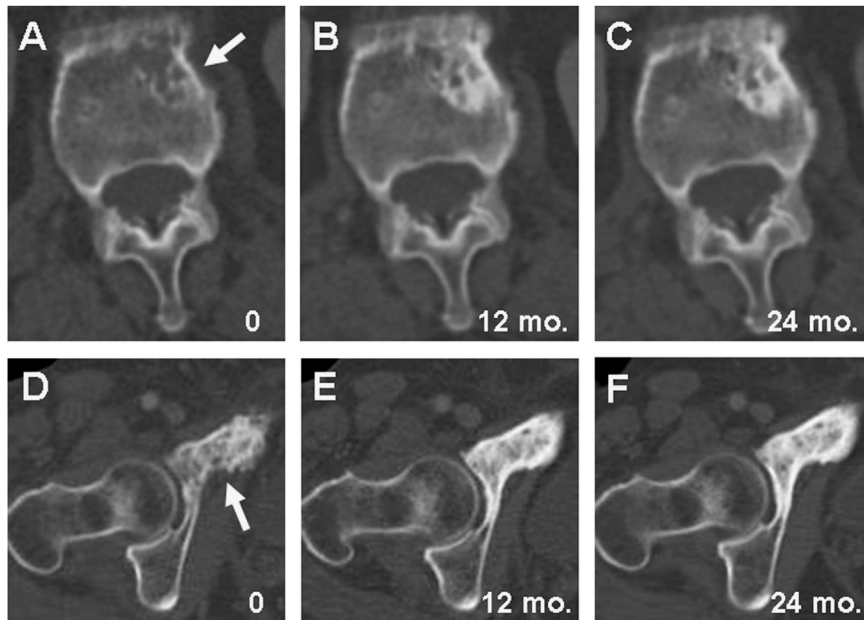


Fig. 3. (A–C) A 65-year-old woman with diagnosis of ductal breast carcinoma. CT images of a predominantly lytic lesion (arrow in A) observed at baseline (A), 12 months (B) and 24 months (C) after initiation of ZA treatment; (D, E) A 75-year-old man with diagnosis of prostate adenocarcinoma. CT images of a predominantly blastic lesion (arrow in D) observed at baseline (D), 12 months (E) and 24 months (F) after initiation of ZA treatment.

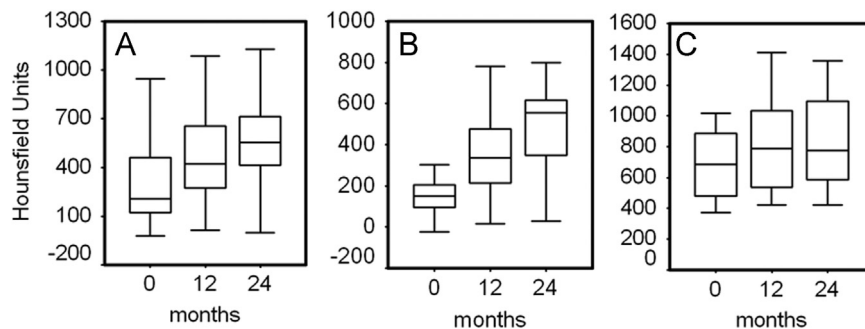


Fig. 4. Box plots represent median, quartiles, minimal and maximal values of absolute Hounsfield units of all (A), predominantly lytic (B) and predominantly blastic (C) bone metastases measured at baseline, 12 months and 24 months after initiation of ZA treatment.

metastases in our patients. This effect is also supported by several reports showing that bisphosphonate administration may significantly decrease levels of osteolytic [35,36] and osteoblastic [37,38] bone markers. Moreover, up-regulation of NF- κ B ligand (RANKL) and osteoprotegerin levels [39], critical for the regulation of osteoclasts maturation, function, and survival, are known to occur following bisphosphonates treatment [40,41].

Osteosclerosis was also significant at the level of osteoblastic bone metastases. The effect, in this case, seemed to reach a plateau level since the difference between the 24 and the 12 months time point was not significant in this subgroup. Residual osteoclastic activity is present even in sclerotic metastases as it is suggested by some evidence of increased bone resorption in osteosclerotic metastases of prostate cancer [42,43]. Yi et al. [44] have shown in an animal model of osteoblastic metastases that an initial phase of bone destruction is followed by extensive formation of bone. Their data suggest that bone resorption precedes bone formation in the development of osteoblastic metastases and that osteoclast activation plays an important role even in the course of osteoblastic metastases [44,45]. The plateau of the increment of density, indeed, could be explained either by a minor effect of ZA on osteoblastic metastases or even by the reaching of HU saturation levels.

A limitation of the study is represented by the lack of quantitative computed tomography measurements [29]. Moreover, given the thin thickness of the cortical bone at the femoral neck, analysis at this site may be biased. However, on this regard, our method of analysis can be applied on routine CT images to evaluate responses to ZA therapy in oncologic patients without needs of additional softwares or phantoms. As such, measurements of response to therapy may be conducted in the standard clinical setting without additional tools.

In conclusion, these results suggest that long-term treatment with ZA increases the bone density both at the level of the normal bone and at the level of bone metastases, with a potential effect on reduction of the incidence of SRE in oncologic patients.

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