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Pathological features of breast cancer response following neoadjuvant treatment with either letrozole or tamoxifen

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Abstract

Morphological characteristics, grading features, proliferation marker MIB1, apoptosis (by Tdt-mediated duTP-biotin nick-end labelling (TUNEL)), Bcl-2 expression, oestrogen receptor (ER) and progesterone receptor (PgR) status were compared in ER-positive breast cancers before and after 3 months of neoadjuvant therapy with either letrozole or tamoxifen. Daily treatment was with letrozole 2.5 mg (12 patients) or 10 mg (12 patients), or with tamoxifen 20 mg (24 patients). Letrozole treatment was associated with a pathological response in 17 of 24 (71%) patients. The predominant change in grading features was a decrease in mitosis, and the expression of MIB1 was reduced in all of the 22 evaluable cases. Whilst only marginal changes were observed in ER expression following letrozole therapy, PgR reactivity was reduced in 20 of 21 evaluable cases which were initially PgR-positive, becoming undetectable in 16 patients. Tamoxifen treatment was associated with pathological response in 15 of 24 (63%) tumours. In contrast to letrozole, the dominant change in grading feature was an increase in tubule formation, ER score was markedly reduced in most cases, and the most common effect on PgR was an increased expression. Following treatment with either tamoxifen or letrozole, variable effects were observed on the apoptotic index and expression of Bcl-2. These results indicate that both letrozole and tamoxifen have marked influences on the pathological features of breast cancer during neoadjuvant therapy. However, the effects of the two agents varied such that the phenotypes of letrozole- and tamoxifen-treated tumours differ markedly. Effects on clinical, pathological and biological endpoints were frequently disconcordant—future studies will therefore require the evaluation of multiple parameters in order to fully assess tumour response.

Keywords: Breast cancer; Neoadjuvant; Histopathology; Letrozole; Tamoxifen

1. Introduction

Management of breast cancer by primary (neoadjuvant) systemic therapy prior to surgery has long been a major focus of the Edinburgh Breast Unit [1]. This approach can provide clinical benefits by downsizing tumours to allow more conservative surgery and more effective adjuvant treatment [2–4]. Study designs in which therapy is given with the primary cancer still *in situ* also offer the opportunity to study the effects of treatment in individual tumours. Our experience relating to biological effects of tamoxifen on primary breast cancer has already been reported [5,6].

Recently, novel endocrine agents in the form of a new generation of aromatase inhibitors have been used successfully in therapeutic trials of postmenopausal women with advanced breast cancer [7,8]. One agent, letrozole, has been shown to be more effective than tamoxifen when used as neoadjuvant therapy [9]. The effects of aromatase inhibitors on tumour pathology in the neoadjuvant setting have not been formally reported. The aim of the present paper was to document the effects of 3 months of therapy with letrozole on tumour histopathological features and biological markers, and to compare these results with the same parameters measured after comparable treatment with tamoxifen.

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2. Patients and methods

2.1. Patients

Forty-eight postmenopausal women with primary breast carcinomas that were >3 cm in diameter, clinically and radiologically, were studied. An initial tumour biopsy was performed at the time of recruitment, immediately before treatment was initiated. This biopsy was used to confirm the presence of invasive breast cancer and to establish Oestrogen Receptor (ER) status (only tumours with >20 fmol/mg cytosol ER protein were eligible for study). Daily treatment for 3 months consisted of either letrozole 2.5 mg (12 patients) or 10 mg (12 patients), or tamoxifen 20 mg (24 patients). Caliper and ultrasound measurements of the breast lesion were then made at 4-weekly intervals. Volume measurements were calculated as previously reported in Ref. [10], with a >25% reduction between the initial and final volume being considered a clinical response. Patients in the letrozole cohort were recruited over the time period 1995–1996, and those for tamoxifen treatment from 1994 to 1996.

2.2. Tissues histology

Sections of the same tumours from pretreatment and final biopsies were assessed for changes in cellularity and degree of fibrosis. Pathological response was categorised as: complete when there was no evidence of malignant cells at the original tumour site; microscopic residual when only scattered foci of malignant cells were identified microscopically; partial response when clear decreases in cellularity and/or increases in fibrosis were seen; or no change. The same specimens were evaluated independently for combined histological grade [11].

2.3. Immunohistochemistry (IHC)

IHC staining with antibody to MIB1 (Ki-67) antigen (Europath Ltd., UK) diluted ×50 was used as a measure of tumour cell proliferation. Reactivity was detected by an ABC–peroxidase–antiperoxidase (PAP) method, and the percentage of cells staining in a minimum of 10 representative high-power microscope fields was used to quantify expression [12]. The Tdt-mediated dUTP-biotin nick-end labelling (TUNEL) technique of Lebat-Moleur and colleagues [13] was used to detect apoptotic cells, employing digoxygenin labelling. The apoptotic index was defined as the number of apoptoses per 100 cancer cells and was adapted from methodology originally used to assess the mitotic index [14]. Reactivity for ER or Progesterone Receptor (PgR) was performed by the PAP method, after microwave antigen retrieval, using antibodies ID5 (Dako Labs, UK) and PG88 (Biogenix, San Ramon, CA, USA), respectively, according to the manufacturers' instructions, and diaminobenzidine was used as the chromogen. Results were scored on a scale of 0–3 for staining intensity (with each successive score denoting increasing intensity), and on a score of 0–5 for increasing proportion of positive cancer nuclei (0 = none, 1 = <1%, 2 = 1–10%, 3 = 11–33%, 4 = 34–66%, and 5 = >66%). The values were then summed into a category score within a range of 0–8 [15]. Staining for Bcl-2 employed a monoclonal antibody (Dako, Ely, UK) diluted ×100, and reactivity was detected as for MIB1. Scoring was assessed semi-quantitatively as for ER and PgR, but for cytoplasmic location only and without regard for the staining intensity.

2.4. Statistics

Clinical responses were analysed using Fisher's Exact test. Differences in tumour histopathological parameters were compared statistically using the Chi-square (χ^2) for trend or Wilcoxon rank tests.

3. Results

3.1. Clinical responses

Of the 24 patients treated with letrozole, 23 (96%) had a >25% reduction in tumour volume over the 3-month study period. The corresponding result for tamoxifen was 16 of 24 (67%). This difference between the two agents was statistically significant. ($P = 0.023$).

3.2. Morphological changes

Following letrozole treatment, marked morphological changes were evident in the majority of tumours (17 of 24; 71%). The category changes are shown in Table 1a. Responses included both decreased cellularity and increased fibrosis, and were equivalent in both dose subgroups (data not shown). After tamoxifen treatment, marked morphological changes were also seen in the majority of cases (15 of 24; 63%). Categories of pathological responses are also shown in Table 1a. The differences between letrozole and tamoxifen treatment for these parameters were not statistically significant.

Histological grade was reduced following letrozole treatment in 9 of 22 (41%) evaluable tumour pairs (2 cases were not evaluable because of lack of tumour in the second biopsy), largely due to fewer mitoses. Thus, as shown in Table 1b, in the majority of cases there was a reduced mitotic score following therapy. Tamoxifen reduced tumour grade in a similar proportion of cases (10 of 24; 42%), but in this instance it was largely on account of increased tubule formation (Table 1b). The differences between letrozole and tamoxifen treatment

Table 1
Histopathological changes in tumours following neoadjuvant treatment with letrozole or tamoxifen

(a) Morphology[a]

	CR	MR	PR	NC
Letrozole ($n=24$)	1 (4%)	3 (13%)	13 (54%)	7 (29%)
Tamoxifen ($n=24$)	0 (0%)	4 (17%)	11 (46%)	9 (38%)

(b) Features of grade[b]

	Tubules			*Nuclear Pleomorphism*			*Mitosis*		
	D	NC	I	D	NC	I	D	NC	I
Letrozole ($n=22$)	2	18	2	5	17	0	12	10	0
Tamoxifen ($n=24$)	0	11	13	3	19	2	4	18	2

[a] CR, complete pathological response; MR, minimal residual disease; PR, partial response (reduced cellularity and/or increased fibrosis; NC = no change. Differences between the letrozole and tamoxifen groups were not statistically significant ($\chi^2=1.96$; $P=0.67$).

[b] D/NC/I = number of tumours showing decrease/no change/increase. Differences between the letrozole and tamoxifen groups were statistically significant for tubule formation ($\chi^2=11.69$; $P=0.003$), non-significant for nuclear pleomorphism ($\chi^2=1.83$; $P=0.4$), and significant for mitosis ($\chi^2=8.21$; $P=0.017$).

were statistically significant for tubule formation and for mitotic index, but not for nuclear pleomorphism.

3.3. MIB1 staining

After treatment with letrozole, immunoreactivity for MIB1 was reduced in all 22 evaluable tumour pairs, with percentage reductions ranging from 17 to 100% (four tumours completely lost MIB1 reactivity) (Fig. 1a and b). An example is shown in Plate 1. The degree of reduction was significantly greater in tumours with a pathological response compared with those with no response, irrespective of the dose of letrozole administered (median reduction 77% versus 51%, $P=0.014$; Wilcoxon rank test). Following tamoxifen treatment, MIB1 reactivity was reduced in 18 of 22 (82%) evaluable cases, the decreases ranging from 22 to 100% (one tumour completely lost reactivity) (Fig. 1). Of the four tumours not showing a reduction in MIB1staining after treatment, three had evidence of reduced cellularity. Quantitative changes in MIB1 score between the two agents were not significantly different.

3.4. TUNEL

Changes in apoptotic index were evident following letrozole treatment (Fig. 2). However, the direction of change was not consistent, decreasing in 10 of 19 (53%) assessable cases and increasing in 9 (47%) cases. Furthermore, the pattern of change was not related to the

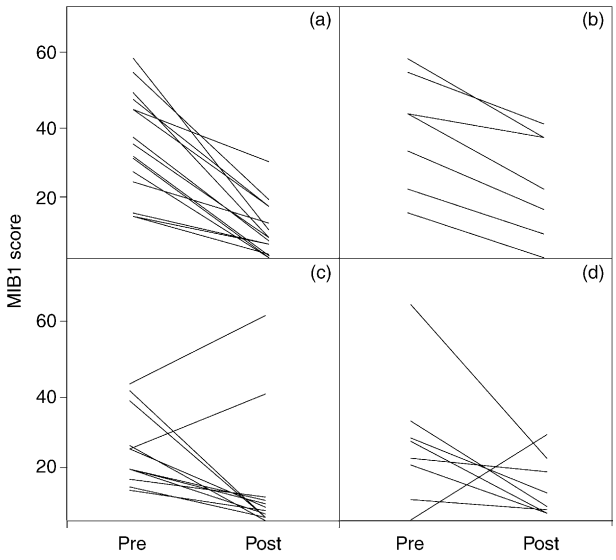

Fig. 1. Tumour MIB1 scores before (pre) and after (post) neoadjuvant treatment with either letrozole or tamoxifen. Scores were subdivided according to whether there was evidence of pathological response versus no change. (a) 15 individual tumour pairs that showed pathological response to letrozole; (b) seven tumour pairs that showed no pathological changes after letrozole treatment. In all 22 of these cases, treatment resulted in a decrease in staining score, and in a complete loss of staining in four tumours; (c) 14 individual tumour pairs that showed pathological response to tamoxifen; (d) eight tumour pairs that showed no pathological changes after tamoxifen treatment. Among these 22 cases, the staining score increased in three tumour pairs (in 1 case after no initial reactivity), and one tumour had no visible staining before or after treatment. The differences between the two treatment groups were not significant, although for letrozole the degree of staining reduction in tumours with pathological change was significantly greater than the degree of reduction in tumours with no pathological change ($P=0.014$; Wilcoxon rank test).

pathological response or to dose of letrozole administered (data not shown). With tamoxifen treatment, IHC staining scores also changed: of 16 evaluable cases, 13 (81%) decreased and 3 (19%) increased. This pattern of change, which was not related to pathological response (data not shown), was not significantly different from the pattern observed with letrozole.

3.5. Staining for Bcl-2 and steroid receptors

Following letrozole treatment, there was a variable pattern of changes in Bcl-2 IHC staining: in 22 assessable cases, scores decreased in 9 (41%), increased in 4 (18%), and were unchanged in 9 (41%). These changes were not related to either the pathological response, changes in the apoptotic index, or dose of letrozole (data not shown). The corresponding results with tamoxifen, for 22 evaluable tumour pairs, were a decreased score in 10 (45%) cases, an increase in two (9%), and no change in 10 (45%). This pattern of change was not significantly different from that obtained with letrozole.

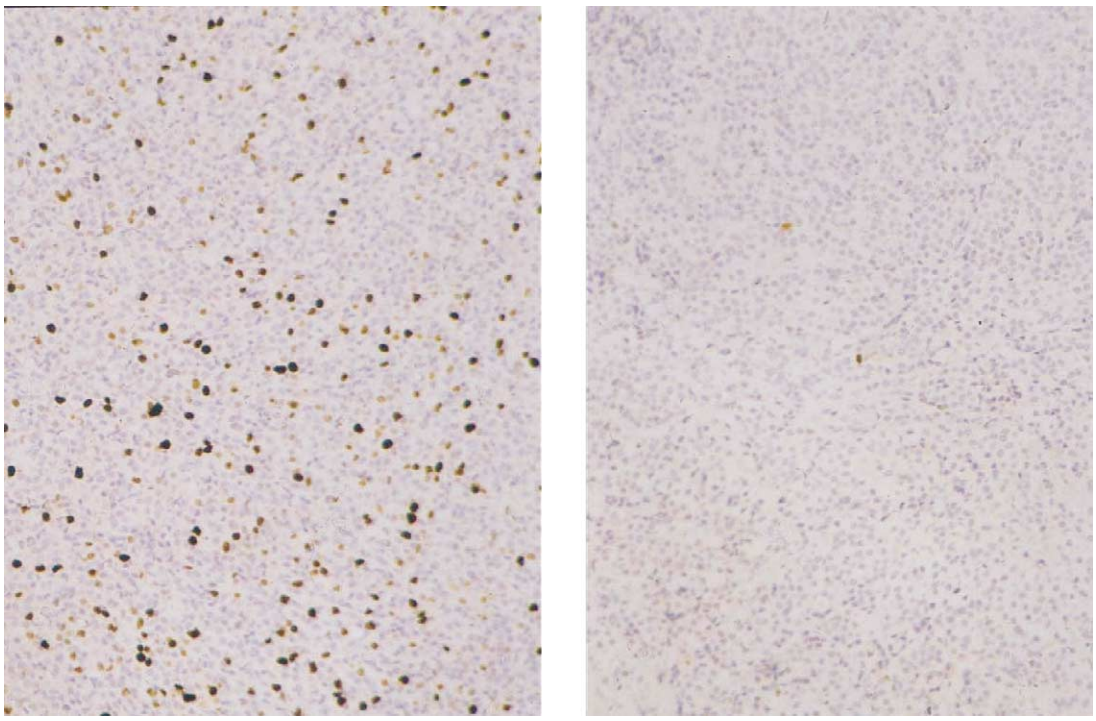

Plate 1. MIB1 staining of the same tumour before (left) and after (right) 3 months of treatment with letrozole, showing a major reduction in staining after treatment (*original magnification×200*).

ER status did not change with letrozole therapy in any of 23 tumours, and the IHC staining score was reduced only marginally (by one category) in 9 (39%) cases, increased by a similar increment in 2 (9%), and unchanged in 12 (52%) tumours (Table 2). Following tamoxifen treatment, all 23 tumours remained positive for ER throughout therapy, but major changes in the intensity of staining were observed: the staining score decreased in 17 (74%) cases, increased in 2 (9%), and was unchanged in only 4 (17%) cases (one tumour pair was unassessable) (Table 2). This pattern of changes did not correlate with either the pathological or clinical responses but was significantly different from that associated with letrozole treatment ($P=0.04$)

Table 2
Changes in staining of steroid receptors following neoadjuvant treatment with letrozole or tamoxifen, based on a semi-quantitative scoring system

	Decrease	No change	Increase
Oestrogen receptor (ER)[a]			
Letrozole ($n=23$)	9 (39%)	12 (52%)	2 (9%)
Tamoxifen ($n=23$)	17 (74%)	4 (17%)	2 (9%)
Progesterone receptor (PgR)[b]			
Letrozole ($n=23$)	20 (87%)	3 (13%)	0 (0%)
Tamoxifen ($n=23$)	4 (17%)	9 (39%)	10 (43%)

[a] Changes in ER score with treatment were significantly different between the letrozole and tamoxifen groups ($\chi^2=6.46$; $P=0.04$).

[b] Changes in PgR score with treatment were significantly different between the letrozole and tamoxifen groups ($\chi^2=23.67$; $P=<0.0001$).

PgR status was positive in 22 of 24 (92%) tumours treated with letrozole (the two PgR-negative tumours subsequently responded clinically). Paired measurements were assessable for 23 tumours treated with letrozole. Therapy was associated with reduced staining in 20 (87%) cases, becoming absent in 16 (70%); staining in the remaining three tumours (including the two PgR-negative tumours) was unchanged. Reductions in staining with treatment (Plate 2) were irrespective of the pathological response (data not shown) or the dose of letrozole administered.

PgR status was positive in 17 of 24 (71%) tumours treated with tamoxifen. Of these tumours, 14 (82%) responded clinically and 10 (59%) pathologically; of the seven negative tumours, two (29%) subsequently responded both clinically and pathologically. The difference in response rate to tamoxifen between PgR-positive and -negative tumours was significant for clinical response ($P=0.02$ by Fishers Exact test), but not for pathological response ($P=0.37$ by Fisher's Exact test). Of the 23 assessable tumour pairs, tamoxifen treatment was associated with reduced IHC staining in 4 (17%) cases (in a single tumour there was a total loss of expression), no change in 9 (39%) (including four negative tumours), and an increase in 10 (43%). Interestingly, two of the six (33%) assessable PgR-negative tumour pairings became positive following tamoxifen treatment. Changes in the pattern of staining were irrespective of the clinical or pathological response. The changes in pattern of PgR expression with tamoxifen

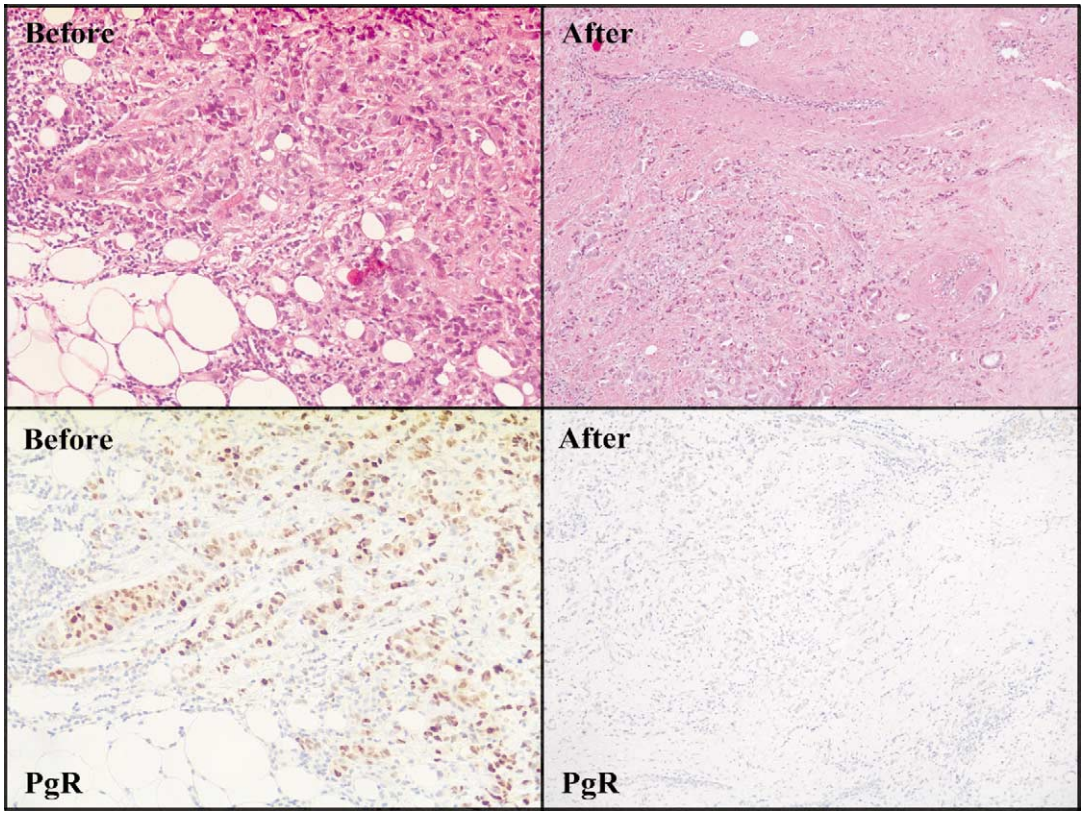

Plate 2. The same tumour, before (*original magnification×200*) and after 3 months of treatment with letrozole (*original magnification×100*), stained with haematoxylin and eosin (top panels) or for PgR (bottom panels). There is a substantial reduction in PgR staining in this tumour after treatment.

were significantly different from letrozole treatment ($\chi^2 = 23.67$; $P < 0.0001$).

4. Discussion

Letrozole is one of the new-generation aromatase inhibitors and has marked endocrine effects on circulating and tissue levels of oestrogen in postmenopausal women [16,17]. When given to patients with ER-positive breast cancer in clinical trials, letrozole has produced response rates and clinical endpoints of disease outcome which are superior to the activities of more established endocrine agents [8,9]. However, effects of letrozole on tumour histopathology are not well-documented and it was the purpose of the present study to fill this gap and to compare effects with those of tamoxifen. The neo-adjuvant design of our protocol meant that direct comparison of the same tumours could be made before and after 3 months of treatment with letrozole or tamoxifen.

Our observations indicate that letrozole can have marked effects on histopathological features within breast tumours. These include changes in cellularity, histological grading features, markers of proliferation and cell death, and hormone receptor expression, which reflect letrozole's potent endocrinological and antitumour properties. However, specific details of many of the observations, which did not differ significantly between the two doses of the drug, merit further consideration.

One of the striking findings in this study was that letrozole reduced the expression of MIB1 in all 22 assessable tumours. Interestingly, the reduction in proliferation index (MIB1 expression) in pathological reponders to letrozole was significantly greater than in those without evidence of response. This effect is consistent with both the clinical reduction in tumour size and the clear pathological responses in terms of reduced cellularity and mitosis seen in the majority of tumours in the present report. However, it should be noted that the relationships between these parameters were by no means absolute. For example, reduction in proliferation failed to translate into: (i) a clinical response in 1 patient; (ii) a reduction in tumour cellularity in 7 cases; and (iii) a decrease in mitosis in 10 cases. These inconsistencies probably reflect the inherent heterogeneity of breast cancer and the differing sensitivity of the measurements. This issue has implications for the use of these parameters as predictors and monitors of response and suggests that they may be best employed in combination.

Measurements of Bcl-2 expression and apoptosis were undertaken because they are indices of cell survival and death. Interestingly, changes were often observed with letrozole treatment, but the direction of the effect was variable, neither interrelated nor significantly associated

with other pathological markers of response. The probable reason for this is that these endpoints (as measured after 3 months of treatment) reflect the interplay of multiple and differing processes. For example, one could envisage that successful treatment might increase apoptosis and reduce cell survival. However, a reduction in cell proliferation, as was observed to follow letrozole use, would ultimately reduce apoptosis [18], and increase cell survival. Since the chronology and degree of these processes may differ in individual cases [19], diverse results might be expected. These considerations also impact on the timing of obtaining study biopsies. In the present investigation, a 3-month interval was chosen because it was considered essential to be able to evaluate clinical response, even though this timing is likely to be suboptimal for the assessment of earlier markers of response. Indeed, we would stress the need for earlier time-points of evaluation in future studies.

The relatively minor and inconsistent effect of letrozole on ER expression observed in this study is similar to results reported with other aromatase inhibitors [20,21]. This was in contrast to effects of letrozole on PgR, the expression of which was reduced in 87% of tumours and was totally lost with treatment in 70% of tumours. Comparable findings have been observed with anastrozole, another non-steroidal aromatase inhibitor [21]. These findings provide clear evidence of the anti-oestrogenic properties of this family of agents, PgR being an oestrogen-inducible protein. Interestingly, loss of PgR expression occurred independently of the pathological response.

While the effects observed with letrozole were remarkably similar to findings with other aromatase inhibitors [21], they are different from previously reported results with the anti-oestrogen tamoxifen [5,6,22–24]. Because of this, we thought it important to identify a group of patients who had been recruited to neoadjuvant treatment with tamoxifen over a similar time-period. Although it is acknowledged that this does not constitute a blind randomised trial, the cohorts did not differ significantly in baseline clinical and tumour parameters except for there being only two PgR-negative tumours in the letrozole group, compared with seven in the tamoxifen cohort.

The clinical response rate to tamoxifen of 67% in this study was similar to our overall experience in a large number of patients treated with tamoxifen in the neoadjuvant setting [6,23] and was less than that observed with letrozole. In contrast, the pathological response rate was similar in the two treatment groups. However, a potentially important difference between the two agents was the nature of the histopathological change when it occurred. Thus, while letrozole was most frequently linked with a reduction in mitosis, the most common change with tamoxifen was an increase in tubule formation. This latter effect has been previously reported in Ref. [25] and is likely to be the result of selective deletion of less-differentiated cell populations although a direct inductive action on a sub-population of cells can not be excluded. The molecular mechanism whereby two different forms of endocrine deprivation therapy have differing effects on tumour pathology can only be a matter for conjecture. It may be that the process of cell proliferation is more readily influenced by levels of oestrogen and an aromatase inhibitor which effectively suppresses tumour oestrogens is more likely to reduce proliferation than a partial oestrogen agonist such as tamoxifen (see below).

Clear differences between the effects of letrozole and tamoxifen were particularly evident for changes in steroid receptor expression. Thus, while letrozole had generally only minor effects on ER staining, major changes (usually marked reduction in expression) were seen following tamoxifen treatment. This latter effect has been previously shown in Ref. [26] and reflects the direct interaction of tamoxifen with ER [27]. Conversely, while letrozole had a dramatic effect on reducing PgR expression, the most frequent change for tamoxifen was an increased staining for the receptor, and only a minority of tumours showed a decreased expression. These disparate effects are clear evidence of the different mechanism of action of the two agents. Letrozole's sole action appears to be to reduce endogenous levels of oestrogen, i.e. it is anti-oestrogenic, whereas tamoxifen is known to behave not only as an anti-oestrogen, but also to have some oestrogen agonist activity. The increase in PgR is consistent with this latter property. Interestingly, the pattern of changes did not relate to the clinical or pathological responses, so that these short-term effects do not appear to influence tumour behaviour. Whether they are predictive of longer-term endpoints such as time to recurrence or survival remains to be determined. Nevertheless, the phenotypes of tumours following treatment with letrozole and tamoxifen clearly differ, the former being more likely to be ER-positive and PgR-negative, whereas the latter are commonly ER-poor and PgR-rich. These differences may have implications with regard to the sequence of endocrine treatments and cross-resistance to those treatments [28–30].

In summary, neoadjuvant letrozole has been shown to have clear effects on histopathological features of breast tumours. These influences, in particular effects on steroid receptor expression, differ from those observed following comparable treatment with tamoxifen. However, clinical, pathological and biological responses are not always consistent, and this emphasises the need to evaluate multiple parameters when comparing different biological agents.

References

1. Forrest AP, Levack PA, Chetty U, *et al*. A human tumour model. *Lancet* 1986, **2**, 840–842.
2. Bonadonna G, Veronesi U, Brambilla C, *et al*. Primary

chemotherapy to avoid mastectomy in tumors with diameters of three centimeters or more. *J Natl Cancer Inst* 1990, **82**, 1539–1545.
3. Powles TJ, Hickish TF, Makris A, *et al*. Randomized trial of chemoendocrine therapy started before or after surgery for treatment of primary breast cancer. *J Clin Oncol* 1995, **13**, 547–552.
4. Cameron DA, Anderson ED, Levack P, *et al*. Primary systemic therapy for operable breast cancer—10-year survival data after chemotherapy and hormone therapy. *Br J Cancer* 1997, **76**, 1099–1105.
5. Keen JC, Dixon JM, Miller EP, *et al*. The expression of Ki-S1 and BCL-2 and the response to primary tamoxifen therapy in elderly patients with breast cancer. *Breast Cancer Res Treat* 1997, **44**, 123–133.
6. Miller WR, Anderson TJ, Hawkins RA, *et al*. Neoadjuvant endocrine treatment: the Edinburgh experience. In Howell A, Dowsett M, eds. *Primary Medical Therapy for Breast Cancer*. *ESO Scientific Updates,* vol. 4. Amsterdam, Elsevier Science BV, 1999, 1–11.
7. Bonneterre J, Thürlimann B, Robertson JFR, *et al*. Anastrozole versus tamoxifen as first-line therapy for advanced breast cancer in 668 postmenopausal women: results of the tamoxifen or Arimidex randomized group efficacy and tolerability study. *J Clin Oncol* 2000, **18**, 3748–3757.
8. Mouridsen H, Gershanovich M, Sun Y, *et al*. Superior efficacy of letrozole versus tamoxifen as first-line therapy for postmenopausal women with advanced breast cancer: results of a phase III study of the International Letrozole Breast Cancer Group. *J Clin Oncol* 2001, **19**, 2596–2606.
9. Ellis MJ, Coop A, Singh B, *et al*. Letrozole is more effective neoadjuvant endocrine therapy than tamoxifen for ErbB-1- and/or ErbB-2-positive, estrogen receptor-positive primary breast cancer: evidence from a phase II randomized trial. *J Clin Oncol* 2001, **19**, 3808–3816.
10. Forouhi P, Dixon JM, Leonard RC, Chetty U. Prospective randomized study of surgical morbidity following primary systemic therapy for breast cancer. *Br J Surg* 1995, **82**, 79–82.
11. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology* 1991, **19**, 403–410.
12. Gerdes J, Becker MH, Key G, Cattoretti G. Immunohistological detection of tumour growth fraction (Ki-67 antigen) in formalin-fixed and routinely processed tissues. *J Pathol* 1992, **168**, 85–86.
13. Lebat-Moleur F, Guillermet C, Lorimier P, Robert C, Lanteujoul S. TUNEL apoptotic cell detection in tissue sections: critical evaluation and improvement. *J Histochem Cytochem* 1998, **46**, 327–334.
14. Simpson JF, Dutt PL, Page DL. Expression of mitoses per thousand cells and cell density in breast carcinomas: a proposal. *Hum Pathol* 1992, **23**, 608–611.
15. Allred DC, Harvey JM, Berardo M, Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. *Mod Pathol* 1998, **11**, 155–168.
16. Demers LM, Lipton A, Harvey HA, *et al*. The efficacy of CGS 20267 in suppressing estrogen biosynthesis in patients with advanced breast cancer. *J Steroid Biochem Mol Biol* 1993, **44**, 687–691.
17. Miller WR, Telford J, Smith H, Dixon JM. Effect of neoadjuvant treatment with the aromatase inhibitor letrozole on peripheral synthesis of oestrogen, uptake by the breast and in situ production of oestrogen within mammary tissues. *Breast Cancer Res Treat* 1998, **50**, 228.
18. Cameron DA, Keen JC, Dixon JM, *et al*. Effective tamoxifen therapy of breast cancer involves both antiproliferative and proapoptotic changes. *Eur J Cancer* 2000, **36**, 845–851.
19. Cameron DA, Ritchie AA, Miller WR. The relative importance of proliferation and cell death in breast cancer growth and response to tamoxifen. *Eur J Cancer* 2001, **37**, 1545–1553.
20. Sasano H, Sato S, Ito K, *et al*. Effects of aromatase inhibitors on the pathobiology of the human breast, endometrial and ovarian carcinoma. *Endocr Relat Cancer* 1999, **6**, 197–204.
21. Miller WR, Dixon JM, Cameron DA, Anderson TJ. Biological and clinical effects of aromatase inhibitors in neoadjuvant therapy. *J Steroid Biochem Mol Biol* 2001, **79**, 103–107.
22. Makris A, Powles TJ, Allred DC, Dowsett M. Changes in hormone receptors and proliferation markers in tamoxifen-treated breast cancer patients and the relationship with response. *Breast Cancer Res Treat* 1998, **48**, 11–20.
23. Chang J, Powles TJ, Allred DC, *et al*. Prediction of clinical outcome from primary tamoxifen by expression of biologic markers in breast cancer patients. *Clin Cancer Res* 2000, **6**, 616–621.
24. Johnstone RD, Smith IE, Dowsett M. Place of aromatase inhibitors in the endocrine therapy of breast cancer. In Miller WR, Santen RJ, eds. *Aromatase Inhibition and Breast Cancer*. New York, Marcel Dekker, 2001, 29–50.
25. Anderson TJ, Dixon JM, Miller WR. Is phenotypic drift in breast cancer disclosed in neoadjuvant endocrine therapy? *Breast Cancer Res Treat* 1999, **57**, 137.
26. Kenny FS, Willisher PC, Gee JM, *et al*. Change in expression of ER, bcl-2 and MIB1 on primary tamoxifen and relation to response in ER positive breast cancer. *Breast Cancer Res Treat* 2001, **65**, 135–144.
27. Katzenellenbogen BS, Choi I, Delage-Mourroux R, *et al*. Molecular mechanisms of estrogen action: selective ligands and receptor pharmacology. *J Steroid Biochem Mol Biol* 2000, **74**, 279–285.
28. MacGregor JI, Jordan VC. Basic guide to the mechanisms of antiestrogen action. *Pharmacol Rev* 1998, **50**, 151–196.
29. Hu XF, Veroni M, De Luise M, *et al*. Circumvention of tamoxifen resistance by the pure anti-estrogen ICI 182,780. *Int J Cancer* 1993, **55**, 873–876.
30. Bajetta E, Zilembo N, Dowsett M, *et al*. Double-blind, randomised, multicentre endocrine trial comparing two letrozole doses, in postmenopausal breast cancer patients. *Eur J Cancer* 1999, **35**, 208–213.