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Original article

# Bone turnover markers in postmenopausal breast cancer treated with fulvestrant – A pilot study

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ABSTRACT

*Background:* Tamoxifen has a protective effect on bone metabolism in breast cancer; aromatase inhibitors deleterious and that of fulvestrant is unknown.

*Methods:* Fourteen locally advanced breast cancers with clinical benefit on fulvestrant (250 mg/month) as first-line primary endocrine therapy had sequential serum bone-specific alkaline phosphatase (BAP), N-terminal propeptide of procollagen type 1 (PINP) and C-terminal telopeptide (CTX) at 0, 1, 6, 12, and 18 months. Mean percentage changes (95% CI) were calculated.

*Results:* Changes from baseline at 1, 6, 12, and 18 months with BAP (3.9–46.8 ng/ml) were +1.5 (−9.8 to +12.9), +2.2 (−22.1 to +26.6), +17.6 (−12.4 to +47.6), +10.8 (−29.9 to +51.7); with PINP (20.6–82.1 ng/ml) were +3.4 (−12.0 to 19.0), +18.8 (−36.7 to +74.2), +47.5 (−21.4 to 116.3), +33.3 (−49.5 to +116.1) and with CTX (0.14–1.35 ng/ml) were +30.8 (0.1 to +61.6), +13.9 (−22.3 to +50.2), +42.9 (−12.7 to +98.5), +45.2 (−28.3 to +118.8).

*Conclusions:* Long-term (18 months) stability of bone markers may be exploited by using fulvestrant earlier in sequence of endocrine therapies particularly in adjuvant setting in those with pre-existing decreased bone mass.



## Background

The increased bone turnover that accompanies declining estrogen levels at the onset of menopause in women leads to decreased bone mass and increased risk of fracture. In postmenopausal women with breast cancer this may be further aggravated by treatment with antiestrogen. Aromatase inhibitors such as anastrozole (Arimidex™, AstraZeneca), letrozole (Femara™, Novartis) or exemestane (Aromasin™, Pfizer) do not have any estrogenic agonistic activity and cause increased bone turnover resulting in significant loss in bone mass.[1] Tamoxifen, however, affords some protection by virtue of its partial agonistic activity.[2–4]

Fulvestrant (Faslodex™, AstraZeneca) is a new estrogen receptor (ER) antagonist with no estrogen agonist effects[5] and has a novel mode of action; it binds, blocks and increases degradation of ER protein, leading to an inhibition of estrogen signaling through the ER.[6,7] In a prospectively planned combined analysis of the data from two randomized trials of similar design (Trials 20 and 21) fulvestrant was reported to be at least as effective as anastrozole in terms of time to progression (TTP; 5.5 months vs. 4.1 months, respectively).[8] A subsequent prospectively planned, combined analysis of survival data reported that the median overall survival was not significantly different between the two treatments.[9] In a further double-blind, randomized phase III trial (Trial 0025) fulvestrant (250 mg/month) was compared with tamoxifen (20 mg/day) in the first-line treatment of postmenopausal women with advanced breast cancer.[10] Prospective planned analysis of patients with ER and/or progesterone receptor (PgR) positive tumours (~80% of the population) showed median TTP of 8.2 months for fulvestrant and 8.3 months for tamoxifen with similar clinical benefit (CB) and objective response (OR) rates and overall survival between groups. However, to date there has been no data of the effect of fulvestrant on bone metabolism in humans.

Bone is constantly renewed by the process of bone remodelling, in which old bone is resorbed by osteoclasts and replaced by new bone, which is laid down by osteoblasts. Markers of bone resorption

*Abbreviations:* LAPC, locally advanced primary breast cancer; BAP, bone-specific alkaline phosphatase; PINP, N-terminal propeptide of procollagen type 1; CTX, C-terminal telopeptide; ER, estrogen receptor; TTP, time to progression; PgR, progesterone receptor; CB, clinical benefit; OR, objective response; MBC, metastatic breast cancer; SD, stable disease; CV, coefficient of variation; CIs, confidence intervals.

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and formation, measured in serum or urine, reflect the activity of osteoclasts and osteoblasts, respectively. This study is the first to report the effect of fulvestrant on markers of bone turnover when used in postmenopausal women with locally advanced primary breast cancer (LAPC) in whom there was no evidence of overt metastatic disease.

## Materials and methods

### *Patients*

Postmenopausal women with LAPC or metastatic breast cancer (MBC) received fulvestrant (250 mg) as their first-line primary endocrine therapy (so patients were endocrine naïve) as part of an open-label prospective clinical trial that had received approval of the institutional Ethics Committee. Patients underwent staging investigations as per study protocol and included blood tests (full blood count, liver function tests, calcium, phosphate, CA15.3 and CEA), chest X-ray and pelvic X-ray for potential skeletal metastases. Bone scintigram was used if plain radiography was not definitive in diagnosing or ruling out metastases. Patients gave written informed consent for the trial including sequential serum samples and tissue biopsies. Twenty-five of 30 patients with LAPC/MBC who were recruited in this study had clinical benefit (CB). The remaining 5 patients progressed within 6 months and were not included in the study. Of the 25 patients with CB, 2 males and 4 MBC patients were not included in the analysis. Thus, a series of 19 post-menopausal women with endocrine-naïve LAPC (primary breast cancer > 5 cm and/or skin involvement) who had CB during fulvestrant therapy were included. Patients with CB were selected so that any bone marker changes would reflect likely the activity of fulvestrant on bony tissue and not disease progression including bone metastasis (and so the MBC patients were excluded).

Patients with LAPC had tumours of TNM stage IIb, IIIa or IIIb (Table 1). Fulvestrant (250 mg) was administered as a once-monthly intramuscular injection into the gluteus muscle. Patients had regular 3 monthly clinical examinations along with CA15.3 and CEA assessments. CB was defined as objective response (complete or partial response) or stable disease [SD] for ≥6 months' duration.[11,12]

### *Bone marker assessments*

Sequential blood samples were taken at baseline and after 1, 6, 12 and 18 months of fulvestrant treatment with majority of patients still being on treatment at 18 months. Patients were not strictly fasting though the large majority of samples were taken at the same time of the day (late mornings).

The clotted blood samples were centrifuged (1000 g for 15 min), and the serum suitably aliquoted and stored at −20 °C. All samples taken from the same patient were analyzed in the same batch at the end of the study. Serum was analyzed for the following markers of bone formation and resorption.

The bone formation markers, bone alkaline phosphatase (BAP) and N-terminal propeptide of procollagen type 1 (PINP), and the bone resorption markers were measured. Bone ALP, an isoenzyme of alkaline phosphatase, was measured using an automated chemiluminescent immunoenzymatic assay (Beckman Access Ostase™ 37300). Intra-assay coefficient of variation (CV) was <2.6% and the normal reference range for postmenopausal women was 3.9–46.8 ng/ml. PINP, a by-product of type I collagen synthesis, was measured by a quantitative radioimmunoassay (Orion Diagnostica UniQ™ PINP RIA). The intra-assay CV was 6.0% and the normal reference range for postmenopausal women was 20.6–82.1 ng/ml.

Serum CTX, a degradation product of crosslinked type I collagen, was measured by an enzyme-linked immunoassay (Serum Cross-laps™, Nordic Bioscience Diagnostics). The intra-assay CV was 3.9% and the normal reference range for postmenopausal women was 0.14–1.35 ng/ml.

### *Statistical analysis*

Data were analyzed using Statgraphics Plus™ version 5 (Herndon, VA) statistical software. Data are presented as mean percentage change (from baseline) in marker level with 95% confidence intervals (CIs).

## Results

The patient and disease characteristics are shown in Table 1. The median duration of CB for patients receiving fulvestrant was 28.0+ months (range: 10.9–55.4 months; treatment ongoing in 15 patients at 18 months and in 11 patients at the time of analysis).

There were no 'baseline' data for 5 patients in whom a sample of blood at baseline was not available. Therefore, 14 patients had bone marker measurements at baseline, 1, 6, 12 and 18 months. Mean percentage change (from baseline) in serum PINP, bone ALP and CTX levels in these 14 patients is shown in Table 2. Wilcoxon signed rank test did not show any significant difference from baseline at any time-point for any of the 3 markers in these patients.

Of the 5 patients who did not have baseline sample available, the marker assessment was over a 17-month period from 1 to 18 months. Kruskal–Wallis analysis revealed no significant changes in bone markers between any of the time-points over this 17-month period in these patients. Similarly, in all 19 patients with LAPC, no significant changes were apparent over the 18-month period.

## Discussion

LAPC patients who had shown CB were selected for this study so that bone turnover marker levels being estimated were not confounded by the presence of overt or occult progressive bony metastases. Furthermore since median time to progression of disease was about 24 months, only samples collected in the first 18 months of the trial were used for marker assessments. This was to avoid as far as possible confounding the results with any early biochemical evidence due to undiagnosed progression of occult bony metastases or the development of new overt bony metastases.

The chosen bone formation and resorption markers are established markers of bone turnover which have been validated in several studies.[13] Although bone markers have high intra-individual variability and diurnal variation (especially CTX)[14] they provide more dynamic and earlier measurement of the skeletal status when compared with bone mineral density measurement.[15,16] Serum markers, however, exhibit less intra-individual variation than urinary markers.[13]

**Table 1**
Patient and disease baseline characteristics.

| | LAPC ($n = 19$) |
|---|---|
| Median age, years (range) | 73.6 (54.9–90.9) |
| Tumour grade, $n$ (%) | |
| 1 | 4 (21.1) |
| 2 | 13 (68.4) |
| 3 | 2 (10.5) |
| Estrogen receptor (ER) status | |
| Median ER H-score | 220 |
| % Cells staining positive | 100 |

**Table 2**
Mean and CI (95% confidence interval) for LAPC ($n = 14$) patients.

| Marker: pre-treatment ($n = 14$) | 1 Month ($n = 13$) | 6 Months ($n = 14$) | 12 Months ($n = 11$) | 18 Months ($n = 10$) |
|---|---|---|---|---|
| Bone ALP | +1.5 (−9.8 to +12.9) | +2.2 (−22.1 to +26.6) | +17.6 (−12.4 to +47.6) | +10.8 (−29.9 to +51.7) |
| PINP | +3.4 (−12.0 to 19.0) | +18.8 (−36.7 to +74.2) | +47.5 (−21.4 to +116.3) | +33.3 (−49.5 to 116.1) |
| CTX | +30.8 (0.1 to +61.6) | +13.9 (−22.3 to +50.2) | + 42.9 (−12.7 to +98.5) | +45.2 (−28.3 to +118.8) |

In these LAPC patients with no demonstrable bony metastases, the stability of bone turnover markers over 17–18 months period (Table 2) suggests the apparent lack of effect of fulvestrant on bone turnover. This was further supported by data in the further 5 patients with unavailable baseline serum sample, in whom there was no significant difference between any of the time-points over 17 months period. To the best of our knowledge, there is no known data in literature of long-term effect of fulvestrant on bone turnover in human studies. Reports of its effect in animals do exist but the data are conflicting. In an experiment by Gallagher et al.[17] in adult female intact rats, fulvestrant reduced cancellous bone volume by increasing bone resorption and decreasing bone formation by abolishing protective effect of estrogen. The increase in bone formation indices was not seen in ovariectomised rats. However, it did not affect longitudinal or periosteal tibial growth in either ovary-intact or ovariectomised rats given estradiol or vehicle. Sibonga et al.[18] in a study of cancellous bones in adult rats found that fulvestrant increased skeletal indices of bone turnover in ovary-intact rats with a reduction in cancellous bone area. However, in ovariectomised rats there was a reduction in bone turnover that was associated with an increase in bone area. Thus if the data from the above experiments in ovariectomised rats are extrapolated to postmenopausal women as in our study then there is no clear evidence for negative influence of fulvestrant on bone tissue.

In yet another study in rats, Lea et al.[19] administered fulvestrant alone and in combination with the anti-androgen, bicalutamide (Casodex™, AstraZeneca, US) and compared the effects on the skeleton with those of ovariectomy. They reported that ovariectomised rats lost significantly greater cancellous bone volume compared with those treated with fulvestrant alone. The combination of fulvestrant and bicalutamide, however, resulted in bone loss equivalent to that in ovariectomised animals. The study authors concluded that ovarian androgens possibly protect against bone loss in rats made estrogen deficient otherwise by fulvestrant. This again if extrapolated to our postmenopausal women may mean that even if there was bone loss induced by fulvestrant by virtue of it being a pure antiestrogen with no agonistic activity (unlike tamoxifen), ovarian androgens may alone have protected against significant bone loss. In contrast to fulvestrant, possible protective effect of androgens on bone is lost on treatment with aromatase inhibitors due to blockage of conversion of circulating androgens into estrogens (by aromatase inhibitors).

In a multi-centre randomized study by Donnez et al.,[20] 50 premenopausal women had short-term exposure to 3 doses of fulvestrant (50 mg, 125 mg, or 250 mg) as an intramuscular injection over 12 weeks period and compared with goserelin and placebo in reduction of uterine fibroid growth before planned hysterectomy. The primary safety end-point of bone resorption measured by urinary crosslinked N-telopeptide and free deoxypyridinolone were measured at baseline, 5, 9 and at 13 weeks (completion of study). There was little change in median bone resorption indices from baseline and in fact no statistical difference between various doses of fulvestrant and placebo. A recent phase II neoadjuvant trial (NEWEST) in 211 postmenopausal women with ER positive large primary breast cancers randomised patients into those receiving approved dose of fulvestrant (250 mg) versus loading dose (500 mg including additional 500 mg on day 14 of first month) over a period of 16 weeks. This trial compared serum bone markers (BAP, PINP, CTX) besides the main tissue tumour indices. The study investigators reported no change in bone markers with either dose.[21] This recent presentation of the NEWEST results at the San Antonio Breast Cancer Conference supports the findings of this study. However, our study remains the only long-term data on the effect of fulvestrant on markers of bone metabolism.

Journe et al.[22] showed that ibandronate (a bisphosphonate) enhanced the growth inhibitory action of tamoxifen and fulvestrant in estrogen-sensitive MCF-7 breast cancer cells. The combination analysis identified additive interactions between ibandronate and ER antagonists. However, in the clinical setting it remains to be seen whether or not there is additive efficacy of fulvestrant plus a bisphosphonate in the treatment of bony metastases.[23] On the other hand the apparently neutral effect of fulvestrant on bone metabolism makes either a higher dose of fulvestrant alone or fulvestrant plus anastrozole combination, a potentially attractive option for future adjuvant endocrine therapy.

## Conclusions

In this small patient series and within the limitations of interpreting variability of response of bone markers, there was a lack of change in markers equating to long-term stability of bone turnover markers in postmenopausal women with LAPC treated with fulvestrant for over a period of 18 months. This is in contrast to the increase in bone markers (serum BAP, PINP and CTX) at 12 months compared to the baseline seen in 58 patients who received anastrozole in a sub-protocol study of patients in ATAC trial.[1]

Data from both animal and now human experiments portray a favourable profile of fulvestrant on bone tissue. While this is the first published report of the effects of fulvestrant on bone metabolism in humans, the recent San Antonio presentation has confirmed that fulvestrant appears neutral in respect of bone metabolism. Furthermore the present study is the only one which has assessed the long-term effects of fulvestrant on bone metabolism. The possible lack of effect on bone turnover may be exploited clinically in the future especially in the adjuvant setting. However, larger randomized studies including head-to-head comparison of long-term bone turnover effects of fulvestrant with tamoxifen and aromatase inhibitors are required to confirm these findings. The comparison could be more robust with inclusion of bone mineral density measurements along with serum samples as radiographs and tumour markers alone may not be sensitive enough.

## Conflict of interest statement

Prof. Robertson and Dr. Cheung have received honoraria and funding from AstraZeneca. Prof. Eastell is a consultant to AstraZeneca. Prof. Eastell and Dr. Hannon have received honoraria and funding from AstraZeneca. Dr. Agrawal has been sponsored by AstraZeneca for Scientific Meetings in the past.

## Authors' contributions

Laboratory tests were arranged and provided by Dr. RA Hannon and Prof. R Eastell. Dr. RA Hannon provided statistical help. Dr. A Agrawal drafted the manuscript which has been revised and approved by Prof. JFR Robertson, Dr. RA Hannon, Prof. R Eastell and Dr. KL Cheung.


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## Ethical approval

The original clinical trial and subset studies were approved by the Local Research Ethics Committee, Nottingham, UK.


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