Optimising preoperative systemic therapy for breast cancer

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Chapter 3

Addition of zoledronic acid to neoadjuvant chemotherapy does not enhance tumor response in patients with HER2-negative stage II/III breast cancer: the NEOZOTAC trial

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Annals of Oncology,
2014 May;25(5):998-1004

Abstract

Purpose
The role of zoledronic acid (ZA) when added to the neoadjuvant treatment of breast cancer (BC) in enhancing the clinical and pathological response of tumors is unclear. The effect of ZA on the antitumor effect of neoadjuvant chemotherapy has not prospectively been studied before.

Patients and methods
NEOZOTAC is a national, multicenter, randomized study comparing the efficacy of TAC (docetaxel, adriamycin and cyclophosphamide i.v.) followed by G-CSF on day 2 with or without ZA 4 mg i.v. q 3 weeks inpatients withstage II/III, HER2-negative BC. We present data on the pathological complete response (pCR in breast and axilla), on clinical response using MRI, and toxicity. Post-hoc subgroup analyses were undertaken to address the predictive value of menopausal status.

Results
Addition of ZA to chemotherapy did not improve pCR rates (13.2% for TAC+ZA vs 13.3% for TAC). Postmenopausal women (n=96) had a numerical benefit from ZA treatment (pCR 14.0% for TAC+ZA vs. 8.7% for TAC, P=0.42). Clinical objective response did not differ between treatment arms (72.9 % vs. 73.7%). There was no difference in grade III/IV toxicity between treatment arms.

Conclusion
Addition of ZA to neoadjuvant chemotherapy did not improve pathological or clinical response to chemotherapy. Further investigations are warranted in postmenopausal women with BC, since this subgroup might benefit from ZA treatment.

Introduction

The role of bisphosphonates (BPs) when added to the neoadjuvant treatment of breast cancer for enhancing the efficacy of antitumor therapy is unknown. One of the proposed mechanisms for the antitumor effect of BPs is a modulatory effect on the bone microenvironment, by inhibition of secretion of tumor-stimulating growth factors released during bone turnover.(1;2) Furthermore, an antitumoral effect has been suggested based on an inhibitory effect on angiogenesis (3;4), and modulation of the immune system.(5-8) Preclinically, a synergistic antitumoral effect of BPs and chemotherapy on breast tumors was found, especially with the most potent bisphosphonate zoledronic acid (ZA).(9;10).
Clinical studies, however, have shown discordant results concerning the antitumor effect of BPs when added to adjuvant treatment breast cancer. Two recent meta-analysis showed benefit of adjuvant BPs. Yan et al. concluded that overall adjuvant ZA did not result in better outcome in all breast cancer patients, but that it provides better disease free survival (DFS) and reduced risk of recurrences in postmenopausal patients (11-14) and in a meta-analysis with individual patient data from a small 20.000 patients Coleman et al revealed a beneficial effect of the addition of BPs to adjuvant therapy on breast cancer death and bone recurrence. (15) In the neoadjuvant setting, a retrospective evaluation of a subpopulation of patients (n=205) treated with neoadjuvant chemotherapy in the AZURE study, which evaluated the effect of chemotherapy and/or hormone therapy with or without ZA in treating women with stage II/III breast cancer, has shown a significant improvement in pathological complete response (pCR) of 10.9% vs. 5.8% when therapy was complemented with ZA.(16) Possibly, the addition of ZA to neoadjuvant therapy can also have a beneficial effect on DFS.(17)
Based on clinical and preclinical findings, we hypothesized that combining ZA to chemotherapy might improve pathological and clinical tumor response. The aim of our current prospective randomized study, was to investigate in a homogenous study population the efficacy of neoadjuvant chemotherapy (TAC; docetaxel, adriamycin and cyclofosfamide) with ZA in patients with stage II or III breast cancer.

Methods

Study population
Patients were eligible for inclusion in the study if they were at least 18 years of age, had stage II-III breast cancer (T2>2 cm and/or positive lymph nodes, T2 > 3cm, T3, T4, any N, M0), and measurable disease. Patients also had to have a WHO 0-2 status and adequate bone marrow, liver and renal function. HER2-negativity of the breast cancer had to be histologically proven. Both, hormone receptor positive and negative tumor status was allowed. Pre- and postmenopausal patients were eligible. Informed consent was obtained from all patients. Patients were excluded from the study if there was evidence for distant metastases (M1), a history of breast cancer or another malignancy within 5 years, with exception of a history of previous basal cell carcinoma of the skin or pre-invasive carcinoma of the cervix. Furthermore there should be no prior bisphosphonate usage and no poor dental health. Menopause was defined as one year without menstrual activity, previous bilateral oophorectomy, age older than 60 years or baseline FSH>20 U/L and estradiol <110 pmol/L).

The study was ethically approved by the Ethical Committee of the Leiden University Medical Center.

Treatment
After randomisation patients received TAC (docetaxel 75mg/m2, adriamycin 50mg/m2 and cyclophosphamide 500 mg/m2 i.v.) chemotherapy on day 1, with or without ZA (4 mg i.v. within 24 hours after infusion of chemotherapy) followed by G-CSF on day 2, every 21 days during 6 cycles. ZA therapy was combined with daily supplements of 500 mg calcium and 400 IU vitamin D. Patients did not receive neoadjuvant endocrine therapy.

Randomisation
Patients were centrally randomized at the LUMC Datacenter of the department of Surgery, through the online ALEA randomisation program. Randomisation was done using Pocock's minimization technique stratified by center, cT classification, cN-classification and estrogen receptor status.

Primary endpoint
Pathological response was evaluated using the Miller and Payne grading system (grade 1 = no response, grade 2 = 0-30% response, grade 3 = 30-90% response, grade 4 = more than 90% response, grade 5 = pCR.(18) Cases were scored as a pCR in case of a grade 5 response in combination with a negative pathological nodal status after neoadjuvant chemotherapy. When only DCIS was present, specimens were revised as a pCR. Response was evaluated on diagnostic slides of specimens from the primary tumor bed. Response was graded by both pathologists in the local study centers as well as by an experienced pathologist (VS) responsible for central revision.

Secondary endpoints
Secondary endpoints included clinical response and treatment tolerability. Clinical response was evaluated using breast MRIs based on to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 after 3 and 6 cycles of chemotherapy.(19) All adverse events grade 2 or more (according to NCI-CTCv4) reported spontaneously by the patient or observed by the treating physician or investigator, were recorded on an adverse event case report form.(20) The relationship to the study drug(s) was recorded. Two post-hoc subgroup analyses were done. First, the effect of ZA administration directly after chemotherapy vs. later (within 24 hours after chemotherapy administered by homecare) was compared based on preclinical results that showed increased benefit of ZA when administered 24 hr after chemotherapy. Subgroup analyses of postmenopausal women were undertaken based on newly reported study findings since the start of the study, suggesting a more pronounced anti-tumor effect of ZA in these patients.(14)(21)Menopausal status was assessed by the local physician. Revision of menopausal status was done, if baseline data on estradiol and FSH was known (postmenopausal: FSH>20 U/L and estradiol <110 pmol/L).(22)

Statistics
The primary endpoint was pCR. pCR rates found in similar studies range from 6-16% for ER-positive breast cancers patients and from 21.6-45% for triple-negative patients.(23-27) Based on the size of the studies reported and the similarity of the patients involved to our patient population, we expected a pCR of 10% for hormone-positive patients and 30% for triple-negative patients. Since we expected 2/3 of our population to be hormone-positive and 1/3 triple-negative, the overall pCR for our population in the control arm (arm B) was expected to be 17%. Our sample size calculation was based on the aim to detect a doubling of the pCR rate for the experimental arm (arm A), based on the results of the neoadjuvant AZURE trial.(16) A total number of 228 patients (114 in each treatment arm) is sufficient to achieve 80% power to detect a difference of 17 vs. 34% in pCR, using a significance level of 5%, based on the two-sided Fisher's exact test. Taking a 10% ineligibility rate into account, it was decided to enroll 250 patients.
All main analyses were done on the intention-to-treat population, defined as all patients randomized and having started with the study medication. The primary endpoint was analyzed using the Cochran-Mantel-Haenszel test, adjusting for the stratification factors (cT-classification, cN-classification, ER-status). Additional explorative analyses involved the analysis of prognostic and predictive factors for achieving the primary endpoint, which was investigated by using logistic regression. Factors considered were cT-classification, cN-classification, hormone receptor status and menopausal status. For the secondary endpoints, categorical data were tested using Pearson's Chi-squared test and Fisher's Exact test. The continuous covariates were tested using the Mann-Whitney test. Cases with missing pCR data were excluded from analysis.

Results

Patient characteristics
From July 2010 to April 2012, 250 patients from 26 participating sites from the Dutch Breast Cancer Trialists' Group (BOOG) were recruited for the study. Of these patients 126 were randomized to TAC alone and 124 to TAC+ZA (Figure 1).Two patients were ineligible: one because of liver metastases, one because of a positive HER2-status. Two patients withdrew informed consent during the study period. Pathologic response data of the breast were available for analysis of the primary endpoint in 242 cases (97%), of which 122 were randomized to TAC alone and 120 to TAC+ZA. 5 cases could not be evaluated for pathological response of the breast and lymph nodes. Patient characteristics are summarized in table 1.

Pathological response
There was strong inter-observer agreement between the pathologists in the local pathology laboratories and the revision pathologist (κ=0.89) as regards pCR. In the total study population pCR in the breast tissue specimen did not differ between the two study arms (13.2% for TAC+ZA vs. 13,3% for TAC only, P=1.00, Mantel-Haenszel O.R. 1.00; 95% C.I. 0.44-2.30) [Figure 1a]. This finding persisted after the prespecified multivariate analysis (OR 1.02; 95% C.I. 0.45-2.32In a subgroup analysis of postmenopausal women there was only a numerical benefit in favor of treatment with ZA. However, this did not reach statistical significance

	TAC alone	TAC + ZA	P value*
	N=124 (%)	N=122 (%)	
Age at randomisation			
Mean	48.9	49.5	0.43
Range	29 - 67	34 - 70	
T stage			
cT1	2 (1.6)	0	0.53†
cT2	69 (55.6)	74 (60.2)	
cT3/cT4+	53 (42.7)	49 (39.8)	
N-stage			
cN +	67 (54.0)	68 (55.7)	0.80•
cN -	57 (46.0)	54 (44.3)	
ER-status			
ER +	103 (83.1)	100 (82.0	0.87•
ER -	21 (16.9)	22 (18.0)	
PR-status			
PR +	83 (66.9)	71 (58.2)	0.21†
PR -	40 (32.3)	51 (41.8)	
Unknown	1 (0.8)	0 (-)	
Menopausal status			
Pre/perimenopausal	74 (59.7)	72 (59.0)	0.36
Postmenopausal	48 (38.7)	50 (41.0)	
Unknown	2 (1.6)	0 (-)	

Table 1. Baseline demographics. P values based on Mann-Whitney U test. P values based on Fisher's Exact test

(14.0% vs. 8.7%, P=0.42, Mantel-Haenszel OR 0.59; 95% C.I. 0.16-2.15) [Figure 1b]. Of two patients the menopausal status was unknown. Of 115 patients data on the timing of the first zoledronic acid administration was available. We did not find a different effect in pCR between patient treated with ZA directly in the hospital (N=94) or the day after chemotherapy administration (N=21; pCR=14.9% vs. 9.5%, P=0.73).

Clinical response

Clinical MRI response after 6 treatment cycles could be evaluated in 221 cases. 19 women received less than 6 cycles and MRI data of 7 patients was missing. Treatment with ZA did not result in improved objective response rates (72.0% for TAC+ZA % vs. 73.7% for TAC only) (Table 2). After six cycles, no significant difference in decrease of the sum of diameters of tumor lesions (as assessed with MRI) was observed between the both treatment arms, although mean decrease was greater in the ZA arm (-30.7 mm for ZA arm vs. -25.2 mm for TAC only arm, P=0.13). There was no difference in performed breast conserving surgery rate (38.5% for TAC+ZA vs. 45.2% for TAC only, P=0.30). Timing of ZA administration, directly after chemotherapy (n=93) vs. one day later but within 24 hr by homecare (n=28) had no influence on the amount of pathological complete response in the breast (pCR 18.3% vs 18.5%, respectively).

	6x TAC + ZA	6x TAC	P values
	N=122 (%)	N=124 (%)	
Miller-Payne grades			0.96†
1	20 (16.4)	18 (14.5)	
2	35 (28.7)	31 (25.0)	
3	24 (19.7)	25 (20.2)	
4	20 (16.4)	25 (20.2)	
5	21 (17.2)	23 (18.5)	
Unknown	2 (1.6)	2 (1.6)	
Clinical response			0.28†
cCR	23 (18.9)	19 (15.3)	
cPR	54 (44.3)	65 (52.4)	
cSD	23 (18.9)	18 (14.5)	
cPD	0 (-)	3 (2.4)	
NE	7 (5.7)	9 (7.3)	
Unknown	15 (12.3)	10 (8.1)	

Table 2. Pathological response in the breast only and clinical response in both treatment arms. †P values based on Chi-square test

Grade III/IV toxicity	6 x TAC + ZA (%)	6 x TAC (%)	P value
Hematological			
Neutropenia	9 (7.4)	10 (8.1)	1.00
Febrile neutropenia	7 (5.7)	12 (9.8)	0.34
Thrombocytopenia	1 (0.8)	2 (1.6)	1.00
Non-hematological			
Diarrhea	2 (1.6)	5 (4.1)	0.44
Nausea	4 (3.3)	1 (0.8)	0.21
Vomiting	1 (0.8)	1 (0.8)	1.00
Fatigue	4 (3.3)	8 (6.5)	0.38
Fever	1 (0.8)	2 (1.6)	1.00
Dyspnea	0 (0)	2 (1.6)	0.50
Hypertension	3 (2.5)	2 (1.6)	0.68
Hypotension	1 (0.8)	0 (-)	0.50
Bone pain	1 (0.8)	0 (0)	0.50
Myalgia	1 (0.8)	0 (0)	0.50
Hypotension	1 (0.8)	0 (0)	0.50
Edema	0 (-)	1 (0.8)	1.00
Increased ALAT	0 (0)	1 (0.8)	1.00
Anorexia	0 (-)	1 (0.8)	1.00
Peripheral sensory neuropathy	1 (0.8)	2 (1.6)	1.00
Cough	0 (-)	1 (0.8)	1.00

Table 3. Grade III/IV toxicities in both treatment arms. P values based on Fisher's Exact test.

Safety

There was no difference in both hematological and non-hematological toxicities between both treatment arms (Table 3). Main grade 3 and/or 4 NCI-CTCv4 toxicity was neutropenia (8%) followed by febrile neutropenia (7%), fatigue (5%), diarrhea (3%), hypertension (2%), nausea (3%) and vomiting (1%). Bone pain, myalgia, peripheral sensory neuropathy and hypocalcaemia all occurred in only one patient in the ZA arm (0.8%). None of the patients developed osteonecrosis of the jaw. Five patients stopped ZA therapy premature due to (possible) toxicity. Eight patients did not complete all 6 chemotherapy cycles because of adverse events (n=5) and progressive disease (n=3).

Discussion

To our knowledge, this is the first reported study, which prospectively investigates the effect of ZA on breast cancer with pCR after neoadjuvant chemotherapy as primary endpoint. Our study shows that in patients with HER2-negative breast cancer, the addition of ZA to neoad-

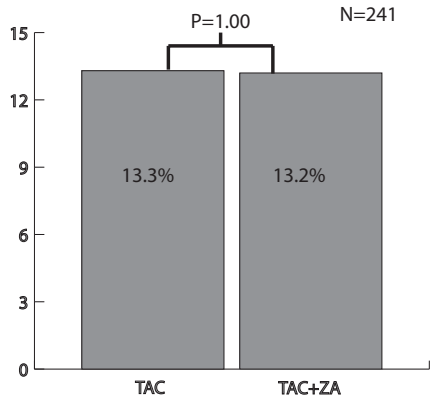
juvant chemotherapy did not enhance pathological or clinical response, except for a small subgroup of postmenopausal women, in whom it provided numerical benefit. Although these results did not reach statistical significance, these observations might be of clinical relevance, since they are supported by evidence from clinical and preclinical studies showing a better anti-tumor effect in the postmenopausal context.(28)(29)(11;13;14)
Recently, a large meta-analysis showed a beneficial effect of the addition of BPs to adjuvant therapy in patients with early breast cancer.(15) Several adjuvant studies, with mainly endocrine adjuvant systemic therapy, have reported a beneficial effect of ZA in breast cancer patients in the presence of low estrogen levels. In the AZURE study, 3360 pre- or postmenopausal patients were randomized to standard adjuvant systemic therapy (i.e. chemotherapy and/or hormonal therapy) with or without ZA.(14) No difference in DFS was observed in the total study population, but women who were postmenopausal benefited significantly more from ZA than their premenopausal counterpart. Recently, even a detrimental effect of ZA in terms of extra-skeletal recurrence was reported in premenopausal women in addition to adjuvant chemotherapy in this study.(30) In the ZO-FAST study, randomizing 1065 postmenopausal women receiving adjuvant letrozole to immediate ZA or delayed ZA until there was measurable decline in bone mineral density, at 60 months follow-up, there was an improved DFS in patients treated immediately with zoledronic acid.(21) These observations are supported by data from the Austrian Breast and Colorectal Cancer Study Group 12 (ABSCG-12) trial, in which 1803 premenopausal patients with early stage breast cancer were treated with or without ZA added to adjuvant goserelin with tamoxifen or anastrozole.(13) In this study, the DFS benefit seemed to be driven by a subgroup of patients who were older than 40 years at baseline and might have achieved more complete estrogen deprivation.

In the neoadjuvant setting both preclinical and clinical data suggested a synergism between chemotherapy and ZA.(9)(15)(31) Preclinically, a sequence dependent synergism was found, with the most beneficial effect when ZA was administered 24 hours after the chemotherapy. This sequence dependency could not be confirmed in our clinical trial; we did not find a different effect in pCR between patients treated with ZA directly or later, within 24 hours after chemotherapy. In mice with castration-induced postmenopausal status, ZA clearly prevented tumor progression through inhibition of growth of disseminated tumor cells in bone. Clinically, a retrospective analysis of the AZURE subgroup study on 205 patients treated with neoadjuvant chemotherapy regimens with or without ZA showed a significant increase of pCR for the zoledronic arm.(16) Additionally, Aft et al. reported a randomized phase II study in which 120 patients with stage II/III breast cancer (46% postmenopausal) were allocated to a combined neoadjuvant and adjuvant chemotherapy schedule (four neoadjuvant and two adjuvant cycles of epirubicin and docetaxel) with or without ZA (4 mg i.v. every 3 weeks), for 1 year.(32) The primary endpoint was the number of patients with detectable disseminated tumor cells (DTCs) at 3 months. Although no significant difference in pCR was found, ZA administered with chemotherapy resulted in a decreased proportion of patients with DTCs detected in the bone marrow at the time of surgery, suggesting that neoadjuvant treatment with ZA might affect long-term outcome by preventing metastasis.

The precise mechanism of the anti-tumor effect of ZA – particularly in postmenopausal women - is still unclear. Various mechanisms have been proposed to explain this effect. First, the modifying role of ZA on the microenvironment, with bone as 'soil' for the growth of the cancer 'seed', by inhibition of bone turnover and thereby cytokine and growth factor release (e.g. TGF-beta and IGF-1) known to stimulate proliferation, migration and angiogenesis of tumor cells.(33) Postmenopausal women are known to have increased bone turnover and might therefore benefit more from the inhibition of bone turnover by bisphosphonates.(2) Second, an explanation related to the microenvironment might be in terms of the immunomodulatory effect of ZA. Low estrogen levels induce an inflammatory response with activation of the innate and adaptive immune cells (macrophages, T cells).(34;35) Tumor associated macrophages (TAM) or M2 macrophages assist tumor progression. Bisphosphonates are known to reverse the TAM phenotype from pro-tumoral M2 to tumoricidal M1 and to deplete these M2 macrophages.(8;36) Therefore, in a microenvironment with estrogen deficiency and with increased macrophages, bisphosphonates might deplete the tumor promoting M2 macrophages. Additionally, Estrogen deficiency amplifies T-cell activation resulting in increased INF-γ, TNF-α and IL-1 enhancing bone loss and antigen presentation.(34) Chinault et al. adequately hypothesized that effect of ZA on host cells, such as immune cells, might be a more plausible explanation for the anti-tumor effect of ZA than a direct influence on tumor cells.(37) In their bioluminescence reporter-based study, protein prenylation was not inhibited in bone- or fat pad located breast cancer cells after ZA administration, suggesting that there is no direct effect of ZA on tumor cells. However, in another study by Rogers et al. ZA did have an effect on prenylation in macrophages after sequential short-term administration of zoledronic acid and doxorubicin, contributing to the suggestion that the reason for anti-tumor effect of ZA lies in the "soil" rather than in the "seed" in the breast cancer patient.(38)

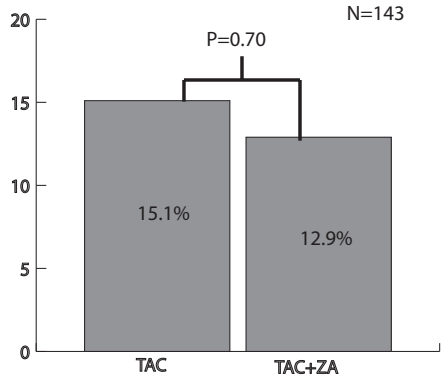
Preclinically, a sequence dependent synergism was found, with the most beneficial effect when ZA was administered 24 hours after the chemotherapy.(9) This sequence dependency could not be confirmed by our trial; we did not find a different effect in pCR between patient treated with ZA directly or within 24 hours after chemotherapy. Unfortunately, our study was not powered to study the role of menopausal status in relation to pathological response and was therefore not powered to detect a significant difference between patient groups. However, a meta-analysis with individual data from thus far existing prospective studies with neoadjuvant chemotherapy and pCR as an endpoint is in progress. Hopefully, this meta-analysis will give an indication of the relevance for further clinical research on addition of bone-targeted medicines to neoadjuvant chemotherapy.

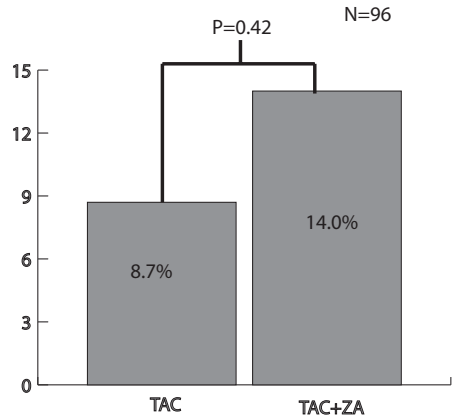
In summary, neoadjuvant TAC chemotherapy with ZA was safe but did not improve pCR as compared to chemotherapy alone in patients with stage II/III breast cancer. Therefore, it is premature to add ZA to neoadjuvant chemotherapy in the standard care of these patients. Further studies are warranted to elucidate the differential effect of adding bisphosphonates to neoadjuvant systemic treatment in postmenopausal women with breast cancer.



Left:
Fig 1A. pCR in the total study population in both treatment arms. P value is based on two-sided Fisher's Exact test.

Under:
Fig 1B. pCR in both treatment groups in menopausal subgroups with left pre/perimenopausal patients and right postmenopausal patients. P value is based on two-sided Fisher's Exact test.





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