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REDUCTION IN NEW METASTASES IN BREAST CANCER WITH ADJUVANT CLODRONATE TREATMENT

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ABSTRACT

Background Bisphosphonates are effective against the increased bone resorption caused by certain diseases because they inhibit the activity of osteoclasts. In patients who have breast cancer and metastatic bone disease, the bisphosphonate clodronate (clodronic acid) reduces the frequency of skeletal complications. Experiments in animals and preliminary clinical observations indicate that early clodronate therapy reduces the incidence of new bony metastases in breast cancer. We investigated the effects of clodronate on the incidence and extent of new metastases in patients with breast cancer.

Methods Between 1990 and 1995, 302 patients with primary breast cancer and tumor cells in the bone marrow (the presence of which is a risk factor for the development of distant metastases) were randomly assigned to receive clodronate at a dose of 1600 mg per day orally for two years (157 patients) or standard follow-up (145 patients). The median length of observation was 36 months. All patients in both groups received standard surgical treatment and customary hormonal therapy or chemotherapy.

Results Distant metastases were detected in 21 patients in the clodronate group and in 42 patients in the control group ($P < 0.001$). The incidence of both osseous and visceral metastases was significantly lower in the clodronate group than in the control group ($P = 0.003$ for both osseous and visceral metastases). Six patients in the clodronate group died, as did 22 in the control group ($P = 0.001$). The mean number of bony metastases per patient in the clodronate group was roughly half that in the control group (3.1 vs. 6.3).

Conclusions Clodronate can reduce the incidence and number of new bony and visceral metastases in women with breast cancer who are at high risk for distant metastases. (N Engl J Med 1998;339:357-63.)

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BISPHOSPHONATES are pyrophosphate analogues that inhibit the formation and dissolution of calcium phosphate crystals in vitro. In vivo, bisphosphonates bind strongly to hydroxyapatite on the bone surface. Used therapeutically, bisphosphonates inhibit osteoclast-mediated bone resorption by mechanisms that are not fully understood.¹⁻³ Clinical trials have demonstrated the anti-osteolytic effect of bisphosphonates in patients with breast cancer and bone metastases. In such patients, bisphosphonates significantly reduced the incidence of hypercalcemia, bone pain, and pathologic fractures, but overall survival was not prolonged.⁴⁻⁶ In experiments in animals, bisphosphonates have been shown to produce a significant reduction in the appearance of new metastases to bone.^{7,8} Clodronate (clodronic acid), a bisphosphonate, was also shown to reduce the number of new skeletal metastases in patients with breast cancer who had advanced local or distant disease without preexisting bony metastases.⁹

In the present study, we evaluated the effect of treatment with oral clodronate (Ostac, Boehringer Mannheim, Mannheim, Germany) during a period of two years in patients with primary breast cancer. The primary end points were the incidence and number of new bony and visceral metastases and the length of time to their appearance; skeletal complications were not included in these end points. Only patients in whose bone marrow tumor cells were found at the time of surgery were enrolled in the study. Even if there is no involvement of the axillary

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lymph nodes, the presence of tumor cells in the bone marrow (minimal residual cancer) portends a high risk of subsequent metastasis.^{10,11}

METHODS

Patients

This prospective, randomized, and non–placebo-controlled study was carried out at the University Hospital Heidelberg between February 1990 and April 1997 (enrollment of patients ended in November 1995). The criteria for inclusion were primary breast cancer that was classified as being at stage T1, T2, T3, or T4 and histologically classified as stage N0, N1, or N2 (i.e., tumor size ranged from less than 2 cm to more than 5 cm, and ipsilateral lymph-node metastases were either absent or present and, if present, either movable or fixed) (Table 1); immunocytochemical detection of at least one tumor cell in a bone marrow aspirate; and provision of written informed consent. Criteria for exclusion were confirmed distant metastasis, previous or simultaneous secondary malignant disease, neoadjuvant chemotherapy or hormone therapy, skeletal disease, serious functional disorders of the liver or kidneys, and pregnancy. The study design was reviewed and approved by the ethics board of the Faculty of Clinical Medicine of the University of Heidelberg.

The inclusion criteria were met by 302 patients, who were randomly assigned either to treatment with clodronate or to the control group. Randomization was carried out postoperatively after the results of the histologic examination, assessment of prognostic factors, and tumor-cell immunocytologic studies had been received, but before a decision was made regarding adjuvant systemic treatment. No stratification according to the type of adjuvant treatment was undertaken. The control group consisted of 145 patients, 3 of whom refused to undergo follow-up examinations, leaving 142 patients who could be evaluated. Of the 157 patients in the bisphosphonate group, 13 were excluded because therapy was stopped for unknown reasons (4 patients), because of side effects (7), or because there was insufficient compliance with therapy in the first two to three months (2). Two additional patients were lost to follow-up, leaving 142 patients in the bisphosphonate group who could be evaluated. All 302 patients were included in the intention-to-treat analysis.

Therapy

The primary surgical therapy consisted of either mastectomy or breast-conserving surgery (lumpectomy or segmental resection plus 50 Gy of radiotherapy to the breast). Axillary lymphadenectomy (levels I and II) and iliac-crest bone marrow aspiration were carried out in all patients. The marrow was processed, stained, and evaluated according to a standardized procedure, as described elsewhere.^{10,11} Tumor cells in bone marrow were identified immunocytologically by the presence of the tumor-associated glycoprotein TAG 12.

Adjuvant systemic therapy was based on the recommendations of the German Adjuvant Breast Cancer Group and the guidelines of the St. Gallen Consensus Conferences. All patients with axillary-lymph-node involvement and all node-negative patients with other factors indicating a poor prognosis received adjuvant systemic treatment. Menopausal status, steroid hormone–receptor status, and tumor size were taken into account in making therapeutic decisions, but the presence or absence of tumor cells in bone marrow was not a factor in the decision. Patients were treated with 30 mg of tamoxifen daily for 2 years (92 patients); standard cyclophosphamide (500 mg per square meter of body-surface area), methotrexate (40 mg per square meter), and fluorouracil (600 mg per square meter) on day 1 and day 8 of every 28-day cycle for six cycles (63 patients); six cycles of cyclophosphamide (600 mg per square meter) and epirubicin (90 mg per square meter) every 21 days, with or without 600 mg of fluorouracil per square meter (17 patients); 3.6 mg of goserelin monthly for

TABLE 1. CLINICAL AND PATHOLOGICAL FACTORS IN THE 302 PATIENTS WITH BREAST CANCER IN WHOM TUMOR CELLS WERE DETECTED IN BONE MARROW.

FACTOR*	ALL PATIENTS (N=302)	CLODRONATE GROUP (N=157)	CONTROL GROUP (N=145)
			no. (%)
Tumor stage†			
T1	113	59 (38)	54 (37)
T2	138	71 (45)	67 (46)
T3	35	17 (11)	18 (12)
T4	16	10 (6)	6 (4)
Nodal status‡			
N0	143	77 (49)	66 (46)
N1 or N2	159	80 (51)	79 (54)
Estrogen-receptor status§			
Positive	188	104 (75)	84 (71)
Negative	69	35 (25)	34 (29)
Progesterone-receptor status§			
Positive	157	85 (62)	72 (63)
Negative	93	51 (38)	42 (37)
Menopausal status			
Premenopausal	113	56 (36)	57 (39)
Postmenopausal	189	101 (64)	88 (61)
Histologic grade¶			
I or II	185	93 (68)	92 (73)
III	78	44 (32)	34 (27)
S-phase fraction			
<5%	111	59 (50)	52 (51)
≥5%	109	60 (50)	49 (49)

*There were no significant differences between groups for any of the factors listed.

†Tumors and axillary lymph nodes were staged according to criteria of the Union International contre le Cancer. T1 denotes tumor ≤2 cm in greatest dimension, T2 tumor >2 cm to 5 cm in greatest dimension, T3 tumor >5 cm in greatest dimension, and T4 tumor of any size with direct extension to chest wall or skin.

‡N0 denotes no regional lymph-node metastasis, N1 metastasis to one or more movable ipsilateral axillary lymph nodes, and N2 metastasis to one or more ipsilateral axillary lymph nodes fixed to another node or to other structures.

§Positive status was defined as ≥20 fmol of protein per milligram, and negative status as <20 fmol of protein per milligram. A total of 257 tumors were tested for estrogen-receptor status, and 250 for progesterone-receptor status.

¶A total of 263 tumors were graded histologically. Tumors were graded according to the Scarf–Bloom–Richardson classification. Grade I indicates a well-differentiated tumor, grade II a moderately well differentiated tumor, and grade III a poorly differentiated tumor.

||The S-phase fraction was measured in 220 tumors.

2 years (27 patients); or a combination of tamoxifen plus cyclophosphamide, methotrexate, and fluorouracil (47 patients). Fifty-six patients received no further systemic treatment.

Patients assigned to the clodronate group received 1600 mg of oral clodronate per day for two years. They were instructed to take four capsules of clodronate (400 mg each) every morning at least one hour before breakfast and were instructed to take the capsules only with water and never with meals containing calcium.

Various endocrine therapies (antiestrogen agents, luteinizing hormone–releasing hormone analogues, aromatase inhibitors, and progestins) were used in patients with confirmed metastases. Antineoplastic agents were given in the event of rapid progression or extensive metastases. Clodronate therapy was continued in all patients with metastatic disease in the clodronate group and was started in patients in the control group in whom metastases to bone were identified. Osteolytic lesions were irradiated in the

event of bone pain or the threat of pathologic fractures. Intravenous clodronate (1500 mg over a period of two hours) was administered to patients with hypercalcemia.

Follow-up

Follow-up investigations were carried out in the University Women's Hospital for all patients according to a standard protocol. The interval between investigations was three to four months during the first two years. At every visit, a history was taken and a physical examination was performed; chest radiography, bone scanning, ultrasonographic examination of the liver, and mammography were performed yearly. Laboratory tests (blood counts and measurements of tumor antigens in serum) were carried out every three months. If there was evidence of metastases to bone, additional x-ray films were obtained of the affected areas. The pattern of metastasis was analyzed at the end of the study. Bone lesions seen on radiographs were assessed by two independent radiologists. Skeletal complications were recorded as events, but they were not included in the statistical analysis.

Statistical Analysis

The initial statistical projection was that, after 36 months of follow-up, there would be a difference of 10 percent between the groups in the rate of bony metastasis. This assumption was based on our previous studies of tumor-cell detection. The planned size of the sample was 300 patients. The data were last updated in March 1997. The chi-square test was used to assess differences in the frequency of individual prognostic factors between the groups. Kaplan-Meier analyses and the log-rank test were used to investigate differences in overall and metastasis-free survival. All P values were two-sided. Statistical data processing was carried out with SAS software (SAS Institute, Cary, N.C.) and Systat software (Systat, Evanston, Ill.).

RESULTS

Characteristics of the Patients

The median age of the patients was 51 years (range, 24 to 78). Tumors in stage T2 were most common (46 percent of patients), followed by tumors smaller than 2 cm (37 percent) and stage T3 or T4 tumors (17 percent) (Table 1). The nodal status was negative in 143 patients (47 percent), whereas the axillary lymph nodes were involved in 159 (53 percent). Of 257 primary tumors tested, 188 (73 percent) were estrogen-receptor-positive; 157 of 250 tumors tested (63 percent) were progesterone-receptor-positive. A total of 113 patients (37 percent) were premenopausal, and 189 (63 percent) were postmenopausal. With regard to grading, 185 of the 263 primary tumors we assessed were grade I or II (70 percent) and 78 were grade III (30 percent) (Table 1). The chi-square test showed that there were no significant differences in the distribution of the individual characteristics and prognostic factors between the clodronate and control groups.

Adjuvant Systemic Treatment

Of the 302 patients who were initially enrolled, 246 (81 percent) received adjuvant systemic treatment (Table 2). There was no significant difference in the proportion of patients receiving adjuvant therapy between the control and clodronate groups.

TABLE 2. ADJUVANT SYSTEMIC TREATMENT IN PATIENTS WITH BREAST CANCER.*

THErapy	ALL PATIENTS (N=302)	CLODRONATE GROUP (N=157)	CONTROL GROUP (N=145)
		no. (%)	
CMF (standard)	63 (21)	31 (20)	32 (22)
EC or FEC	17 (6)	8 (5)	9 (6)
Tamoxifen	92 (30)	49 (31)	43 (30)
Goserelin	27 (9)	16 (10)	11 (8)
Chemotherapy and endocrine therapy†	47 (16)	25 (16)	22 (15)
No adjuvant treatment	56 (19)	28 (18)	28 (19)

*CMF denotes cyclophosphamide, methotrexate, and fluorouracil; EC, epirubicin and cyclophosphamide; and FEC, fluorouracil, epirubicin, and cyclophosphamide. See the Methods section for details of all regimens.

†Chemotherapy and endocrine therapy consisted of tamoxifen plus standard CMF.

TABLE 3. INCIDENCE OF METASTATIC DISEASE AND DEATH IN THE CLODRONATE AND CONTROL GROUPS AFTER A MEDIAN FOLLOW-UP OF 36 MONTHS.

OUTCOME	ALL PATIENTS (N=302)	CLODRONATE GROUP (N=157)	CONTROL GROUP (N=145)	P VALUE*
		no. (%)		
Distant metastasis	63	21 (13)	42 (29)	<0.001
Bony metastasis†	37	12 (8)	25 (17)	0.003
Visceral metastasis†	40	13 (8)	27 (19)	0.003
Death	28	6 (4)	22 (15)	0.001
		mean		
No. of bony metastases per patient		3.1	6.3	0.004‡

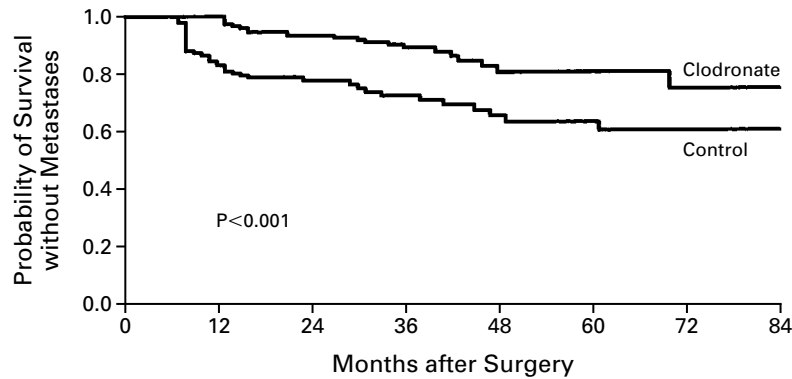
*P values were derived by the log-rank test, unless otherwise specified.

†Patients with synchronous visceral and bony metastases are included in both groups.

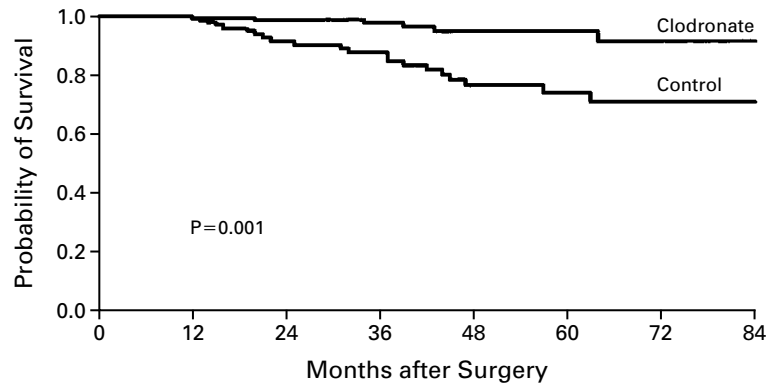
‡The P value was derived by the Mann-Whitney-Wilcoxon test.

Follow-up and Pattern of Metastasis

During the median observation period of 36 months, distant metastases (bone or visceral) were detected in 21 women in the clodronate group and in 42 women in the control group (Table 3). In the clodronate group, metastases to bone developed in 12 women and visceral metastases in 13 women, whereas in the control group there were 25 women with bony metastases and 27 with visceral metastases. Twenty-two patients (15 percent) in the control group died, as compared with six patients (4 percent) in the clodronate group. Furthermore, the mean number of bony metastases per patient was about



NO. OF PATIENTS		0	12	24	36	48	60	72	84
Clodronate	157	146	119	71	36	21	13	8	
Control	145	120	87	49	31	22	7	5	



NO. OF PATIENTS		0	12	24	36	48	60	72	84
Clodronate	157	146	126	80	45	28	17	10	
Control	145	142	100	62	38	28	10	5	

Figure 1. Kaplan–Meier Curves for Metastasis-free Survival and Overall Survival after Primary Surgery among 157 Patients Treated with Clodronate and 145 Controls.

P values were derived with the log-rank test. The numbers of patients below the panels are the numbers at risk.

twice as high in the control group (6.3) as in the clodronate group (3.1, $P=0.004$).

The Kaplan–Meier curves showed significant differences between the two groups in metastasis-free survival ($P<0.001$) and overall survival ($P=0.001$) (Fig. 1). The differences were also significant with regard to the proportion of patients with bony metastases ($P=0.003$) and visceral metastases ($P=0.003$) (Fig. 2).

DISCUSSION

In this study of women with breast cancer, we found that oral treatment with 1600 mg of clodronate per day for two years in addition to standard surgical and systemic therapy significantly reduced the incidence of osseous and nonosseous metastases. There was an even distribution of possible confounding factors such as the receipt of chemotherapy, en-

docrine therapy, or no therapy, in the two groups of patients, perhaps because of the randomization procedure. After a median follow-up of 36 months, the number of metastatic bony lesions per woman was twice as high in the control group as in the clodronate group. All the women in this study had a high risk of distant metastases, as indicated by the presence of tumor cells in the bone marrow. We and others have shown that the presence of tumor cells in the bone marrow is a strong predictor of early hematogenous metastasis.¹¹⁻¹³ Tumor cells were found in the bone marrow in 55 percent of node-positive patients with breast cancer and 31 percent of node-negative patients, and their presence was significantly associated with larger tumors, positive lymph nodes, and other unfavorable prognostic factors. There was a strong correlation between the presence of tumor cells in the bone marrow and the subse-

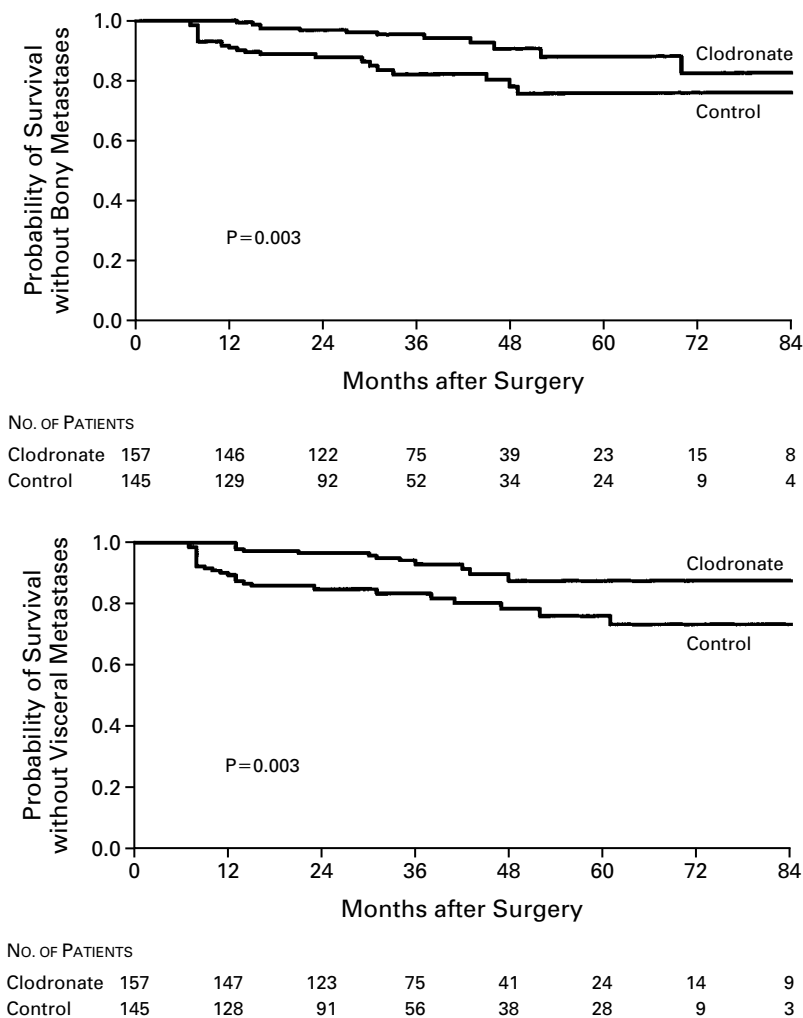


Figure 2. Kaplan–Meier Curves for Survival Free of Bony Metastases and Survival Free of Visceral Metastases among 157 Patients Treated with Clodronate and 145 Controls.

P values were derived with the log-rank test. The numbers of patients below the panels are the numbers at risk.

quent detection of distant metastasis. Indeed, the presence of tumor cells in the marrow was found to be the most powerful predictor of outcome, superior even to nodal status in predictive value.¹¹

In about three quarters of women who eventually die of breast cancer, bony metastases develop. Patients with skeletal metastases have relatively favorable survival times, but the course of their diseases is complicated by bone pain, pathologic fractures, spinal cord compression, and hypercalcemia.¹⁴⁻¹⁶

Bisphosphonates such as clodronate are potent inhibitors of bone resorption. They have been used for years to reduce skeletal complications of manifest bony metastasis, both in patients with breast cancer and in those with other types of solid tumor. Although only 4 to 5 percent of clodronate is absorbed when it is given orally, the efficacy of the drug in disease with metastases to bone is very good.

Furthermore, oral therapy is safe and has a low rate of side effects.^{6,17-19}

In the early phase of the metastasis of breast cancer to bone, osteolytic factors produced by the metastatic cells, such as parathyroid hormone–related protein, activate osteoclasts. There is evidence that growth factors, such as transforming growth factor and insulin-like growth factor, are released when bone matrix is degraded. These growth factors may promote the proliferation of tumor cells.^{20,21} Bisphosphonates can influence this interaction between tumor cells and bone cells at a very early stage. Like other bisphosphonates, clodronate inhibits bone destruction by inhibiting the activity of osteoclasts. The therapeutic effect of the bisphosphonates is more likely to be due to a protective effect on uninvolved skeleton than to recalcification of existing lytic lesions.^{19,22}

In experiments in animals, the timely use of etidronate, clodronate, pamidronate, and risedronate markedly reduced both the extent of tumor-induced osteolysis and the metastatic tumor burden.²³⁻²⁸ In rats that were simultaneously inoculated with tumor cells and treated with risedronate, significantly fewer bony metastases developed than was the case in untreated control animals.⁷

Elomaa et al.¹⁷ reported that the use of clodronate in patients with breast cancer who already had metastases to bone led to a reduction in the number of new metastases. This observation was confirmed by Kanis et al.⁹ in a study of patients with advanced local or distant breast cancer but without skeletal involvement. In their study, Kanis et al. found that oral treatment with 1600 mg of clodronate per day for three years significantly reduced the number of new bony metastases.

Our results also show that clodronate significantly reduces the incidence of new skeletal metastases in women with breast cancer. The mechanism of this effect is uncertain. There are no reports of cytotoxic effects of bisphosphonates at therapeutic doses, but these compounds can induce apoptosis, at least in osteoclasts and macrophage-like cells, and they can suppress the paracrine activity of macrophages.²⁹⁻³¹ There are also reports that adhesion molecules on breast-cancer cells and the surface of the bone matrix are altered by bisphosphonates.^{32,33} Yoneda et al.³⁴ showed that the combination of the bisphosphonate ibandronate (ibandronic acid) and the tissue inhibitor of matrix metalloproteinase 2 reduced bony metastases when human breast-cancer cells were inoculated into immunodeficient mice.

Because the observation period was only 36 months in the present study, the results may simply have been due to a drastic reduction in the growth of bony metastases, rendering them undetectable by conventional means. Later follow-up will show whether clodronate merely prolonged bony-metastasis-free survival or whether bony metastases were actually prevented from developing.

The reduction in nonosseous metastases in the group of women given clodronate was unexpected. This effect has never been seen in experiments in animals. In fact, some investigators feared that bisphosphonates could lead to a shift in the pattern of metastasis toward a greater frequency of visceral metastases. The processes relevant to osseous metastasis (apoptosis and alteration of adhesion molecules and proteases) could, however, also have a role in the development of visceral metastasis. Another factor may be synergy between cytotoxic agents and bisphosphonates. There is little evidence of such synergy, but investigations in animal models have shown that combinations of paclitaxel and alendronate³⁵ and of ibandronate and doxorubicin (Yoneda T, Mundy GR: personal communication) are more effective than

the compounds given individually in preventing the occurrence and development of new osseous and nonosseous metastases. We assume that bisphosphonates interfere with the adhesion and invasiveness of tumor cells by changing the microenvironment, whereas cytotoxic drugs suppress cell proliferation. We suggest that by attacking tumor cells and changing the microenvironment at the metastatic site, the combination of bisphosphonates and cytotoxic agents produces a "two-hit" effect that reduces the development of metastases in patients with breast cancer.

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REFERENCES

1. Fleisch H. Bisphosphonates: a new class of drugs in diseases of bone and calcium metabolism. In: Brunner KW, Fleisch H, Senn H-J, eds. Bisphosphonates and tumor osteolysis. Vol. 116 of Recent results in cancer research. Berlin, Germany: Springer-Verlag, 1989:1-28.
2. Rodan GA, Fleisch HA. Bisphosphonates: mechanisms of action. *J Clin Invest* 1996;97:2692-6.
3. Averbuch SD. New bisphosphonates in the treatment of bone metastases. *Cancer* 1993;72:Suppl:3443-52.
4. Hortobagyi GN, Theriault RL, Porter L, et al. Efficacy of pamidronate in reducing skeletal complications in patients with breast cancer and lytic bone metastases. *N Engl J Med* 1996;335:1785-91.
5. O'Rourke N, McCloskey E, Houghton F, Huss H, Kanis JA. Double-blind, placebo-controlled, dose-response trial of oral clodronate in patients with bone metastases. *J Clin Oncol* 1995;13:929-34.
6. Paterson AHG, Powles TJ, Kanis JA, McCloskey E, Hanson J, Ashley S. Double-blind controlled trial of oral clodronate in patients with bone metastases from breast cancer. *J Clin Oncol* 1993;11:59-65.
7. Hall DG, Stoica G. Effect of the bisphosphonate risedronate on bone metastases in a rat mammary adenocarcinoma model system. *J Bone Miner Res* 1994;9:221-30.
8. Krempien B. Experimental findings on the osteoprotective potential of bisphosphonates against bone metastases and tumor-induced osteopathy: a pleading for an early and preventive administration. In: Orr FW, Singh G, eds. Bone metastasis — mechanisms and pathophysiology. New York: Springer, 1996:221-45.
9. Kanis JA, Powles T, Paterson AHG, McCloskey EV, Ashley S. Clodronate decreases the frequency of skeletal metastases in women with breast cancer. *Bone* 1996;16:663-7.
10. Diel IJ, Kaufmann M, Goerner R, Costa SD, Kaul S, Bastert G. Detection of tumor cells in bone marrow of patients with primary breast cancer: a prognostic factor for distant metastasis. *J Clin Oncol* 1992;10:1534-9.
11. Diel IJ, Kaufmann M, Costa SD, et al. Micrometastatic breast cancer cells in bone marrow at primary surgery: prognostic value in comparison to nodal status. *J Natl Cancer Inst* 1996;88:1652-8.
12. Redding WH, Coombes RC, Monaghan P, et al. Detection of micrometastases in patients with primary breast cancer. *Lancet* 1983;2:1271-4.
13. Mansi JL, Easton D, Berger U, et al. Bone marrow micrometastases in primary breast cancer: prognostic significance after 6 years' follow-up. *Eur J Cancer* 1991;27:1552-5.
14. Coleman RE, Rubens RD. Bone metastases and breast cancer. *Cancer Treat Rev* 1985;12:251-70.
15. *Idem*. The clinical course of bone metastases from breast cancer. *Br J Cancer* 1987;55:61-6.
16. Theriault RL, Hortobagyi GN. Bone metastasis in breast cancer. *Anticancer Drugs* 1992;3:455-62.
17. Elomaa I, Blomqvist C, Porkka L, Lamberg-Allardt C, Borgström GH. Treatment of skeletal disease in breast cancer: a controlled clodronate trial. *Bone* 1987;8:Suppl 1:S53-S56.
18. Elomaa I, Blomqvist C, Porkka L, et al. Clodronate for osteolytic metastases due to breast cancer. *Biomed Pharmacother* 1988;42:111-6.
19. Kanis JA, O'Rourke N, McCloskey EV. Consequences of neoplasia induced bone resorption and the use of clodronate. *Int J Oncol* 1994;5:713-31.
20. Mundy GR. Mechanisms of osteolytic bone destruction. *Bone* 1991;12:Suppl 1:S1-S6.
21. Mundy GR, Martin TJ. Pathophysiology of skeletal complications of

- cancer. In: Mundy GR, Martin TJ, eds. *Physiology and pharmacology of bone*. Berlin, Germany: Springer-Verlag, 1993:641-71.
22. Kanis JA. Bone and cancer: pathophysiology and treatment of metastases. *Bone* 1995;17:Suppl 2:S101-S105.
23. Nemoto R, Uchida K, Tsutsumi M, Koiso K, Satou S, Satou T. A model of localized osteolysis induced by the MBT-2 tumor in mice and its responsiveness to etidronate disodium. *J Cancer Res Clin Oncol* 1987;113:539-43.
24. Krempien B, Manegold C. Prophylactic treatment of skeletal metastases, tumor-induced osteolysis, and hypercalcemia in rats with the bisphosphonate CL2MBP. *Cancer* 1993;72:91-8.
25. Krempien B, Wingen F, Eichmann T, Müller M, Schmähl D. Protective effects of a prophylactic treatment with the bisphosphonate 3-amino-1-hydroxypropane-1,1-bisphonic acid on the development of tumor osteopathies in the rat: experimental studies with the Walker carcinosarcoma 256. *Oncology* 1988;45:41-6.
26. Wingen F, Eichmann T, Manegold C, Krempien B. Effects of new bisphosphonic acids on tumor-induced bone destruction in the rat. *J Cancer Res Clin Oncol* 1986;111:35-41.
27. Kostenuik PJ, Orr FW, Suyama K, Singh G. Increased growth rate and tumor burden of spontaneously metastatic Walker 256 cancer cells in the skeleton of bisphosphonate-treated rats. *Cancer Res* 1993;53:5452-7.
28. Sasaki A, Boyce BF, Story B, et al. Bisphosphonate risedronate reduces metastatic human breast cancer burden in nude mice. *Cancer Res* 1995;55:3551-7.
29. Mönkkönen J, Heath TD. The effects of liposome-encapsulated and free clodronate on the growth of macrophage-like cells in vitro: the role of calcium and iron. *Calcif Tissue Int* 1993;53:139-46.
30. Mönkkönen J, Taskinen M, Auriola SOK, Urtti A. Growth inhibition of macrophage-like and other cell types by liposome-encapsulated, calcium-bound, and free bisphosphonates in vitro. *J Drug Target* 1994;2:299-308.
31. Rogers MJ, Chilton KM, Coxon FP, et al. Bisphosphonates induce apoptosis in mouse macrophage-like cells in vitro by a nitric oxide-independent mechanism. *J Bone Miner Res* 1996;11:1482-91.
32. van der Pluijm G, Vloedgraven H, van Beek E, van der Wee-Pals L, Löwik C, Papapoulos S. Bisphosphonates inhibit the adhesion of breast cancer cells to bone matrices in vitro. *J Clin Invest* 1996;98:698-705.
33. Boissier S, Magonetto S, Frappart L, et al. Bisphosphonates inhibit prostate and breast carcinoma cell adhesion to unmineralized and mineralized bone extracellular matrices. *Cancer Res* 1997;57:3890-4.
34. Yoneda T, Sasaki A, Dunstan C, et al. Inhibition of osteolytic bone metastasis of breast cancer by combined treatment with the bisphosphonate ibandronate and tissue inhibitor of the matrix metalloproteinase-2. *J Clin Invest* 1997;99:2509-17.
35. Stearns ME, Wang M. Effects of alendronate and taxol on PC-3 ML cell bone metastases in SCID mice. *Invasion Metastasis* 1996;16:116-31.