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# Anastrozole demonstrates clinical and biological effectiveness in oestrogen receptor-positive breast cancers, irrespective of the erbB2 status

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**Abstract**

Overexpression of erbB2 in breast tumours can predict resistance to tamoxifen therapy. We conducted a small trial to determine if erbB2 status correlates with tumour response and biochemical changes in postmenopausal women receiving neoadjuvant therapy with the aromatase inhibitor, anastrozole. Twenty-four postmenopausal women with oestrogen receptor (ER)-rich, large, operable breast tumours received three months of neoadjuvant anastrozole, 1 or 10 mg daily, then surgery, followed by another five years of anastrozole 1 mg daily. Response to the treatment was based on changes in clinical and ultrasound measurements of tumour volume and changes in tumour proliferation and progesterone receptor (PgR) status. After follow-up for a median duration of four years therapy, there was no apparent difference between erbB2 0/1+ and erbB2 3+ tumours in clinical response or changes in proliferation and PgR expression. In conclusion, anastrozole appears to be an effective endocrine option in this patient population, irrespective of the erbB2 status.


*Keywords:* Anastrozole; erbB2; Breast tumours; Endocrine; Postmenopausal; Oestrogen

## 1. Introduction

Human epidermal growth factor receptor-2 (erbB2) is a member of the Type 1 tyrosine kinase growth factor family, which is one of the best-studied growth factor systems in breast cancer. Overexpression of erbB2 has been recorded in approximately 20–30% of all breast cancers; however, only approximately 10% of oestrogen receptor (ER)-positive tumours overexpress erbB2 [1]. ErbB2 is also associated with more aggressive forms of breast cancer and a worse prognosis [2–8]. Early breast cancer patients with erbB2 overexpression were reported to have a median overall survival 50% shorter than patients with erbB2-negative tumours (three years compared with approximately 6–7 years) and, therefore erbB2 may have a greater prognostic value in breast cancer than ER status [2].

Some hormonal therapies and cytotoxic chemotherapies may not be as efficacious in erbB2-positive tumours compared with those that are erbB2-negative. In particular, tumours overexpressing erbB2 were found to be less responsive to CMF (cyclophosphamide, methotrexate, 5-fluorouracil) compared with erbB2-negative tumours and, in node-positive patients, disease-free survival was greater for patients with erbB2-negative tumours compared with erbB2-positive tumours [9]. When comparing adjuvant CMF with placebo following surgery, women with erbB2-negative tumours had a much

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larger increase in median overall survival (12.7 vs 7.3 years, respectively) than women with erbB2-positive tumours (6.1 vs 4.4 years, respectively), although it was noted that all patients benefited from CMF [10]. Letrozole, a third-generation aromatase inhibitor, has been shown to produce a better response rate than tamoxifen (88% vs 22%, respectively) in the neoadjuvant setting in patients with erbB2-positive breast tumours ($P = 0.0004$) [11].

Since patients with erbB2-positive breast cancer are more likely to have a worse prognosis than those with erbB2-negative tumours, it is important to find new treatments that are effective and improve survival in these patients. Although tamoxifen has been the established endocrine treatment for breast cancer for over 30 years, showing good efficacy and tolerability in both the adjuvant and advanced settings, some clinical data have indicated that tumours that are positive for erbB2 may be resistant to tamoxifen [12–15]. However, it should be noted, that not all studies concur with this finding [16].

Anastrozole is a potent, orally-active, non-steroidal aromatase inhibitor that has shown superior efficacy to tamoxifen in the advanced [17,18] and adjuvant settings [19,20] in postmenopausal women with ER-positive breast cancer. It has been previously demonstrated that anastrozole has clinical and biological effectiveness in both erbB2-positive and -negative breast cancers, suggesting its suitability in all hormone receptive-positive patients, irrespective of the erbB2 status [21].

Anastrozole has also been shown to be highly effective as neoadjuvant therapy in a small study of postmenopausal women with large, operable breast cancers, as assessed by tumour volume [22]. The aim of this study was to evaluate tumour response and biological changes in this series of postmenopausal patients with ER-positive breast cancer treated for three months with neoadjuvant anastrozole, and to correlate these with the erbB2 status of the cancer.

## 2. Patients and methods

### 2.1. Patients

Postmenopausal women with ER-rich (histoscore > 80, Allred score $\geqslant 6$, assessed by open wedge biopsy removing <1 g of tumour), large operable or locally advanced breast cancer were included in the study. Postmenopausal status was defined as those patients aged $\geqslant 50$ years who had not menstruated in the last 12 months, or women of any age with follicle-stimulating hormone levels >40 IU/l [22].

Patients were screened for eligibility and to provide baseline assessments four weeks prior to randomisation. This included a medical history, full physical examination including a 12-lead electrocardiogram (ECG and laboratory determinations), assessment of baseline evaluable disease clinically by callipers, ultrasound and mammogram, and a bone scan and chest X-ray to confirm the absence of overt metastases. Assessments that could not be repeated at short intervals (e.g. bone scan, chest X-ray, mammogram) were accepted within 12 weeks prior to randomisation.

Exclusion criteria included drug-induced menopause, abnormal liver function tests (more than three times the upper limit of the reference range), other abnormal laboratory test results (that would place the patient at risk or confound the results of the study), patients with a history of systemic malignancy other than breast cancer or satisfactorily controlled basal cell/squamous cell carcinoma of the skin or cancer of the cervix, and patients with an expected survival time of less than three months from the start of the study. All patients in the study gave their written, informed consent.

### 2.2. Study design

The study was randomised, double-blind and single centre in design. Patients were randomised 1:1 to receive either 1 or 10 mg anastrozole for three months (see Fig. 1). A total of 24 patients were recruited for this trial. This number was based on feasibility rather than any formal statistical technique. Further details of the study design and assessments have been published elsewhere [22].

### 2.3. Assessments

Tumour volume was assessed by calliper, ultrasound and mammography at baseline and after three months of treatment. After three months, all patients underwent

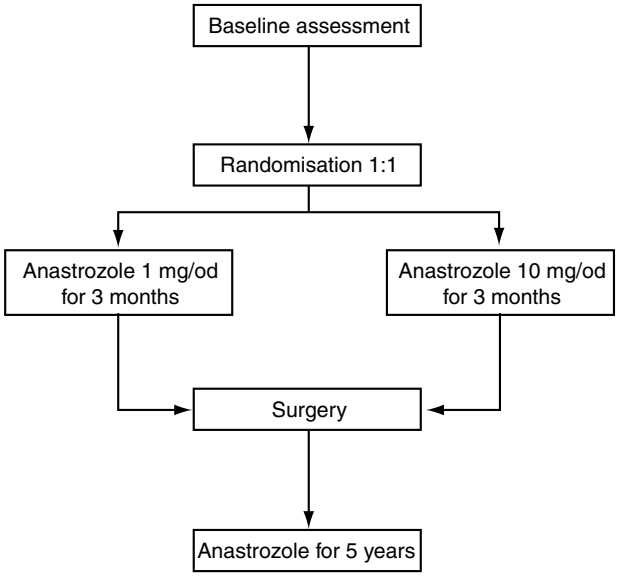


Fig. 1. Study design. od = once day.

surgery and then continued on anastrozole for five years or until recurrence [22].

Tissue samples were taken before and after three months of anastrozole treatment for assessment of tumour proliferation, progesterone receptor (PgR) status and erbB2 status. Proliferation was assessed using Ki67 antibody and was scored as the percentage of stained cells, while PgR status was assessed using Dako antibody and an Allred score (0–8) was allocated. ErbB2 status was assessed in a United Kingdom (UK) registered laboratory (at the Royal Marsden Hospital, London, UK) using the HercepTest. Each tumour was scored as 0, 1+, 2+ or 3+.

All samples scored as 2+ for erbB2 status were tested by fluorescent in situ hybridisation (FISH). Overexpression of erbB2 was defined as tumours staining 2+ or 3+, which also tested positive by FISH, while all patients with tumours scored as 0 or 1+ were considered not to overexpress erbB2.

### 2.4. Analysis of the results

The data on tumour volumes were not subjected to any formal statistical analysis. The differences between patients with erbB2-positive tumours and those with negative erbB2 status following anastrozole treatment (1 and 10 mg groups combined), for tumour volume, node status, disease recurrence (local or metastatic) were assessed using two-way ANOVA (Analysis of Variance). The percentage changes in proliferation and PgR expression following anastrozole treatment were assessed separately in the erbB2 0/1+ and erbB2 3+ tumours using a one-way ANOVA. For all tests, $P < 0.05$ was deemed as statistically significant.

## 3. Results

Patient characteristics for the eligible patients are shown in Table 1. The patient demography was comparable between the two treatment groups.

Twenty-four patients were enrolled into the study. One patient who received 10 mg of anastrozole withdrew due to the side-effects; the remaining 23 continued on treatment and had surgery as per protocol after three months. Sufficient tissue was available before and after three months of anastrozole treatment for assessment of proliferation, PgR status and erbB2 status in 22 patients. After surgery, patients were followed up for a median duration of therapy of four years. The response rate did not differ between the 1 and 10 mg of anastrozole groups; therefore, the results for the two doses of drug have been combined.

### 3.1. ErbB2 and nodal status

Ten patients had erbB2 status scored as 0, six had erbB2 status scored as 1+ and six had erbB2 status scored as 3+. None had erbB2 status scored as 2+.

Table 1
Patient demographics and baseline tumour characteristics

| | Anastrozole (1 mg/day) ($n = 12$) | | Anastrozole (10 mg/day) ($n = 12$) | |
|---|---|---|---|---|
| Age (years) (mean ± SD) | 74.1 ± 8.7 | | 68.8 ± 8.9 | |
| Weight (kg) (mean ± SD) | 69.6 ± 9.6 | | 77.5 ± 14.2 | |
| Height (cm) (mean ± SD) | 156.3 ± 12.2 | | 157.8 ± 11.7 | |
| Tumour stage | *n* | (%) | *n* | (%) |
| $T_2$ | 11 | (92) | 10 | (83) |
| $T_3$ | 0 | (0) | 2 | (17) |
| $T_{4B}$ | 1 | (8) | 0 | (0) |
| Lymph node metastases | 2 | (17) | 1 | (8) |
| Tumour volume assessment | | | | |
| *Calliper ($cm^3$)* | | | | |
| Median | | 23.7 | | 27.3 |
| Maximum | | 42.4 | | 161.0 |
| Minimum | | 12.4 | | 10.9 |
| *Ultrasound ($cm^3$)* | | | | |
| Median | | 4.8 | | 4.9 |
| Maximum | | 9.9 | | 14.3 |
| Minimum | | 2.4 | | 1.4 |
| *Mammography ($cm^3$)* | | | | |
| Median | | 7.3 | | 8.4 |
| Maximum | | 25.5 | | 47.7 |
| Minimum | | 1.4 | | 0.7 |

Nine of 22 patients had node-positive disease (five had erbB2 status scored as 0/1+, and four scored 3+). Thirteen patients had node-negative disease (eleven had erbB2 status scored as 0/1+ and two scored 3+). Although only a small number of patients were included in the trial, a formal analysis was performed (Fisher's exact test). The difference in nodal status between the erbB2 0/1+ and the 3+ groups did not reach statistical significance (erbB2 0/1+ vs erbB2 3+, $P = 0.18$).

Most patients receiving anastrozole in both groups had not recurred at four years of follow up (13/16 in the erbB2 0/1+ group and 4/6 in the erbB2 3+ group are alive without recurrence). Two patients in the erbB2 0/1+ and two patients in the erbB2 3+ groups recurred within four years. No patients had died from breast cancer; one unrelated death occurred at 22 months in the erbB2 0/1+ group.

### 3.2. Clinical response

The percentage reductions in tumour volume assessed by calliper or by ultrasound were equivalent for the two groups, subdivided according to the erbB2 status (see Figs. 2(a) and (b)).

### 3.3. Proliferation

Changes in proliferation related to erbB2 status were comparable for both the erbB2-positive and -negative groups (Fig. 3); all erbB2 3+ patients showed a reduction in proliferation ($P < 0.017$) of similar magnitude to the 0/1+ patients ($P < 0.0001$). Median (range) erbB2 0/1+: pre-anastrozole, 25 (16.25–38.75); post-anastrozole, 5.0 (1.0–14.25). Median (range) erbB2 3+: pre-anastrozole, 22.5 (15.0–33.75); post-anastrozole, 7.5 (2.0–12.5).

### 3.4. Changes in progesterone receptor expression compared with erbB2 status

Seventeen patients were PgR-positive at baseline. The changes in PgR expression over the three-month treatment period are shown for individual patients in Fig. 4, and were similar in the erbB2 0/1+ and erbB2 3+ groups.

## 4. Discussion

Anastrozole has been found to be highly effective as neoadjuvant therapy in postmenopausal women with ER-rich, large, operable breast cancers, with a median reduction in tumour volume of 75.5% after 12 weeks of treatment (combined data for patients receiving 1 and 10 mg anastrozole) [22]. Following on from these data, this study has indicated that anastrozole is clinically and biologically effective in both erbB2-positive and -negative breast cancers. In terms of clinical response to treatment, changes in proliferation or PgR status appear equivalent for both erbB2 0/1+ and erbB2 3+ tumours. Similarities were also observed in terms of nodal status and clinical outcome between the two groups. However, patient numbers were very small and further larger-scale studies are required to confirm these findings.

It is clear that erbB2 is a useful prognostic factor regarding certain therapies such as tamoxifen and chem-

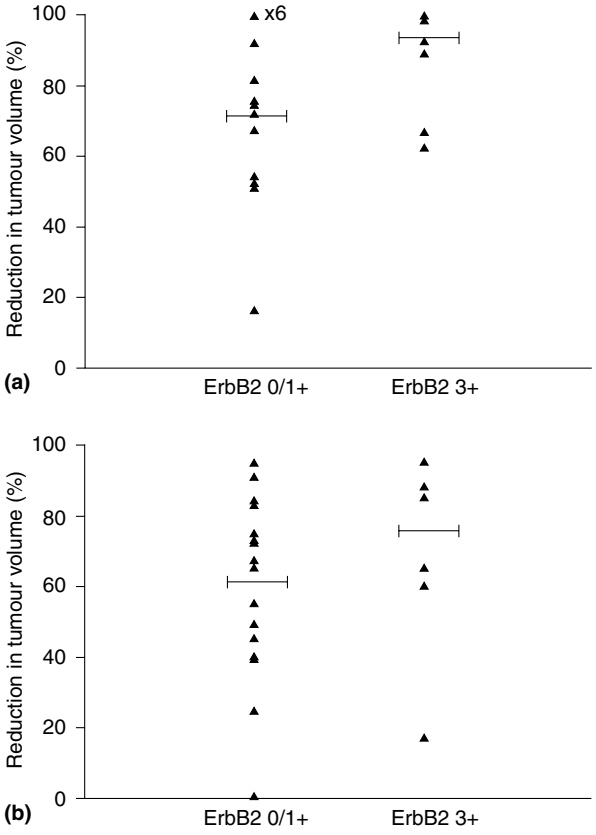


Fig. 2. (a) Percentage change in tumour volume, as assessed by calliper, following three months of treatment with anastrozole according to the erbB2 status. (b) Percentage change in tumour volume, as assessed by ultrasound, following three months of treatment with anastrozole according to the erbB2 status.

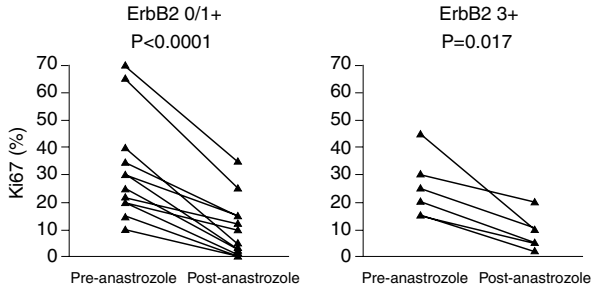


Fig. 3. Percentage change in proliferation (Ki67) following three months of treatment with anastrozole according to the erbB2 status.

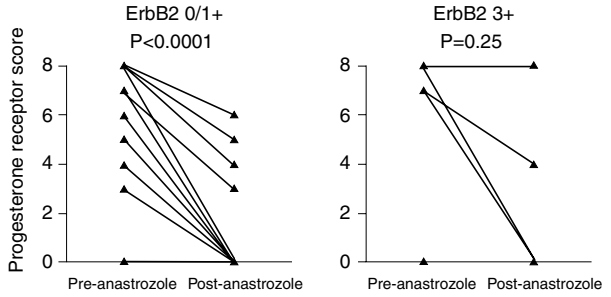


Fig. 4. Change in progesterone receptor (PgR) expression following three months of treatment with anastrozole according to the erBb2 status. Note: three out of 16 erbB2 0/1+ patients were PgR-negative; two out of 6 erbB2 3/+ patients were PgR-negative.

otherapy; however, it may not useful for predicting the response to anastrozole. Although this study was performed in only a small number of patients, results show that most patients with both erbB2-positive tumours (4/6) and erbB2-negative tumours (13/16) had not recurred after four years of follow-up. These results indicate that anastrozole may be a valuable treatment option in patients with ER-positive breast cancer, irrespective of the erbB2 status.

Results of previous studies indicate that the relationship between aromatase inhibitors and erbB2 overexpression is not fully understood. A randomised study in 324 postmenopausal women with metastatic breast cancer showed that letrozole had a higher response rate than tamoxifen in patients with erbB2- and erbB1-positive tumours [11]. In another small study ($n = 112$) correlating tumour response and biological changes with erbB-2 status, anastrozole was shown to be effective in all hormone receptor-positive patients, irrespective of the erbB-2 status [21].

Pre-clinical models have shown that ER-positive and erbB2-positive tumours are highly oestrogen-dependent. Benz and colleagues [23] have demonstrated that MCF-7 breast cancer cells transfected with an erbB2 expression vector grew as rapidly as xenografts in nude mice supplemented with oestrogen. However, no erbB2-positive tumours formed in the absence of oestrogen, indicating that oestrogen dependence was maintained despite erbB2 overexpression. In this model, erbB2-transfected cells continued to grow in the presence of tamoxifen with no signs of regression, even after weeks of treatment. A molecular explanation for these findings is suggested by the recent observation that a downstream mediator of erbB1/2 signalling, MEKK1, activates the ER and stimulates the agonist activity of tamoxifen [24]. The erbB1/2 tamoxifen resistance pathway may be circumvented by letrozole because, by removing oestrogen, the ER becomes monomeric and unable to bind DNA. Under these circumstances, it is speculated that the ER is incapable of transcription and is not a productive target for erbB1/2-activated protein kinases.

The results of the present study are particularly important considering that expression of the erbB2 gene in breast cancer may predict resistance to tamoxifen therapy. It must be noted that patients with erbB2-negative tumours generally have a better prognosis than those with erbB2-positive tumours regardless of the treatment recieved; however, the demonstration in this study (in agreement with the data of Milla-Santos and colleagues [24]) that anastrozole is effective in erbB2-overexpressing tumours suggests that it is an effective endocrine option for patients with erbB2-negative and erbB2-positive disease.

## Conflict of interest statement/role of funding source

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