Elevated Bone Turnover Predicts for Bone Metastasis in Postmenopausal Breast Cancer: Results of NCIC CTG MA.14

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A B S T R A C T

Purpose
We investigated the association of bone-only relapse with a pretreatment marker of bone resorption: serum beta C-terminal telopeptide (B-CTx) of type I collagen.

Methods
Pretreatment serum B-CTx concentrations were determined from 621 of 667 patients with primary breast cancer enrolled onto the NCIC CTG MA.14 phase III adjuvant trial of tamoxifen with or without octreotide. Recurrence-free survival (RFS) was a secondary end point; the focus here was bone-only relapse. We analyzed continuous or categorical (.71 ng/mL cut point) serum B-CTx in stepwise forward multivariate Cox regression, adjusted for trial stratification factors. We also examined B-CTx and bone relapse by pretrial chemotherapy status.

Results
At median 7.9 years follow-up, 123 of 621 patients experienced recurrence; 19 (3.1%) of 621 had bone-only recurrence, and 47 (7.5%) of 621 had bone plus other sites of recurrence. Larger pathologic tumor size ($P = .001$) and elevated continuous and categorical serum B-CTx were associated with shorter bone-only RFS (both $P = .02$) when added to a model with factors significant in the main trial analyses (hazard ratio [HR], 3.43 and 3.50, respectively; 95% CI, 1.20 to 9.77 and 1.26 to 9.75, respectively). The univariate HR for B-CTx was 2.80 (95% CI, 1.05 to 7.48; $P = .03$). Elevated serum B-CTx was also associated with shorter bone-only RFS ($P = .02$) when added to a model with factors significant in the main trial analyses. Serum B-CTx level was not associated with any other type of recurrence. Serum B-CTx was not significantly different for patients who underwent pretrial chemotherapy, compared with those who did not ($P = .27$), nor did pretrial chemotherapy affect bone relapse ($P = .48$ for bone only; $P = .76$ for bone with other relapse).

Conclusion
Higher pretreatment serum B-CTx was a significant predictor of shorter RFS for bone-only metastasis. Increased bone resorption creates an environment that promotes growth of breast cancer cells.

INTRODUCTION

The prevalence of metastatic bone disease is highest in breast and prostate cancers, which account for approximately 80% of metastatic bone disease, with 65% to 75% of patients with advanced breast and prostate cancers developing bone metastases. Additionally, approximately one third of patients with advanced lung, kidney, and bladder cancers develop bone metastases.[1,2] Complications of bone metastases include significant skeletal morbidity, pain, pathologic fracture, spinal cord compression, and hypercalcemia of malignancy.

Healthy bone has a continuous dynamic state between the activity of two primary cell types: osteoblasts (bone-forming cells) and osteoclasts (bone-resorbing cells). Levels and activity of biochemical markers of bone remodeling reflect normal bone turnover occurring in healthy bone. Extreme alterations in bone markers are measured in serum or urine, indicating bone turnover. Bone resorption leads to collagen breakdown, which leads to the release of biochemical markers into the circulation, including C-telopeptide cross-links (CTx) of type I collagen.[3]

The seed and soil hypothesis in the cellular microenvironment of bone was first proposed by Paget.[4] Paget postulated that cancer cells will preferentially grow in an organ or site that provides a fertile environment for growth. Androgen ablation, a standard treatment for prostate cancer, increases osteoclastic bone resorption. Hypogonadal mice, when inoculated with a human prostate cancer cell line or

human bladder cancer cell line, develop accelerated bone metastases because of the presence of tumor cells in the microenvironment of bone.[5] The current study examined whether beta C-telopeptide (B-CTx), a marker of bone resorption, predicts relapse in the setting of a phase III adjuvant breast cancer trial of postmenopausal women who received adjuvant tamoxifen.[6]

METHODS

Patient Population

The NCIC CTG (National Cancer Institute of Canada Clinical Trials Group) MA.14 protocol enrolled 667 postmenopausal women with histologically proven adenocarcinoma of the breast with satisfactory surgical removal of the tumor by lumpectomy or total mastectomy.[6] Patients had no previous or concurrent malignancies except adequately treated carcinoma of the skin (basal cell), cervix, endometrium, colon, or thyroid, treated more than 5 years before study entry and presumed cured.

Patients were to have undergone either segmental or total mastectomy. Patients treated with segmental mastectomy were required to have breast irradiation according to local institutional guidelines. If these patients also had microscopic disease identified at the resection margins during pathologic examination of the tumor, further excision was recommended. If further excision was not undertaken, a boost to the tumor bed had to be delivered in addition to the required breast irradiation. Patients treated with total mastectomy where microscopic disease was identified at the resection margins during pathologic examination of the tumor were required to have chest-wall irradiation.

Patients with disease beyond the regional lymph nodes were considered to have metastatic disease and were not eligible for the study. All patients were required to have a chest x-ray before study entry. If the patient's alkaline phosphatase levels before random assignment were equal to or greater than $2 \times$ ULN and/or there were symptoms of metastatic disease, a bone scan was required. If results of the bone scan were questionable, confirmatory x-rays were required. If the patient's AST/ALT or alkaline phosphatase levels before random assignment were equal to or greater than $2 \times$ ULN, abdominal ultrasound, liver scan, or abdominal computed tomography were required. For patients receiving no chemotherapy or chemotherapy concurrent with protocol treatment, these investigations had to be performed within 16 weeks before randomization. For patients receiving protocol treatment after chemotherapy, these investigations had to be performed within 16 weeks before initiation of chemotherapy. In addition, there were to be no clinical symptoms of metastatic disease.

Adjuvant chemotherapy was allowed before randomization (sequential) or during study treatment (concurrent), but it was not required. All patients had an Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1, or 2. Pathologic stage was T1, T2, T3, or T4 with dermal involvement on pathology only; Nx N0, N1, N2; M0. Tumors could be estrogen receptor (ER) and/or progesterone receptor (PgR) positive (biochemical value > 10 fmol/mg or positive by immunohistochemistry), negative, or unknown.

Osteoporosis was not specifically assessed before randomization. Bisphosphonates and other nonhormonal treatments for osteoporosis were permitted at inclusion and during follow-up; however, bisphosphonate use was not specifically segregated on computerized forms.

Baseline 25-hydroxy (OH) vitamin D was centrally assessed[7] for 607 of the MA.14 patients (91%) and was not associated with bone only ($P = .19$), concurrent bone plus other site ($P = .73$), or all bone relapses ($P = .66$). Continuous baseline 25-OH vitamin D was not associated with recurrence-free survival (RFS) for any relapse ($P = .57$). Continuous B-CTx and 25-OH vitamin D level were not significantly associated in this patient population (Pearson correlation coefficient, -0.08 [$P = .06$]; Spearman correlation coefficient, -0.07 [$P = .08$]).

This report is based on 621 randomly assigned patients, for whom baseline B-CTx assessments were performed; 323 patients were included in the tamoxifen arm and 298 in the tamoxifen plus octreodtide arm. The B-CTx serum biomarker analysis of NCIC MA.14 was approved by the Penn State Hershey Medical Center Internal Review Board (Hershey, PA).

Stratification

Patients were stratified by adjuvant chemotherapy (none, concurrent, or sequential), nodal status (none, one to three, four or more, or unknown), and receptor status (ER and/or PgR positive, ER and PgR negative, or ER and PgR unknown).

Treatment Schema

Patients were randomly assigned to arm 1 (tamoxifen 20 mg orally once daily for 5 years) or arm 2 (tamoxifen 20 mg orally once daily for 5 years plus octreotide long-acting release 90 mg intramuscularly monthly for 5 years by minimization). In July 2000, the duration of octreotide was reduced from 5 to 2 years because of a greater incidence of gallbladder toxicity in the octreotide arm of NSABP (National Surgical Adjuvant Breast and Bowel Project) B29.

End Points

The primary end point of the trial was event-free survival (EFS); events included recurrence of disease, second malignancy, or death as a result of any cause. A secondary end point was recurrence-free survival (RFS), which included relapse of primary disease only. For the purposes of this report, the focus is RFS of the following types: first, any relapse; second, bone as the only site of relapse; third, bone plus other sites of relapse; or four, nonbone sites of relapse. Relapse in bone was of particular interest, because B-CTx is a measure of bone resorption.

Bone metastatic disease must have been confirmed by imaging. By x-ray, relapse was defined as evidence of lytic, blastic, or mixed lytic-blastic lesions on skeletal films with or without bone scan confirmation. Progressive bone scan changes over at least a 4-week period showing development of new lesions were necessary in asymptomatic patients with only bone scan abnormalities. Any positive bone scan in joints or in a recent area of trauma (surgical or otherwise) could not be used as evidence of bone relapse. Biopsy of bone lesions was not required but if done and positive for malignancy, it was used as evidence of bone relapse. The date used for all incidences of recurrence was the first clinical sign (not symptom) of disease, even if confirmed retrospectively.

Systematic screening for bone metastases using imaging was not employed. Bone metastatic relapse may have been identified by investigations triggered by clinically symptomatic disease or incidentally on radiologic investigation performed for another purpose (not scheduled as part of the clinical trial).

Serum Collection and Storage

All blood samples were to be obtained before octreotide injections using routine venipuncture procedures. Serum samples were collected in a red-top tube and allowed to clot for at least 30 minutes then centrifuged at 3,000 rpm for 10 minutes in a clinical centrifuge at room temperature. The serum was transferred into a plastic screw-cap tube and shipped on cold pack to the storage laboratory. The serum was stored in a -70°C freezer.

B-CTx Enzyme-Linked Immunosorbent Assay

The B-CTx assay employed was the Serum CrossLaps Enzyme-Linked Immunosorbent Assay (ELISA; No. 4CRL4000) from Immunodiagnostic Systems (Fountain Hills, AZ). The B-CTx ELISA uses two monoclonal antibodies directed against cross-linked amino acid sequence EKAHD-Beta-GGR, a degradation product of type I collagen.[8,9] The B-CTx ELISA has an assay detection limit of 0.020 ng/mL, intra-assay coefficient of variation of 2% at 0.45 ng/mL, and inter-assay coefficient of variation of 9% at 0.45 ng/mL (manufacturer's instructions for use). A control cohort of 226 premenopausal white women (mean age, 39 years; standard deviation, 6 years) had a serum B-CTx range of 0.066 to 0.919 ng/mL, median of 0.262 ng/mL, and lower and upper quartiles of 0.206 and 0.377 ng/mL, respectively (S. Durham, personal communication, December 2010).

Statistics

Patients were included in this correlative science investigation if they had a serum B-CTx assessment. We used exact Fisher tests to examine whether there were significant differences by treatment arm and stratification factors in

the numbers of patients with serum for B-CTx assessment. Formal testing for adequacy of assuming normality for B-CTx was performed with Shapiro-Wilk, Kolmogorov-Smirnov, Cramer-von Mises, and Anderson-Darling test statistics. With the assumption of normality being inappropriate, a Box-Cox transformation of the data[10] was performed to improve symmetry and stabilize variances. Hereafter, continuous refers to continuous transformed data.

B-CTx data is continuous, and modeling was performed both with continuous and categorical factor data. The pretreatment serum B-CTx dichotomous cutoff value for high patient B-CTx was derived using the upper nonparametric 95% (one-sided 97.5% quantile) for serum B-CTx levels from a cohort of 226 healthy premenopausal white women (0.71 ng/mL; S. Durham, personal communication, December 2010). We examined associations between continuous B-CTx and categorical factors tumor size (T), nodal status (N), ECOG performance status, and race with analysis of variance. We looked at whether there were differences in categorical low or high B-CTx by treatment arm and baseline patient characteristics with exact Fisher tests.

We knew of no prior assessment of B-CTx in a clinical trial of patients with breast cancer and hypothesized that the continuous B-CTx factor would have a progressively continuous increasing or decreasing effect on outcome. To test the biologic effects of B-CTx on outcome, we used continuous B-CTx. Additionally, we were interested in whether high B-CTx values defined by healthy premenopausal normal women were associated with different outcomes in our patients with breast cancer. Finally, we looked for additional relevance for B-CTx in terms of whether it significantly affected outcome when added to a model with significant factors identified in the main trial analyses.

Exploratory univariate and multivariate analyses were performed with stratified Cox models. The trial stratification factors resulted in 36 strata (three levels of chemotherapy, four of nodal status, three of ER/PgR status), which precluded further stratification for any observed factor imbalances in who had B-CTx assessment. We planned a multivariate investigation of the effects of factors with imbalances, with adjustment as required for those with evidence of a significant effect. Continuous and categorical B-CTx were examined for each end point by adding B-CTx to the stepwise Cox model indicated by the main trial EFS analyses (age, tumor size, nodal status, c-peptide).[6] B-CTx multivariate and univariate hazard ratios (HRs) were generated with 95% CIs.

The number of patients in this investigation precluded Kaplan-Meier plots for B-CTx level simultaneously subgrouped by all patient classifications with indication of significant effects on efficacy. We provided a graphical description of adjusted high and low B-CTx effect on outcome with a Cox survivor plot, where each B-CTx level is adjusted at the mean baseline values of stratification factors (adjuvant chemotherapy, nodal status, hormone receptor status) and tumor size.[11,12]

We examined the association between pretrial chemotherapy and baseline B-CTx as well as bone relapse with tests for: first, significant differences in B-CTx by whether women had chemotherapy before the B-CTx serum draw, using analysis of variance, or if $P < .10$ in Levene test for homogeneous variance, with Welch test; two, significant differences in number of women with higher than 97.5% premenopausal level of B-CTx (0.71 ng/mL) by whether women had chemotherapy before the B-CTx serum draw, using exact Fisher test; and three, significant differences in number of women who had

Table 1. Baseline Characteristics of Patients by Treatment Arm

Characteristic	Total		Tamoxifen		Tamoxifen Plus Octreotide Long-Acting Release		*P**
	No.	%	No.	%	No.	%	
Total	621		323		298		
Age, years							
Median	60.1		59.9		60.3		
At allocation							.69
< 60	306	49	162	50	144	48	
≥ 60	315	51	161	50	154	52	
Race							.12
Non-Hispanic white	600	97	316	98	284	95	
Asian, Aboriginal, other (1% each)	21	3	7	2	14	5	
ECOG performance status							.44
0 or unknown	485	78	248	77	237	80	
1 or 2	136	22	75	23	61	20	
T pathologic classification							.94
0, 1, or in situ	362	58	189	59	173	58	
2, 3A, 4, or unknown	259	42	134	41	125	42	
Breast surgery type							.16
Total mastectomy	236	38	114	35	122	41	
Other or segmental mastectomy	385	62	209	65	176	59	
No. of positive axillary nodes							.99
0	322	52	168	52	154	52	
1-3	208	33	109	34	99	33	
≥ 4	78	13	39	12	39	13	
Unknown	13	2	7	2	6	2	
ER/PgR status							.58
Negative or unknown	56	9	27	8	29	10	
Positive	565	91	296	92	269	90	
Adjuvant chemotherapy							1.00
None	414	67	215	67	199	67	
Concurrent or sequential	207	33	108	33	99	33	

Abbreviations: ECOG, Eastern Cooperative Oncology Group; ER, estrogen receptor; PgR, progesterone receptor.
*Exact Fisher *P* value.

bone-only relapse or bone with other relapse by whether women had chemotherapy before the B-CTx serum draw, using exact Fisher test. SAS version 9.1.3 (SAS Institute, Cary, NC) was used for all analyses.

RESULTS

A total of 667 patients were enrolled onto the NCIC CTG MA.14 trial between 1996 and 2000; there was no significant difference ($P = .62$) in EFS between the treatment arms in this trial.[6] Baseline serum B-CTx levels were determined for 621 (93.1%) of 667 patients with the following specifications: minimum, 0.02; lower quartile, 0.28; median, 0.39; upper quartile, 0.59; and maximum, 1.88. Fewer patients in the tamoxifen plus octreotide arm ($P < .001$) had B-CTx assessments: 323 (97%) in the tamoxifen arm and 298 (89%) in the tamoxifen plus octreodtide arm. There were no significant imbalances by the stratification factors (B-CTx was assessed for 93% of those who received no chemotherapy, 94% who received concurrent chemotherapy, and 93% who received sequential [$P = 1.00$]), lymph node status (92% had node-negative disease, 94% with one to three positive nodes, 95% with four or more positive nodes, and 100% with unknown axillary status [$P = .62$]), or hormone receptor status (93% were ER and/or PgR positive; 90% were ER/PgR negative; 100% who had unknown receptor status [$P = .51$]).

Median age for patients in this investigation was 60.1 years. Other baseline patient characteristics were similar between treatment arms regarding age, race, ECOG performance status, tumor pathologic classification, breast surgery type, number of positive nodes, ER/PgR status, adjuvant chemotherapy, and c-peptide (Table 1). Continuous B-CTx was not significantly associated with T ($P = .99$), N ($P = .85$), ECOG performance status ($P = .24$), or race ($P = .91$). The only significant difference in categorical B-CTx (Table 2) was that fewer women who received a total mastectomy had low B-CTx ($P = .01$). Median follow-up for patients in this study was 7.9 years. Nineteen (3.1%) of 621 patients relapsed in bone as the only site of relapse, 47 (7.5%) of 621 concurrently relapsed first in bone and then other site(s), and 57 (9.2%) of 621 relapsed only in nonbone sites. We found that the assumption of normality was inappropriate for serum B-CTx and that the Box-Cox power (0.25) transformation adequately improved symmetry.

Higher pretreatment serum B-CTx was associated with shorter bone-only RFS when the factor was categorized relative to levels observed in healthy women (Fig 1; univariate HR, 2.80; 95% CI, 1.05 to

Table 2. Baseline Characteristics of Patients by B-CTx Level

Characteristic	Total		B-CTx* < 0.71 ng/mL		B-CTx ≥ 0.71 ng/mL		P†
	No.	%	No.	%	No.	%	
Total	621		529		92		
Treatment							.73
Tamoxifen	323	52	277	52	46	50	
Tamoxifen plus octreotide long-acting release	298	48	252	48	46	50	
Age at allocation, years							.65
< 60	306	49	263	50	43	47	
≥ 60	315	51	266	50	49	53	
Race							.53
Non-Hispanic white	600	97	512	97	88	96	
Asian, Aboriginal, other (1% each)	21	3	17	3	4	4	
ECOG performance status							.59
0 or unknown	485	78	415	78	70	76	
1 or 2	136	22	114	22	22	24	
T pathologic classification							.91
0, 1, or in situ	362	58	309	58	53	58	
2, 3A, 4, or unknown	259	42	220	42	39	42	
Breast surgery type							.01
Total mastectomy	236	38	189	36	47	51	
Other or segmental mastectomy	385	62	340	64	45	49	
No. of positive axillary nodes							.71
0	322	52	276	52	46	50	
1-3	208	33	178	34	30	33	
≥ 4	78	13	65	12	13	14	
Unknown	13	2	10	2	3	3	
ER/PgR status							.84
Negative or unknown	56	9	47	9	9	10	
Positive	565	91	482	91	83	90	
Adjuvant chemotherapy							.63
None	414	67	355	67	59	64	
Concurrent or sequential	207	33	174	33	33	36	

Abbreviations: B-CTx, beta C-terminal telopeptide; ECOG, Eastern Cooperative Oncology Group; ER, estrogen receptor; PgR, progesterone receptor.
*B-CTx cut point of 0.71 ng/mL is upper 95% (one-sided 97.5% quantile) for serum B-CTx level from 226 healthy premenopausal women.
†Exact Fisher *P* value.

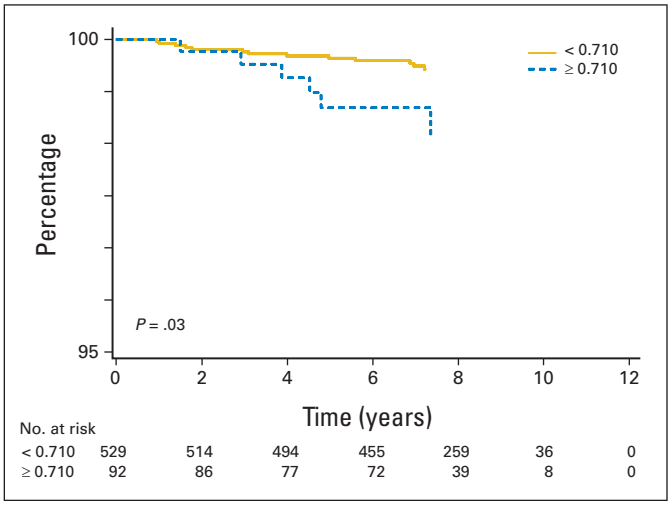

Fig 1. Pretreatment serum beta C-terminal telopeptide (B-CTx) predicts bone-only relapse in NCIC (National Cancer Institute of Cananda) MA.14 trial. B-CTx cut point of 0.71 ng/mL is the upper 95% CI (one-sided 97.5% quantile) for serum B-CTx for healthy premenopausal women. Cox survivor plot: each arm adjusted at the mean baseline values for adjuvant chemotherapy (for B-CTx, < 0.710, 0.327; for B-CTx, ≥ 0.710, 0.348), nodal status (for B-CTx, < 0.710, 0.473; for B-CTx ≥ 0.710, 0.500), hormone receptor status (for B-CTx, < 0.710, 0.905; for B-CTx, ≥ 0.710, 0.902), and pathologic T (for B-CTx, < 0.710, 0.416; for B-CTx, ≥ 0.710, 0.424). Univariate hazard ratio, 2.80 (95% CI, 1.05 to 7.48; $P = .03$). Scale for recurrence-free survival plot is 95% to 100%.

7.48; $P = .03$). Continuous and categorical serum B-CTx was significantly associated with bone-only relapse (both $P = .02$) when it was added to significant factors (age, tumor size, nodal status, c-peptide) identified in event-free survival analyses (HR, 3.43 and 3.50, respectively; 95% CI, 1.20 to 9.77 and 1.26 to 9.75, respectively); larger pathologic tumor size was also associated with bone-only RFS ($P = .001$). There was no significant interaction between B-CTx and trial therapy. Categorical serum B-CTx was not univariately or multivariately associated with any other type of first recurrence: all types of relapse (HR, 1.49; 95% CI, 0.95 to 2.35; $P = .08$ *v* HR, 1.52; 95% CI, 0.96 to 2.41; $P = .08$), concurrent bone and other relapse (HR, 1.29; 95% CI, 0.69 to 2.43; $P = .43$ *v* HR, 1.35; 95% CI, 0.71 to 2.54; $P = .38$), or nonbone relapse (HR, 1.76; 95% CI, 0.91 to 3.40; $P = .09$ *v* HR, 1.84; 95% CI, 0.95 to 3.56; $P = .09$).

We also examined whether the B-CTx results were attributable to chemotherapy. Serum B-CTx was assessed before (any) chemotherapy in 382 (61.5%) of 621 of patients and after chemotherapy in 239 (38.5%) of 621 patients; there were no significant differences in serum B-CTx values ($P = .27$). We found that 92 (14.8%) of 621 patients had high serum B-CTx levels compared with healthy women; a similar proportion of the women with high B-CTx received chemotherapy before and after serum was drawn for B-CTx assessment ($P = .42$). Furthermore, there were no differences in the number of women who had bone-only recurrence ($P = .48$) or bone and other type of relapse ($P = .76$) by whether women had chemotherapy before blood was drawn for the B-CTx serum assessment.

DISCUSSION

More than a century ago, Paget[4] postulated that a favorable soil in the bone marrow microenvironment was an important factor for the growth of cancer cells. Two mouse models of metastasis to bone support this theory. The first employed PC-3 prostate cancer cells resulting in osteolytic bone metastases after intracardiac inoculation in nude mice. In the second model, injection of TSU-Pr1, a bladder cancer cell line, resulted in mixed osteolytic-osteoblastic bone metastases. Hypogonadal mice have accelerated bone turnover because of increased osteoclastic bone resorption and when inoculated with the human prostate cancer cell line, PC-3, or the human bladder cancer cell line, TSU-Pr1, had accelerated bone metastases. These data in animals support the hypothesis that increased bone resorption results in a more fertile environment for the development of bone metastases.[5]

In this study, the ability of pretreatment serum B-CTx of type I collagen, a marker of bone resorption, to predict breast cancer relapse was investigated in a randomized adjuvant breast cancer trial in which 123 of 621 patients experienced recurrence of disease. Higher pretreatment serum B-CTx was significantly associated with shorter RFS in patients with bone-only metastases. As expected, larger tumor size was associated with shorter time to bone metastases, and there was no apparent association with tumor size and B-CTx.

Bone matrix stores a number of immobilized growth factors. For example, transforming growth factor beta (TGF-β) is released in active form during osteoclastic resorption.[13] TGF-β has been demonstrated to directly stimulate the growth of certain cancer cell lines. TGF-β also regulates several genes that are responsible for enhanced bone metastases in MDA-MB-231 cells, such as IL-11, PTHrP, and connective tissue growth factor.[14,15]

Trials of adjuvant zoledronic acid in premenopausal women (ABCSG-12)[16] and postmenopausal women (Z-FAST, ZO-FAST, and EZO-FAST)[17] and in postmenopausal women more than 5 years after menopausal (AZURE)[18] have demonstrated an increase in RFS. Bone marrow can act as a sanctuary for tumor cells that can be the source of subsequent disease relapse. Monthly zoledronic acid reduces the prevalence and persistence of disseminated tumor cells in the bone marrow of women with early breast cancer.[19] Another possible mechanism is that zoledronic acid can inhibit osteoclast activity, which is responsible for a hospitable environment for cancer cell growth.

In summary, bone metastases are the result of complex interactions between tumor cells, bone cells, and the bone microenvironment. Assessing and targeting the bone microenvironment should be part of the strategy to prevent this devastating complication of advanced cancer.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Glossary Terms

Bone resorption: The process of losing bone substance. Bone, when it is remodeled (reshaped), undergoes both new formation and resorption. The cell responsible for the resorption of bone is called an osteoclast, and the bone forming cell is the osteoblast.

Collagen: The principal protein of the skin, tendons, cartilage, bone, and connective tissue.

C-telopeptide cross-links (CTx) of type I collagen: Type I collagen cross-links are covalent chemical links that connect collagen fibrils. Cross-linking between molecules in fibrils produces a very stable protein structure, which contributes to collagen's tissue-strengthening function. The common cross-links of bone resorption markers are pyridinium cross-links, cross-linked N-terminal telopeptides of type I collagen (NTx), and C-terminal telopeptides of type I collagen (CTx) that are found in the circulation and are excreted into the urine. The telopeptides of type I collagen have proven to be more sensitive markers than the pyridinium cross-links.

Enzyme-linked immunosorbent assay (ELISA): An ELISA is a sensitive, quantitative immunochemical test that involves an enzyme linked to an antibody or antigen to allow detection of a specific protein. Generally, the specimen is added to a surface, on which are immobilized antibodies specific to the protein of interest. If the protein is present, it will bind to the attached antibody layer. The presence of the bound protein is then verified with antibodies that have been tagged with an enzyme, which causes the specimen to change color corresponding to the concentration of the target protein.

Osteoclast: A cell that breaks down bone and is responsible for bone resorption. Osteoclasts are large multinucleate cells that differentiate from macrophages.

Type I collagen: the major component of bone, it accounts for more than 90% of the organic matrix of bone.