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Changes in bone and lipid metabolism in postmenopausal women with early breast cancer after terminating 2-year treatment with exemestane: A randomised, placebo-controlled study

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ABSTRACT

Aromatase inhibitors improve relapse-free survival in early breast cancer, but there is concern about possible detrimental effects on bone mineral density (BMD) and plasma lipids. This paper presents the results of a 2-year study evaluating the effects of exemestane versus placebo on BMD, bone markers, plasma lipids and coagulation factors, including a 1-year follow-up after termination of treatment in 147 patients. During treatment, the mean annual rate of loss of BMD in the lumbar spine was 2.17% in the exemestane group versus 1.84% in the placebo group (n.s.) and 2.72% versus 1.48%, respectively, in the femoral neck ($P = 0.024$). A loss of BMD above that expected in both arms of this study could be due to low vitamin D status (88% of all patients had vitamin D levels <30 ng/ml). The changes observed with exemestane were partially reversed during a 1-year follow-up, with no significant difference between the two arms. Similarly, the moderate decrease in high-density lipoprotein (HDL)-cholesterol was reversed. The bone marker values decreased, although a difference at 6 months of follow-up was still recorded, in particular for the markers of bone synthesis.

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1. Introduction

While phase III studies have demonstrated the unequivocal superiority of aromatase inhibitors (AIs) administered as monotherapy or sequentially following tamoxifen over tamoxifen monotherapy as adjuvant treatment for postmenopausal women with hormone-sensitive breast cancers,[1–5] information regarding the long-term toxicity of AIs is lacking.

The two major concerns with respect to the long-term profound oestrogen deprivation resulting from AI administration[6,7] relate to a negative influence on bone metabolism (accelerated bone loss), which could lead to fractures, and to a potential detrimental effect on plasma lipid levels, which could contribute to cardiovascular events. In the latest update of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) study,[1] adjuvant treatment with anastrozole monotherapy increased the total fracture rate by 49% compared with tamoxifen. Similarly, the joint report of the Austrian Breast and Colorectal Cancer Study Group (ABCSG 8) and German Arimidex-Nolvadex (ARNO 95) trials reported a significant increase in the frequency of fractures among patients treated with anastrozole following tamoxifen versus patients on continuous tamoxifen treatment (odds ratio 2.14, 95% CI 1.14–4.17),[5] while the Intergroup Exemestane Study reported a non-significant increase in fracture rate (35%) during treatment with exemestane in comparison with tamoxifen.[3] The first report from the Breast International Group (BIG) 1-98 trial confirmed an increase of 43.5% in fractures, comparing women treated initially with letrozole with those treated initially with tamoxifen.[8] Considering vascular events, a higher incidence of thromboembolic events have been recorded in the tamoxifen treatment arms, with a non-significant increase in cardiovascular events on treatment with the different AIs.[9]

A major challenge in evaluating the toxicity of AIs is the use of tamoxifen as a comparator in the control arm in most studies. Tamoxifen is known to influence bone metabolism, blood lipids and coagulation status in postmenopausal women.[10,11] While letrozole was compared with placebo in the MA17 study,[2] the patients enrolled had all received 5 years of tamoxifen therapy prior to treatment with the AI, and tamoxifen may persist in tissue compartments for months.[12] The beneficial effects of tamoxifen on bone mineral density (BMD) in postmenopausal women have to be considered whenever AIs are compared with the anti-oestrogen in the same study.[10] Thus, to address the issue of potential toxic effects of exemestane on bone and lipid metabolism, we conducted a 2-year double-blind placebo-controlled study evaluating these parameters in patients with early breast cancer who were not candidates for adjuvant endocrine therapy.[13] Because a major issue relates to whether any potential changes may be reversible upon terminating treatment, BMD, bone biomarkers, and plasma lipids were evaluated during a 1-year follow-up after termination of treatment. This paper reports the results of this follow-up with respect to BMD, bone biomarkers, plasma lipids and serum homocysteine levels, coagulation parameters and sex steroids. The key finding is that the modest changes in bone and lipids recorded during 2 years of treatment with exemestane were largely reversible 1-year after the termination of treatment. In addition, vitamin D (25-OHD) and parathormone (PTH) were measured in all patients before and during therapy, looking for potential explanations for the high loss in BMD in both treatment arms that was reported previously.[13]

2. Patients and methods

2.1. Study design

The study was designed and conducted by the Norwegian Breast Cancer Group in collaboration with Pfizer Inc. Postmenopausal women with low-risk, surgically treated early breast cancer ($n = 129$) or ductal carcinoma *in situ* ($n = 18$) were randomised (double-blind) either to exemestane 25 mg daily or to oral placebo for 2 years, to be followed up for 1 year after cessation of treatment for the primary (BMD) and secondary (bone biomarkers, plasma lipids, coagulation factors, homocysteine and serum hormone levels) study end-points. The parameters recorded at each time interval are shown in Fig. 1. The study was carried out at six Norwegian centres, and enrolled patients from January 1999 to October 2001. At that time, postmenopausal patients with low-risk breast cancer, as defined in the inclusion criteria,[13] were not offered routine adjuvant systemic treatment under the Norwegian National Guidelines. The protocol was approved by the Regional Ethical Committees, and each patient gave written informed consent before being enrolled. Randomisation was performed by each centre; patients were randomly assigned to treatment in blocks of four. A Data Monitoring Board, including an oncologist, a lipid expert and a bone expert not involved in the study, monitored the safety of patients.

Relapses and adverse events, together with drug compliance, were monitored at each visit.

2.2. Assessment of BMD

BMD was measured in the lumbar spine (L1 to L4) and in the femoral neck by Hologic densitometry (Hologic Inc., Walthem, MA, United States of America (USA)) in 130 patients and Lunar densitometry (Lunar Corp., Madison, WI, USA) in 17 patients. Absolute BMD, as well as t-scores (which represent standard deviations (SDs) from the mean value in normal young adults), were recorded. Patients were categorised as having normal BMD, osteopenia or osteoporosis according to the World Health Organisation (WHO) definition, applied to either the spine or to the femoral neck.[14]

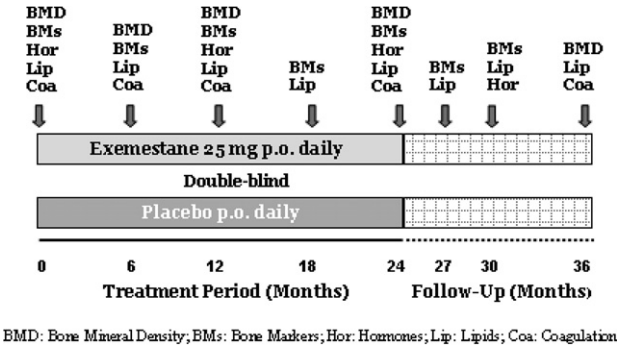

Fig. 1 – Study design.

2.3. Evaluation of bone biomarkers

To assess bone metabolism, the following bone formation markers were measured: serum bone alkaline phosphatase (BAP), osteocalcin and pro-collagen type I amino-terminal propeptide (PINP). As markers of bone resorption, serum C-telopeptide (CTX) and 12-h urinary excretion of CTX and N-telopeptide (NTX) were measured. All bone metabolism markers were determined by Synarc SAS, Lyon, France.

2.4. Measurement of 25-hydroxyvitamin D (25-OHD) and parathormone (PTH)

Assessments of serum 25-OHD and PTH were performed at Synarc SAS (Lyon, France) using specific analytical methods: the 25-OHD ^{125}I-radioimmunoassay (RIA) kit from DiaSorin (Stillwater, MN, USA) and the Elecsys intact PTH assay from Roche Diagnostics (Basel, Switzerland). While there has been a debate about lower limits for vitamin D serum levels, the current view is to consider a serum value below 30 ng/ml 25-OHD (the value at which PTH starts to increase) as suboptimal.[15–17]

2.5. Evaluation of lipids, homocysteine, coagulation factors and hormone status

Plasma lipid profile (total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, apolipoprotein A1, apolipoprotein B and lipoprotein A), serum homocysteine, coagulation profile (fibrinogen, activated partial thromboplastin time (APTT) and prothrombin time (PT)) and other safety parameters were determined centrally by the Haukeland University Laboratory of Clinical Chemistry on fresh samples delivered by courier. Serum sex-steroids (oestradiol, oestrone, oestrone sulphate, androstenedione and testosterone) were measured by a coupled gas chromatography mass spectroscopy (GC/MS/MS) bio-analytical method (Taylor Technology Inc., Princeton, NJ, USA).

2.6. Statistical analysis

The trial was powered to detect a difference in mean annual rate of BMD loss versus the placebo arm equal to or greater than 1.1%. Sixty-four evaluable patients per treatment arm were required to detect such a difference during a treatment time-span of 2 years and given a 1-tailed alpha level of 0.05 and 0.80 power. Statistical analysis of primary (BMD) and secondary end-points during the 24-month treatment interval has been reported elsewhere.[13] Results are reported here for those patients who completed the 24-month treatment period and were followed up post-treatment.

The mean percentage change in BMD at 1 year after terminating treatment (36-month visit) was compared within each treatment arm to the mean percentage change at the end of therapy (24-month visit) using a paired *t*-test. The 36-month mean changes were also compared between treatment groups using a two-sample *t*-test. A significance level of 0.05 was adopted. Bone biomarkers, plasma lipids and serum homocysteine levels during the follow-up (see Fig. 1 for specific parameters assayed at 27, 30 and 36 months), expressed as percentage changes from baseline were analysed by repeated measures analysis of variance. Values were log-transformed before the analysis if specified in the protocol. Due to the exploratory nature of the analyses of these secondary end-points, no adjustment for multiple testing was considered, and a significance level of 0.05 was used except for the correlations between changes in bone biomarkers and BMD measurements. Due to multiple comparisons, a level of statistical significance of 0.01 was used for these estimates.

Coagulation parameters and sex steroids were also evaluated by descriptive statistics. Patients receiving concomitant medication known to influence one or more study parameters (e.g. lipid-lowering drugs, anticoagulants, bisphosphonates, etc.) were considered not evaluable for the analysis of affected parameters. General safety analysis for this report is included for all treated patients who completed the 2-year treatment period and had at least one follow-up assessment. Safety analysis on all treated patients has been reported previously.[13]

3. Results

Of a total of 73 patients initially randomised to exemestane treatment and 74 randomised to placebo, 58 and 65 patients, respectively, completed 24 months of therapy and were available for follow-up. No patient completing 24 months of therapy was lost to subsequent follow-up. The reasons for premature discontinuation in the two arms are given in Table 1.

3.1. Bone mineral density

In the original publication,[13] alterations in BMD status were reported as annual bone loss in all patients having BMD assessed at baseline and at least on one occasion (12 months or later) during therapy. The mean annual rate of BMD loss in the lumbar spine was 2.17% in the exemestane group versus 1.84% in the placebo group (n.s.). In the femoral neck, the corresponding values for the exemestane and placebo groups were 2.72% versus 1.48%, respectively ($P = 0.024$).

For patients completing 24 months of therapy, making them available for follow-up analysis, the BMD expressed in absolute values for the treatment and follow-up periods is shown in Fig. 2, while percentage change from baseline at 24 and 36 months is given in Fig. 3. The average BMD losses after 24 months of therapy compared with before-treatment values were 3.59% and 2.12% in the lumbar spine for exemes-

Table 1 – Reason for premature treatment discontinuation

Reason	Number of patients on exemestane	Number of patients on placebo
Adverse event	9	3
Refused further treatment	3	2
New primary cancer	2	–
Relapse	1	3
Lost to follow-up	–	1

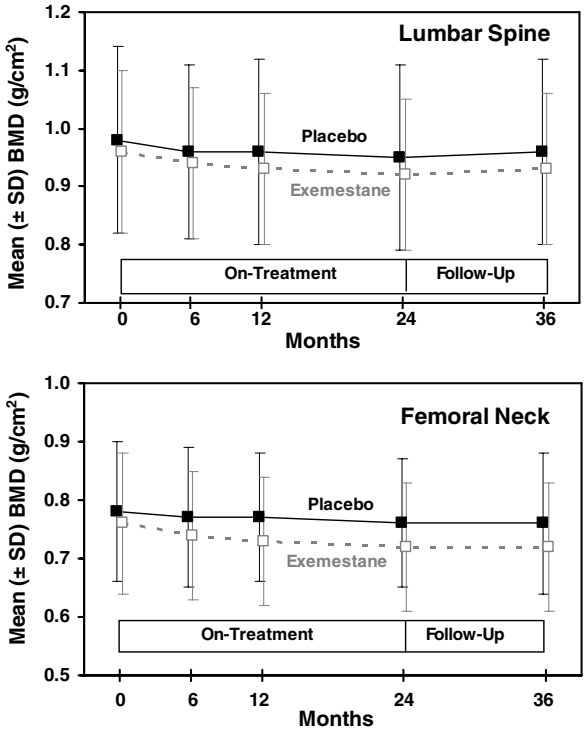

Fig. 2 – Effect of 2-year treatment with placebo or exemestane on bone mineral density (BMD) of the lumbar spine (upper panel) and femoral neck (lower panel). SD, standard deviation.

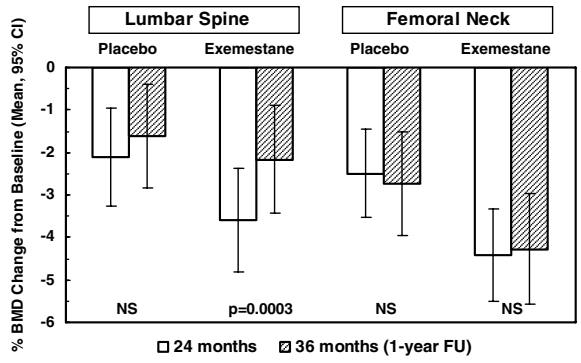

Fig. 3 – Percentage changes from baseline of bone mineral density (BMD) of the lumbar spine and femoral neck at the end of 2-year treatment with placebo or exemestane and at 1 year after treatment discontinuation. CI, confidence interval; NS, not significant; FU, follow-up.

tane versus placebo-treated patients, with corresponding values of 4.42% and 2.50% in the femoral neck for the two treatment groups, respectively. One year after terminating treatment, BMD in the lumbar spine had improved for patients in the exemestane arm (mean percentage bone loss decreased from 3.59% at 24 months to 2.16% at 36 months; $P = 0.0003$; Fig. 3). BMD loss improved slightly in the placebo arm (from 2.12% at 24 months to 1.61% at 36 months; $P = 0.169$), and the difference in lumber spine BMD between the 2 treatment arms at 36 months remained non-significant ($P = 0.538$). Considering the femoral neck, BMD loss remained stable (4.42% at 24 months versus 4.28% at 36 months, respectively) in the exemestane arm, while a minor BMD decrease (from 2.50% at 24 months to 2.73% at 36 months) was observed in the placebo arm. Comparing femoral neck values obtained at 36 months in the 2 arms, the treatment difference was no longer of statistical significance ($P = 0.092$).

3.2. *Bone biomarkers*

For patients completing 24 months of therapy and available for follow-up, markers of bone formation (BAP, PINP and osteocalcin) and bone resorption (serum-CTX, urinary-CTX and urinary-NTX) are shown in Fig. 4 (upper and lower panel, respectively). As seen from the Figures, these biomarkers were all raised in the exemestane treated patients after 24 months of therapy. A correlational analysis looking at the alterations in bone biomarkers at 6 months on treatment[13] and BMD at 24 months showed a significant ($P < 0.01$) negative correlation between BAP and BMD in the lumbar spine during treatment with exemestane (highest increase related to high BMD loss). When BAP values obtained at 12 months on treatment[13] were used, this phenomenon could be shown in both the exemestane and the placebo group. All serum markers for bone formation (BAP, PINP and OC at 6 and 12 months) were found to correlate significantly ($P < 0.01$) and negatively with BMD in the femur measured at 24 months in the placebo group only.

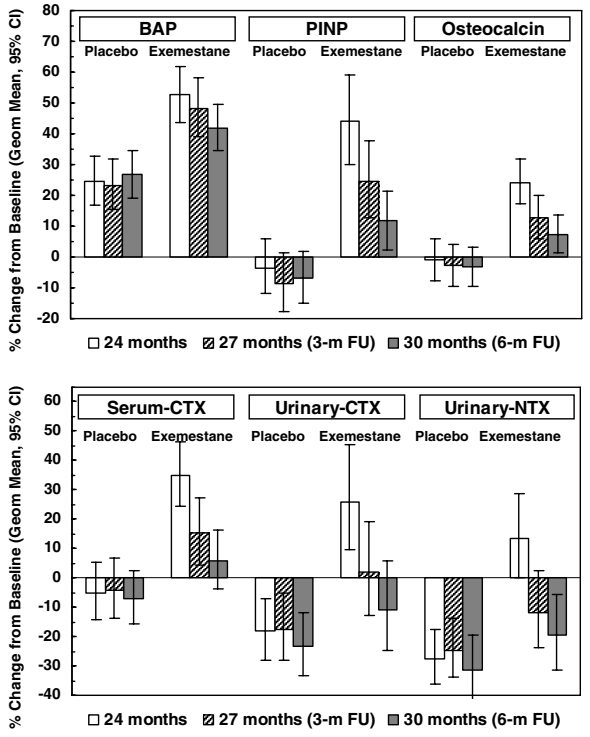

Fig. 4 – Percentage changes from baseline of bone formation (upper panel) and bone resorption markers (lower panel) at the end of 2-year treatment with placebo or exemestane and up to 6 months after treatment discontinuation. BAP, bone alkaline phosphatase; PINP, pro-collagen type I amino-terminal propeptide; CTX, C-telopeptide; NTX, N-telopeptide; CI, confidence interval; m, month; FU, follow-up.

After exemestane withdrawal, bone resorption markers returned to or below baseline values within 6 months, with the exception of serum-CTX. No statistically significant difference was found between treatment arms during the post-treatment follow-up period for all these markers, apart from serum-CTX ($P = 0.018$). Considering markers of bone formation, a successive decrease in values at 3 and 6 months after terminating therapy was recorded for each marker. However, 6 months after terminating treatment with exemestane they all remained elevated compared with baseline and compared with values recorded among placebo-controlled patients. For BAP, PINP and osteocalcin, a statistically significant difference was found between treatment arms during the post-treatment follow-up period ($P < 0.001$, $P < 0.001$ and $P = 0.004$, respectively) (6 months after termination of treatment; Fig. 4). Notably, we did not observe any significant correlation between changes in serum bone markers and changes in BMD during the treatment discontinuation period.

3.3. *Vitamin D and parathormone status*

The mean values of 25-OHD at baseline were 21.8 ng/ml (20.5–23.2) and 21.2 ng/ml (19.7–22.8) in the placebo and exemestane group, respectively (geometric mean values with 95% CI). 128 patients (88%) had 25-OHD levels below 30 ng/ml (67 patients in the placebo group and 61 patients in the exemestane group) reflecting a general vitamin D deficiency in the study population. There was no significant correlation between 25-OHD levels at baseline and BMD measurements at baseline. Neither did we find significant correlations between 25-OHD levels at baseline and loss of BMD during the 2-year treatment period. However, there was a (non-significant) trend toward higher loss of BMD in the femoral neck and in the lumbar spine during treatment with exemestane for 2 years in the subgroup of patients with baseline 25-OHD < 30 ng/ml (data not shown). This trend was not present in the placebo group. The measurement of PTH revealed no differences between the two study groups at baseline (geometric mean levels of 30.7 and 30.8 pg/ml in the placebo and exemestane group, respectively). The changes in serum 25-OHD and PTH during the 2-year treatment period (placebo or exemestane) and the first 6 months after treatment termination are shown in Fig. 5. During the 2-year treatment period, exemestane had no significant effect on 25-OHD levels, whereas a slight decrease was observed in PTH levels of a maximum of 7–8% (compared with a slight increase of 4–12% in the placebo arm, $P = 0.038$). These PTH changes disappeared during the 6 months after exemestane discontinuation (15% increase).

3.4. *Plasma lipid and serum homocysteine levels*

As described in the original publication,[13] in all patients evaluable during the 2-year treatment period, exemestane had no significant effect on lipid fractions, with the exception of a decrease in HDL-cholesterol of 6–9% (versus a 1–2% increase on placebo; $P < 0.001$) and a 5–6% decrease in apolipoprotein A1 (versus a 0–2% decrease on placebo; $P = 0.004$). In addition, during the 24 months of treatment, exemestane caused a minor increase in serum levels of homocysteine (7–18% in the exemestane arm versus 1–12% during placebo; $P = 0.018$).

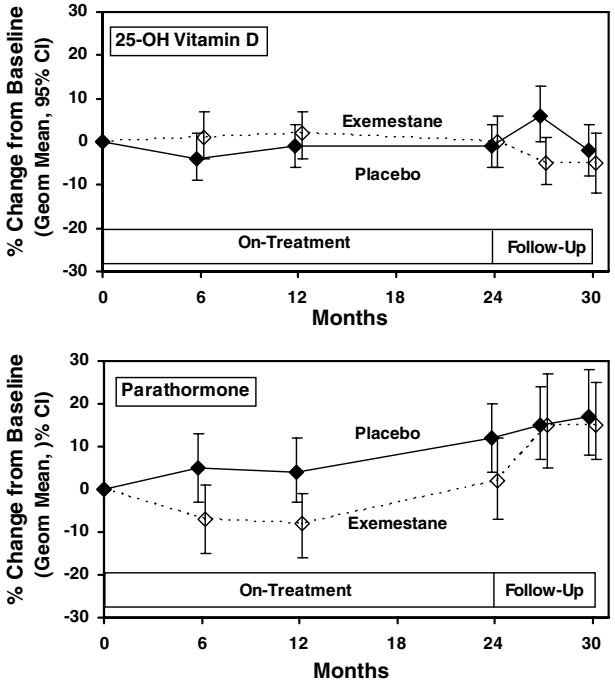

Fig. 5 – Percentage changes from baseline of serum 25-OH Vitamin D (upper panel) and parathormone (lower panel) during 2-year treatment with placebo or exemestane and up to 6 months after treatment discontinuation. CI, confidence interval; FU, follow-up.

For patients completing 24 months of therapy and available for follow-up, the on-treatment changes in lipids were reversed during the 3–12 month follow-up phase (Fig. 6). For homocysteine, the changes from baseline observed at 6–12 months after exemestane withdrawal (14–19% increase) were no longer statistically significant versus placebo (9–12% increase; Fig. 6).

3.5. *Coagulation parameters*

No relevant change in any of the coagulation parameters measured (plasma fibrinogen, PT and APTT) were observed, either during therapy or during the follow-up phase (data not shown).

3.6. *Sex steroids*

During the 2-year treatment period, exemestane has been reported to cause a marked decrease (83–93%) in all serum oestrogens and no relevant changes in androstenedione and testosterone.[13] Oestrogen levels returned to pre-treatment values within 6 months of withdrawal of exemestane treatment. Exemestane withdrawal did not cause any change in androgen levels (data not shown).

3.7. *Reasons for premature discontinuation of the study and adverse events during the follow-up period*

The reasons for premature discontinuation of the study in the two arms are given in Table 1. All in all, 3 patients (4.1%) in the

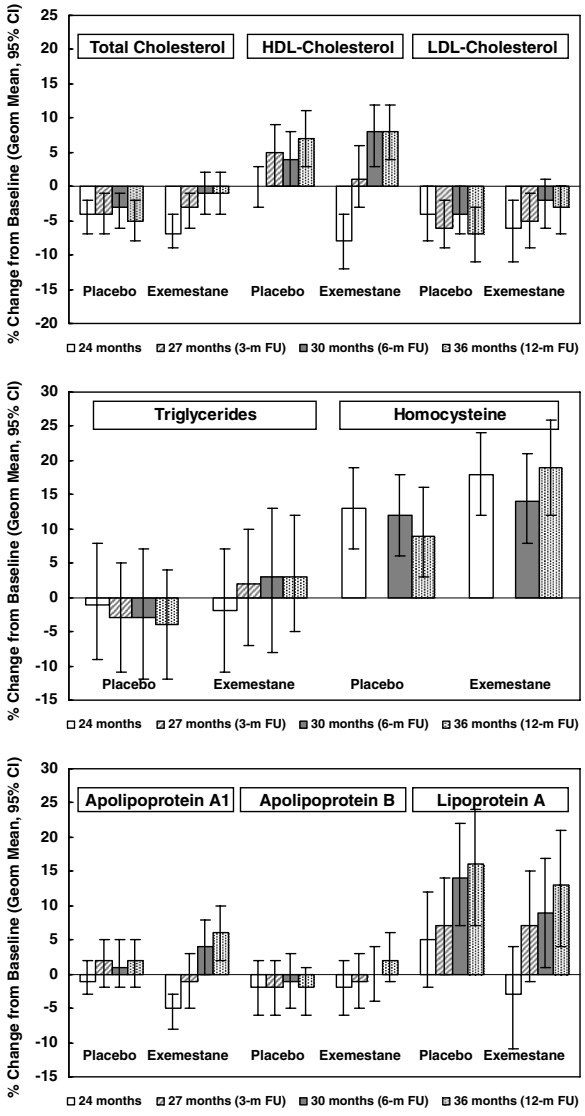

Fig. 6 – Percentage changes from baseline of plasma lipids and serum homocysteine levels at the end of 2-year treatment with placebo or exemestane and up to 1 year after treatment discontinuation. HDL, high-density lipoprotein; LDL, low-density lipoprotein; CI, confidence interval; m, month; FU, follow-up.

placebo arm and 9 patients (12.3%) in the exemestane arm discontinued the study due to adverse events. Side-effects, possibly related to exemestane, causing premature discontinuation of therapy were: hot flushes, nausea, abnormal liver function tests, diarrhoea, and deep vein thrombosis.

No serious adverse events or grade 4 events were reported in either treatment group during the 1-year follow-up period. No fractures were reported during follow-up in either arm. The total number of on-study fractures remains 4 in the exemestane arm and 5 in the placebo arm.

4. Discussion

Potential toxicities with respect to bone and lipid metabolism are major concerns regarding long-term AI treatment of women for early breast cancer. These concerns relate to potential detrimental effects during the treatment period, but also the potential for a sustained impact resulting in long-term detrimental effects. According to official national statistics, the expected life-span for normal healthy Norwegian women aged 50, 60 and 70 years is 33 years, 24 years and 16 years, respectively. The life-time risk for an otherwise healthy woman of having a hip fracture may exceed 20%,[18] and hip fractures, as well as spinal compression fractures on an osteoporotic basis, are associated with significant morbidity as well as increased mortality.[19] Thus, an increased risk of osteoporosis and cardiovascular disease may reduce the benefits of adjuvant treatment with AIs.

In our initial report from this study,[13] we found 2-year treatment with exemestane to have a modest impact on bone loss from the hip area and to cause a non-significant increase in bone loss from the spine compared with placebo. Interestingly, this finding with respect to BMD was accompanied by elevated levels of bone biomarkers associated with bone formation in addition to elevated markers of bone resorption. This may represent a difference between steroidal and non-steroidal compounds.[20] The different impact of exemestane on bone is further supported by the recent report of the bone sub-study of the Intergroup Exemestane Study, in which bone loss stabilised at <1% per year.[21] Considering plasma lipid levels, with the exception of a small (6–9%) decrease in HDL-cholesterol and a concomitant 5–6% decrease in apolipoprotein A1, no significant changes in plasma lipids were recorded.[13]

The results presented here confirm that these changes are, at least in part, reversible. One year after terminating treatment with exemestane, BMD had improved in the lumbar area. A possible explanation for this phenomenon may be a more prolonged effect of exemestane on bone synthesis, reflected by a rise in the markers of bone production during at least the first 6 months after termination of exemestane. In contrast, markers for bone resorption decreased rapidly after termination of exemestane. In the hip, there is a slight improvement in BMD among patients who had received 2-year treatment with exemestane, while there was a further small decrease in the placebo-arm; thus, the difference between the 2 arms was no longer statistically significant. The improvement in BMD was paralleled by a gradual normalisation of all the bone biomarkers over the 6-month follow-up period in which they were recorded. A slight increase in lumbar spine BMD in the placebo group after treatment termination was unexpected, and is probably found by chance.

Previous studies have reported conflicting results regarding the effects of exemestane on plasma lipid fractions in metastatic[22,23] as well as in early[24,25] breast cancer. In our study,[13] we found exemestane to cause a small (6–9%) decrease in HDL-cholesterol and apolipoprotein A1, but no effect on other lipid fractions. While HDL-cholesterol is known to be a cardioprotective factor,[26,27] recent results from large, randomised studies evaluating the effects of hormone replacement therapy have found no significant cardiopreventive effect of such treatment, despite a 8–10% increase in plasma-HDL-cholesterol.[28–32] Thus, it remains unknown whether a small decrease in HDL-cholesterol, as recorded in our study during exemestane therapy, is of clinical importance. The finding reported here, that this minor alteration is completely reversible upon terminating treatment, provides further

assurance of the safety of exemestane with respect to cardiovascular side-effects. This conclusion is further substantiated by the finding of no detrimental effects on coagulation factors during the follow-up phase, as well as the reversion of the mild effect on serum homocysteine levels. Considering non-steroidal AIs, no difference was recorded between letrozole and placebo with respect to lipid parameters in the MA17 study.[33]

The evaluation of the 25-OHD status in the study population revealed that 88% of patients had suboptimal serum levels. While we found no correlation between 25-OHD levels and BMD at baseline, we observed a trend toward higher losses in BMD in the femoral neck and the lumbar spine on treatment with exemestane in the subgroup of patients with suboptimal 25-OHD serum levels. Our findings suggest that vitamin D supplementation may be an important factor in maintaining postmenopausal BMD during treatment with AIs.

In conclusion, the results from this 1-year follow-up after terminating exemestane treatment add information regarding the lack of significant toxicity of this compound. The results now available indicate that exemestane is unlikely to produce durable toxic effects with respect to bone metabolism or plasma lipids. It is noteworthy that exemestane is a steroidal compound with a chemical structure different from anastrozole and letrozole.[34] Clinically, there is a lack of complete cross-resistance between exemestane and the non-steroidal AIs when administered for metastatic breast cancer,[35] probably due to a slight androgen-agonistic effect of its major metabolite.[36] Thus, it is possible that exemestane may have an effect on bone metabolism different from the non-steroidal compounds, and the results obtained here may not be extrapolated to the non-steroidal AIs.

Conflict of interest statement

Per E Lønning received research grants from the Pfizer Co. Jurgen Geisler and Per E Lønning both received speakers honoraria and participated in advisory boards for Pfizer Co. Anna Polli, Enrico Di Salle and Jolanda Paolini are all employed by Pfizer Co.

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REFERENCES

1. Howell A, Cuzick J, Baum M, et al. Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years' adjuvant treatment for breast cancer. *Lancet* 2005;**365**(9453):60–2.
2. Goss PE, Ingle JN, Martino S, et al. A randomized trial of letrozole in postmenopausal women after five years of tamoxifen therapy for early-stage breast cancer. *N Engl J Med* 2003;**349**(19):1793–802.
3. Coombes RC, Hall E, Gibson LJ, et al. A randomized trial of exemestane after two to three years of tamoxifen therapy in postmenopausal women with primary breast cancer. *N Engl J Med* 2004;**350**(11):1081–92.
4. Boccardo F, Rubagotti A, Puntoni M, et al. Switching to anastrozole versus continued tamoxifen treatment of early breast cancer: preliminary results of the Italian Tamoxifen Anastrozole trial. *J Clin Oncol* 2005;**23**(22):5138–47.
5. Jakesz R, Jonat W, Gnant M, et al. Switching of postmenopausal women with endocrine-responsive early breast cancer to anastrozole after 2 years' adjuvant tamoxifen: combined results of ABCSG trial 8 and ARNO 95 trial. *Lancet* 2005;**366**(9484):455–62.
6. Geisler J, King N, Anker G, et al. In vivo inhibition of aromatization by exemestane, a novel irreversible aromatase inhibitor, in postmenopausal breast cancer patients. *Clin Cancer Res* 1998;**4**:2089–93.
7. Geisler J, Haynes B, Anker G, Dowsett M, Lønning PE. Influence of letrozole (Femara) and anastrozole (Arimidex) on total body aromatization and plasma estrogen levels in postmenopausal breast cancer patients evaluated in a randomized, cross-over-designed study. *J Clin Oncol* 2002;**20**:751–7.
8. Thurlimann B, Keshaviah A, Coates AS, et al. A comparison of letrozole and tamoxifen in postmenopausal women with early breast cancer. *N Engl J Med* 2005;**353**(26):2747–57.
9. Geisler J, Lønning PE. Aromatase inhibitors as adjuvant treatment of breast cancer. *Crit Rev Oncol Hematol* 2006;**57**:53–61.
10. Powles TJ, Hickish T, Kanis JA, Tidy A, Ashley S. Effect of tamoxifen on bone mineral density measured by dual-energy X-ray absorptiometry in healthy premenopausal and postmenopausal women. *J Clin Oncol* 1996;**14**:78–84.
11. Love RR, Wiebe DA, Feyzi JM, Newcomb PA, Chappell RJ. Effects of tamoxifen on cardiovascular risk factors in postmenopausal women after 5 years of treatment. *J Natl Cancer Inst* 1994;**86**(20):1534–9.
12. Lien EA, Solheim E, Ueland PM. Distribution of tamoxifen and its metabolites in rat and human tissues during steady-state treatment. *Cancer Res* 1991;**51**:4837–44.
13. Lønning PE, Geisler J, Krag LE, et al. Effects of exemestane administered for 2 years versus placebo on bone mineral density, bone biomarkers, and plasma lipids in patients with surgically resected early breast cancer. *J Clin Oncol* 2005;**23**(22):5126–37.
14. World, Health Organisation. Assessment of fracture risk and application to screening for postmenopausal osteoporosis. Geneva: WHO; 1994.

15. Hollis BW, Wagner CL. Normal serum vitamin D levels. *N Engl J Med* 2005;**352**(5):515–6.
16. Heaney RP, Dowell MS, Hale CA, Bendich A. Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. *J Am Coll Nutr* 2003;**22**(2):142–6.
17. Hollis BW. Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: implications for establishing a new effective dietary intake recommendation for vitamin D. *J Nutr* 2005;**135**(2):317–22.
18. Kanis JA, Johnell O, De Laet C, Jonsson B, Oden A, Ogelsby AK. International variation in hip fracture probabilities: implications for risk assessment. *J Bone Mineral Res* 2002;**17**:1237–44.
19. Cauley JA, Thompson DE, Ensrud KC, Scott JC, Black D. Risk of mortality following clinical fractures. *Osteoporos Int* 2000;**11**(7):556–61.
20. Subar M, Goss P, Thomsen T, Banke-Bochita J. Effects of steroidal and nonsteroidal aromatase inhibitors (Als) on markers of bone turnover and lipid metabolism in healthy volunteers. *Am Soc Clin Oncol* 2004;**23**:734.
21. Coleman R, Banks L, Girgis S, et al. Skeletal effect of exemestane in the Intergropup Exemestane Study (IES) – 2 year bone mineral density (BMD) and bone biomarker data. *Breast Cancer Res Treat* 2005;**94**(S1):S233.
22. Atalay G, Dirix L, Biganzoli L, et al. The effect of exemestane on serum lipid profile in postmenopausal women with metastatic breast cancer: a companion study to EORTC Trial 10951, 'Randomized phase II study in first line hormonal treatment for metastatic breast cancer with exemestane or tamoxifen in postmenopausal patients'. *Ann Oncol* 2004;**15**(2):211–7.
23. Engan T, Krane J, Johannessen DC, Lønning PE, Kvinnsland S. Plasma changes in breast cancer patients during endocrine therapy – lipid measurements and nuclear magnetic resonance (NMR) spectroscopy. *Breast Cancer Res Treat* 1995;**36**:287–97.
24. Markopoulos C, Polychronis A, Zobolas V, et al. The effect of exemestane on the lipidemic profile of postmenopausal early breast cancer patients: preliminary results of the TEAM Greek sub-study. *Breast Cancer Res Treat* 2005;**93**(1):61–6.
25. Markopoulos C, Chrissochou M, Michailidou A, et al. Effect of exemestane on the lipidemic profile of post-menopausal operable breast cancer patients following 5–7 years of adjuvant tamoxifen: preliminary results of the ATENA substudy. *Anti-Cancer Drugs* 2005;**16**(8):879–83.
26. Boden WE. High-density lipoprotein cholesterol as an independent risk factor in cardiovascular disease: Assessing the data from Framingham to the Veterans Affairs high-density lipoprotein intervention trial. *Am J Cardiol* 2000;**86**(12A):19L–22L.
27. Gotto AM. High-density lipoprotein cholesterol and triglycerides as therapeutic targets for preventing and treating coronary artery disease. *Am Heart J* 2002;**144**:533–42.
28. Hulley S, Grady D, Bush T, et al. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. *J Am Med Assoc* 1998;**280**:605–13.
29. Alexander KP, Newby LK, Hellkamp AS, et al. Initiation of hormone replacement therapy after acute myocardial infarction is associated with more cardiac events during follow-up. *J Amer Coll Cardiol* 2001;**38**(1):1–7.
30. Viscoli CM, Brass LM, Kernan WN, Sarrel PM, Suissa S, Horwitz RI. A clinical trial of estrogen-replacement therapy after ischemic stroke. *N Engl J Med* 2001;**345**(17):1243–9.
31. Anderson GL, Limacher M, Assaf AR, et al. Effects of conjugated, equine estrogen in postmenopausal women with hysterectomy – The women's health initiative randomized controlled trial. *Jama* 2004;**291**(14):1701–12.
32. Harman SM, Naftolin F, Brinton EA, Judelson DR. Is the estrogen controversy over? Deconstructing the Women's Health Initiative study: a critical evaluation of the evidence. *Ann NY Acad Sci* 2005;**1052**:43–56.
33. Wasan KM, Goss PE, Pritchard PH, et al. The influence of letrozole on serum lipid concentrations in postmenopausal women with primary breast cancer who have completed 5 years of adjuvant tamoxifen (NCIC CTG MA.17L). *Ann Oncol* 2005;**16**(5):707–15.
34. Geisler J, Lønning PE. Aromatase inhibition – translation into a successful therapeutic approach. *Clin Cancer Res* 2005;**11**:2809–21.
35. Lønning PE, Bajetta E, Murray R, et al. Activity of exemestane in metastatic breast cancer after failure of nonsteroidal aromatase inhibitors: a phase II trial. *J Clin Oncol* 2000;**18**(11):2234–44.
36. Johannessen DC, Engan T, di Salle E, et al. Endocrine and clinical effects of exemestane (PNU 155971), a novel steroidal aromatase inhibitor, in postmenopausal breast cancer patients: a phase I study. *Clin Cancer Res* 1997;**3**:1101–8.