Influence of Neoadjuvant Anastrozole (Arimidex) on Intratumoral Estrogen Levels and Proliferation Markers in Patients with Locally Advanced Breast Cancer\textsuperscript{1}

Jürgen Geisler, Simone Detre, Hildegunn Berntsen, Lars Ottestad, Bernt Lindtjørn, Mitch Dowsett, and Per Eystein Lønning\textsuperscript{2}

Departments of Oncology \cite{JG, HB, PEL, B], and Surgery \cite{B}. Haukeland University Hospital, N-5021 Bergen, Norway; Academic Department of Biochemistry, Royal Marsden Hospital, London, SW3 6JJ, United Kingdom \cite{SDDM]; and Department of Oncology, The Norwegian Radium Hospital, N-0310 Oslo, Norway \cite{LO}.

ABSTRACT

Anastrozole (Arimidex) is a novel, selective, and potent aromatase inhibitor used for the treatment of postmenopausal breast cancer. The drug has been shown to inhibit in vivo aromatization by 96–97\% and to suppress plasma estrogen levels by 84–94\%. However, the effects of anastrozole on intratumoral estrogen levels have not been studied. Here we report the effects of neoadjuvant treatment with anastrozole on intratumoral levels of estrone (E\textsubscript{1}), estradiol (E\textsubscript{2}), and estrone sulfate (E\textsubscript{1}S), measured by a highly sensitive RIA following a multistep purification procedure involving high-pressure liquid chromatography. Tumor tissue was obtained prior to treatment and after 15 weeks on therapy with anastrozole on intratumoral levels by 89.0\% (73.2–95.5\%), and 80.7 (31.4–207.3) fmol/g tissue (geometric mean values with 95\% confidence interval; respectively). Treatment with anastrozole suppressed tissue E\textsubscript{2}, E\textsubscript{1}, and E\textsubscript{1}S levels by 89.0\% (73.2–95.5\%), 83.4\% (63.2–92.5\%), and 72.9\% (47.3–86.1\%), respectively, compared with baseline levels, with no significant difference between responders and nonresponders. Plasma levels of E\textsubscript{2}, E\textsubscript{1}, and E\textsubscript{1}S were suppressed by 86.1, 83.9, and 94.2\%, respectively. Anastrozole caused a decrease in the immunoexpression of the proliferation markers Ki67 and pS2 in all of the patients, with a trend for a more profound suppression in those achieving an objective response. The mean percentage of apoptotic cells was found to be decreased in responders and increased in nonresponders after 15 weeks of anastrozole therapy. Our results reveal anastrozole to cause a significant suppression of tissue estrogen levels and to influence the biology of primary estrogen receptor-positive breast cancers in postmenopausal women.

INTRODUCTION

Aromatase inhibitors are successfully used for the therapy of postmenopausal breast cancer \cite{1, 2}. Although novel, third-generation drugs like letrozole, anastrozole, and exemestane have been shown to cause profound suppression of plasma estrogen levels and to inhibit total body aromatization by 96 to >99\% \cite{3–5}, these changes may not necessarily reflect alterations of tumor estrogen levels. Breast tumor tissue contains sulfatase as well as aromatase and dehydrogenase \cite{6, 7}, allowing synthesis of E\textsubscript{2} from androgens as well as from E\textsubscript{1} and E\textsubscript{1}S. Whereas the aromatase in tumor tissue is the same enzyme as detected in other tissues, its expression may be enhanced by growth factors and interleukins locally synthesized \cite{8}. In addition, recent evidence has suggested active uptake of E\textsubscript{2} from the circulation in tumor cells \cite{9}.

We have recently developed a highly sensitive and specific HPLC-RIA method for the simultaneous measurement of E\textsubscript{2}, E\textsubscript{1}, and E\textsubscript{1}S levels in breast cancer tissue \cite{10}. The aim of this study was to determine the influence of anastrozole treatment on intratumoral estrogen levels in postmenopausal women with locally advanced breast cancer who had not been exposed to previous anticancer therapy, by determining estrogen levels in the same tumors prior to and after 15 weeks of neoadjuvant anastrozole therapy. To evaluate the biological effects of estrogen suppression, we measured changes in the proliferation marker Ki67 and the percentage of apoptotic cells as well as hormone and growth factor receptors (ER, PgR, c-erbB-2, EGF-R) and the estrogen-dependent protein pS2.

PATIENTS AND METHODS

Patients. Postmenopausal women with locally advanced (T\textsubscript{3}, T\textsubscript{4}, and/or N\textsubscript{2}) noninflammatory breast cancer, with or without limited distant metastasis, were eligible. Fourteen patients fulfilled the inclusion criteria and started treatment ac-

\textsuperscript{1}The abbreviations used are: E\textsubscript{2}, estradiol; E\textsubscript{1}, estrone; E\textsubscript{1}S, estrone sulfate; HPLC, high-performance liquid chromatography; CI, confidence interval; ER, estrogen receptor; PgR, progesterone receptor; EGF-R, epidermal growth factor receptor; IHC, immunohistochemistry.
According to protocol. However, one patient refused surgery after having a partial response and another patient died after only 2 weeks on treatment for reasons not related to her breast cancer (cardiopulmonary insufficiency). The remaining 12 patients (median age, 66.5 years; range 55–80) completed the study (Table 1). Postmenopausal status was defined as amenorrhea for ≥1 year duration with luteinizing hormone/follicle-stimulating hormone levels in the postmenopausal range. All of the tumors were ER positive (≥10 fmol/mg tissue or ≥10% of the tumor cells staining positive for ER by IHC) as determined prior to inclusion. To assess the influence of therapy on this parameter, samples collected before and during treatment were re-determined with use of the same immunoassay as described in the method section. Exclusion criteria included hormone replacement therapy, systemic treatment with glucocorticoids, or treatment with other drugs, like phenytoin, carbamazepine, or rifampicin, that are known to influence estrogen metabolism (11, 12), within 3 months prior to protocol inclusion. One patient (no. 8) received treatment with glucocorticoid inhalations because of an obstructive lung disease. The patients were treated at the Departments of Oncology, Haukeland University Hospital, Bergen, and at The Norwegian Radium-hospital, Oslo, Norway.

**Treatment.** Each patient received anastrozole (Arimidex) as an oral dose of 1 mg once daily. In general, treatment was given for 15 weeks, followed by surgery (mastectomy) and radiation. Each breast tumor (and, if present, palpable axillary nodes), was measured prior to therapy and after 4, 8, 12, and 15 weeks on anastrozole therapy. Tumor size was calculated as the product of the largest diameter and its perpendicular. Clinical response was classified according to the Union International Contre Cancer criteria (13), with the exception that tumors reduced by ≥25% but less than 50% in size were classified as “minimal change.” All of the patients who experienced a partial response or a minimal change were defined as respondents, whereas all of the patients with stable disease or a progressive disease were defined as nonresponders. Patients who experienced an objective response to treatment with anastrozole continued with anastrozole as adjuvant therapy for up to 5 years. The protocol was approved by the local ethical committee, and every patient gave her written informed consent.

**Tissue Collection.** Breast tumor tissue was collected prior to treatment (open biopsy), after 2–3 weeks on treatment (true-cut biopsy), and during final surgery (mastectomy). All of the tissue samples were stripped of adhering fat and were divided into several pieces of about 100 mg each and one single piece of about 500 mg. Tissue samples to be used for estrogen measurements were immediately snap-frozen and stored in liquid nitrogen until analysis. For IHC, tissue samples were fixed in formalin and embedded in paraffin.

**Measurement of Intratumoral Estrogen Levels.** Tissue estrogen concentrations were measured using a novel highly sensitive RIA method subsequent to a multiple-step purification process involving HPLC as described elsewhere (10). Briefly, tissue homogenates were incubated with [3H]-labeled estrogens (E1, E2, E1S) as recovery controls and crude fractions were separated by ether extraction. The E1S fraction was hydrolyzed with sulfatase followed by elution on a Sephadex column. HPLC was used to purify the individual estrogen fractions prior to RIA analysis. E1 and E1S were converted into E2, and all three estrogen fractions were finally measured by the same highly sensitive and specific RIA using estradiol-6-carboxymethylxime-[2,125I]-iodohistamine as a ligand. Final estrogen values were corrected for the amount of tissue used in each individual sample (wet weight) as well as for the recovery of the amount of [3H]-hormone added as internal standard. The detection limits for tissue levels of E2, E1, and E1S were 4.3, 19.8, and 11.9 fmol/g tissue, respectively (10).

**Collection of Blood Samples and Measurement of Plasma Estrogen Levels.** Blood samples for hormone measurements were taken into heparinized vials (2 vials containing 10 ml each) immediately before commencing therapy with anastrozole and after 15 weeks on treatment. Each sample was obtained after an overnight fast. Plasma was separated by centrifugation and stored at −20°C until processing. E2 and E1 were determined by RIAs as reported elsewhere (14, 15). Plasma levels of E1S were determined by a novel, highly sensitive assay involving purification and derivatization into E2 and by RIA analysis using estradiol-6-carboxymethylxime-[2,125I]-iodohistamine as tracer ligand (16). The sensitivity limits for plasma levels of E2, E1, and E1S were 2.1, 6.3, and 2.7 pmol/L, respectively.

**Tissue Biomarkers.** All of the biomarkers were analyzed by previously published methodology on histological sections. ER expression was demonstrated with the DAKO DS5 mouse monoclonal (17) and PgR with the Novocastra antibody NCL-PgR clone 1A6 (18). Measurement of Ki67 was performed with the MIB1 mouse monoclonal antibody and of apoptosis by the TUNEL method (terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling; Ref. 19). To ensure acceptable precision, quantitation of apoptosis and Ki67 involved the counting of 3000 and 1000 cells, respectively. For all other analytes, 10 high-powered fields (chosen randomly) were assessed. Notably, the cellularity of the tissue samples obtained after treatment with anastrozole did not vary substantially from pretreatment ones. c-erbB2 and EGF-R were assessed with the ICR12 and Biogenex MU207 antibodies, respectively (20). pS2 antibody was a gift from Prof. Pierre Chambon (Institut de Genetique et de Biologie Moleculaire et Cellulaire, CNRS/INSE Louis Pasteur, College de France, Strasbourg, France). Staining was scored as described previously except for ER and PgR, which were reported as percentage of positively stained cells.

**Statistics.** Plasma and tissue estrogen levels were described by their geometric means with 95% CI limits. Whenever estrogen levels (either in plasma or tissue samples) below the detection limits were found, the value of the respective detection limit was ascribed to the sample for statistical analysis. Before-treatment and on-treatment values were compared using the Wilcoxon matched-pair signed rank test. The mean value of percentage suppression from baseline for a parameter was calculated as 100 − x, where x is the geometric mean value of the individual parameters in the on-treatment situation expressed as percentage of pretreatment values (4). All of the quantitative results generated by IHC (percentage of ER and PgR, Ki67, p52 and percentage of apoptotic cells) are given as their arithmetic mean values. EGF-R and c-erbB-2 status were described as either positive or negative. The Friedman test was used to test for differences between three groups (see Table 2 for details). The comparison of changes in proliferation markers...
and receptor levels between responders and nonresponders was performed using the Mann-Whitney U test.

RESULTS

The mean tissue concentrations of E2, E1, and E1S at baseline were 217.9 fmol/g (95% CI, 69.8–679.9 fmol/g), 173.6 fmol/g (95% CI, 83.9–358.9 fmol/g), and 80.7 fmol/g (95% CI, 31.4–207.3 fmol/g), respectively. There was a nonsignificant trend for a positive correlation (r=0.40) between the percentage of ER positive cells and E2 levels in the tumor tissues.

Treatment with anastrozole suppressed tissue concentrations of E2, E1, and E1S (Fig. 1) to 23.9 fmol/g (95% CI, 12.2–47.0 fmol/g), 28.8 fmol/g (95% CI, 19.7–42.1 fmol/g), and 21.9 fmol/g (95% CI, 12.6–37.9 fmol/g). The percentage suppression of E2, E1, and E1S were 89.0% (95% CI, 73.2–95.5%), 83.4% (95% CI, 63.2–92.5%), and 72.9% (95% CI, 47.3–86.1%), respectively (Fig. 2). It should be noted that one, eight, and seven patients, respectively, had tissue levels of E2, E1, and E1S below the detection limit during treatment. There was no significant correlation between the tissue concentrations of any estrogen fraction before or after therapy with anastrozole.

Pretreatment plasma levels of E2, E1, and E1S were 18.4 pmol/liter (95% CI, 13.2–25.6 pmol/liter), 70.6 pmol/liter (95% CI, 55.3–90.1 pmol/liter), and 578.8 pmol/liter (95% CI, 328.7–1020.5 pmol/liter), respectively. After 15 weeks on anastrozole therapy the plasma levels of E2, E1, and E1S decreased to 2.6 pmol/liter (95% CI, 2.1–3.1 pmol/liter), 11.4 pmol/liter (95% CI, 9.5–13.7 pmol/liter), and 33.3 pmol/liter (95% CI, 16.6–67.0 pmol/liter), which corresponded to a suppression of 86.1% (95% CI, 81.6–89.5%), 83.9% (95% CI, 79.0–87.6%), and 94.2% (95% CI, 90.7–96.5%), respectively. Seven patients had plasma levels of E2, and one patient had plasma levels of E1S below the detection limit while on treatment with anastrozole. The patient (no. 8) receiving glucocorticoids by inhalation had low pretreatment plasma estrogen levels with values of E2, E1, and E1S of 8.4, 32.2, and 330.9 pmol/liter, respectively.

Patient demographics, clinical responses, and individual expression of growth factor receptors are given in Table 1. Whereas true-cut biopsies were obtained from all of the patients after 2–3 weeks on treatment, IHC was evaluable for seven to eight patients only, because amounts of tissue in some biopsies were too small. However, open biopsies were available for all patients before treatment and surgical specimens (obtained directly from the removed breast) after 15 weeks (median) on treatment with anastrozole.

There was a trend for ER levels to be higher in responders than in nonresponders (Tables 1 and 2). Whereas expression of ER did not change during therapy, PgR levels fell significantly. The suppression was similar in responders and nonresponders. A similar pattern of change was seen for pS2, except that in this case, the fall was already significant after 2 weeks on therapy.

For the proliferation marker Ki67, there was a significant decrease at 2–3 weeks and an additional decrease to 37% of baseline values at 3 months. This was quantitatively greater for responders but statistically significant for both responders and nonresponders. Overall, there was no significant change in the mean percentage of apoptotic cells. However, it decreased in

![Fig. 1 A–C, influence of treatment with neoadjuvant anastrozole on intratissue estrogen (fmol/g tissue) levels (●, responders; ○, nonresponders).](image-url)
responder and increased in nonresponders \((P = 0.045, \text{ when comparing the two groups})\).

Two nonresponding tumors showed positive staining for c-erbB-2; one of these tumors, in addition, expressed EGF-R. It was notable that the two patients expressing c-erbB-2 also had the lowest ER levels and the lowest tissue E2 levels of all of the participants in this trial. No change in the expression of either of these receptors was observed during therapy with anastrozole. In addition, no significant correlation was found between staining for c-erbB-2, ER levels, estrogen tissue or plasma levels and clinical response in the whole study group.

**DISCUSSION**

Although several studies have determined plasma estrogen levels and total body aromatization in patients treated with novel aromatase inhibitors \((3–5)\), there is limited information about alterations in tumor estrogen concentrations during therapy. It is well documented that breast cancer E2 concentrations are 10- to 20-fold higher and E1 concentrations 2- to 10-fold higher than the corresponding plasma levels in postmenopausal women \((21–24)\), a finding confirmed in this study. Whether this may be attributable to local synthesis \((6)\) or active estrogen uptake from the circulation \((9)\) is unknown. Studies published by Miller \((25)\) and by James et al. \((26)\), evaluating the origin of intratumoral estrogens, reported a substantial interindividual variation, which suggested that the bulk of intratumoral estrogens occur from local synthesis in some tumors but are derived from the circulation in others. Although the aromatase enzyme is the same in both malignant and normal cells, the local activity of the enzyme may be influenced by growth factors and cytokines \((27, 28)\). Thus, direct measurements are necessary to evaluate the influence of different treatment modalities on intratumoral estrogens.

A key problem associated with intratumoral estrogen measurements during treatment with aromatase inhibitors is the requirement of a detection method with sensitivity limits suitable for estrogen measurements in the low range. We have recently developed a novel, highly sensitive, and specific HPLC-RIA for the simultaneous measurement of E2, E1, and E1S in breast tissue biopsies \((10)\). The sensitivity limits of this method allow the measurement of estrogen levels in the range expected during treatment of postmenopausal women with highly potent aromatase inhibitors. Although several investigators have measured intratumoral estrogen levels in untreated patients \((7, 21–24, 29–35)\), only a few studies have determined the effect of aromatase inhibitor therapy on intratumoral estrogen levels. Reed et al. \((36)\) investigated the influence of the second-generation steroidal aromatase inhibitor 4-hydroxyandrostenedione on tumor E2 levels in four patients and reported a reduction by 62%. de Jong and colleagues measured intratumoral E1 and E2 levels in five patients on treatment with vorozole, an aromatase inhibitor belonging to the triazole class \((37)\). Because of the lack of pretreatment levels, they compared their results with historical data for untreated patients, suggesting a suppression of tissue E1 and E2 levels by 64% and 80%, respectively. Although Miller et al. \((38)\) determined tumor E1 and E2 levels in patients before and during treatment with letrozole, the exact percentage of suppression could not be determined because of the concomitant infusion of tracer steroids used to determine estrogen production rates.

The present study revealed profound suppression of tumor E1 and E2 levels to an extent similar to that of plasma estrogen suppression. The exact proportional decreases were not possible to calculate because a number of patients had levels suppressed below the detection limit of even these highly sensitive assays. The finding of a better suppression of tissue E1 and E2 levels compared with tissue E1S levels may also be partly explained by three of the patients having tissue E1S levels below the detection limit at baseline. In addition, 7 of the 12 patients had on-treatment tissue E1S levels below the detection limit; thus, an underestimation of the E1S suppression is likely in the descriptive statistics. It is notable that patients with high intratumoral E1 and E2 levels experienced, in general, a better estrogen suppression compared with those with lower levels at baseline.

In marked contrast to a previous study \((22)\), which reported tissue E1S levels to be 10-fold higher than plasma levels, we found tissue levels of E1S to be only \(\sim 20\%\) of plasma E1S levels. E1S is the most abundant circulating estrogen in postmenopausal women and conversion of E1S to E1 and E2 has been suggested to be a major pathway of intratumoral estrogen synthesis \((6, 39)\). Because of its hydrophilic nature, E1S is unlikely to pass the cell membrane. However, after hydrolysis of E1S by the ubiquitous sulfatase, E1 may pass through cell membranes and be converted to either E2 or E1S in the cells. Thus, whereas our data are consistent with a lack of uptake of E1S per se and refute major conversion of E2 to E1S in tumors, our findings do not refute the possibility that circulating E1S may contribute to intratumoral estrogens after deconjugation prior to uptake.

We found no correlation between the suppression of any of the tissue estrogen fractions and the clinical response to anastrozole treatment. Thus, our data do not suggest that resistance to neoadjuvant treatment with anastrozole can be explained by an incomplete suppression of tissue estrogens; intrinsic resistance of the tumor to estrogen deprivation therapy is a more probable explanation.

Several studies have investigated biomarkers that are potentially predictive for response to neoadjuvant endocrine treatment in addition to pretreatment ER and PgR levels. The majority of these studies have involved tamoxifen therapy. The numbers of patients in the present study were insufficient to provide data of substantial statistical strength, but these findings...
remain of interest because they are some of the first to be described in terms of the effects of an aromatase inhibitor.

As expected, the two patients (nos. 1 and 7) who were found to have low levels of ER determined biochemically (10.4 and 11.1 fmol/mg, respectively), but who were later found to be negative by immunostaining, did not respond to therapy. These two patients showed expression of the type I growth factor receptors, EGF-R and/or c-erbB2, which is also consistent with the known inverse correlation between these receptors and ER. The lack of effect of anastrozole treatment on ER levels is somewhat surprising, because it is known that estrogen down-regulates its own receptor, and we have previously shown (18) that estrogen deprivation in the MCF7 human xenograft model leads to increased ER expression. The reason for this discrepancy in the human breast cancer data is unclear.

We found a marked decrease of PgR and pS2 expression during treatment. This is consistent with the known estrogen-induced synthesis of these proteins (40, 41). Although our finding that PgR and pS2 were suppressed to the same extent in responders and nonresponders contrasts with observations during treatment with tamoxifen (42), the suppression of Ki67 seems to be more profound in responders compared with nonresponders on both treatments.

The finding of a decrease in the number of apoptotic cells

### Table 1 Patients’ characteristics

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>BMI</th>
<th>TNM</th>
<th>% ER</th>
<th>% PgR</th>
<th>Met.</th>
<th>Resp.</th>
<th>c-erbB-2</th>
<th>EGF-R</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>56</td>
<td>28.0</td>
<td>T1N1M0</td>
<td>2.0</td>
<td>0.0</td>
<td>PD</td>
<td>pos.</td>
<td>pos.</td>
<td>neg.</td>
</tr>
<tr>
<td>2</td>
<td>73</td>
<td>22.4</td>
<td>T1N1M1</td>
<td>98.0</td>
<td>0.0</td>
<td>bone</td>
<td>MC</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td>3</td>
<td>67</td>
<td>30.8</td>
<td>T1N1M1</td>
<td>86.0</td>
<td>53.0</td>
<td>bone</td>
<td>MC</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td>4</td>
<td>67</td>
<td>31.9</td>
<td>T1N1M0</td>
<td>92.0</td>
<td>86.0</td>
<td>MC</td>
<td>MC</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td>5</td>
<td>55</td>
<td>30.5</td>
<td>T1N1M0</td>
<td>87.0</td>
<td>70.0</td>
<td>MC</td>
<td>MC</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td>6</td>
<td>70</td>
<td>29.9</td>
<td>T1N1M0</td>
<td>82.0</td>
<td>7.5</td>
<td>PD</td>
<td>PD</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td>7</td>
<td>58</td>
<td>26.4</td>
<td>T1N1M0</td>
<td>0.0</td>
<td>4.3</td>
<td>MC</td>
<td>MC</td>
<td>pos.</td>
<td>neg.</td>
</tr>
<tr>
<td>8</td>
<td>70</td>
<td>29.2</td>
<td>T1N1M0</td>
<td>93.0</td>
<td>0.0</td>
<td>skin</td>
<td>PR</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td>9</td>
<td>66</td>
<td>25.8</td>
<td>T1N1M0</td>
<td>92.0</td>
<td>79.0</td>
<td>MC</td>
<td>MC</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td>10</td>
<td>80</td>
<td>26.9</td>
<td>T1N1M0</td>
<td>91.0</td>
<td>86.0</td>
<td>MC</td>
<td>MC</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td>11</td>
<td>60</td>
<td>25.4</td>
<td>T1N1M0</td>
<td>n.a.</td>
<td>n.a.</td>
<td>MC</td>
<td>MC</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td>12</td>
<td>65</td>
<td>22.0</td>
<td>T1N1M0</td>
<td>83.0</td>
<td>84.0</td>
<td>MC</td>
<td>MC</td>
<td>neg.</td>
<td>neg.</td>
</tr>
</tbody>
</table>

a BMI, body mass index; TNM, tumor-node-metastasis; Met., metastasis; Resp., response; PD, progressive disease; pos., positive; MC, minimal change; neg., negative; PR, partial response; StD, stable disease; n.a., not available.
b Expressed as percentage of cells staining positively (IHC).
c Staining for c-erbB-2 and EGF-R is given prior to treatment with anastrozole (/) and after 15 weeks (median) on treatment (/x).
d ER levels $\geq 10$ fmol/mg were confirmed by the charcoal method prior to treatment with anastrozole.

### Table 2 Influences of neoadjuvant Arimidex on ER, PgR, Ki67, pS2, and percentage of apoptotic cells (IHC)

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Arimidex (2 wk)</th>
<th>Arimidex (15 wk)</th>
<th>% change (0/15 wk)</th>
<th>Friedman test</th>
<th>P for change (0/2 wk)</th>
<th>P for change (0/15 wk)</th>
<th>P between subgroups</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>73.3</td>
<td>65.9</td>
<td>71.6</td>
<td>-3.4%</td>
<td>0.1210</td>
<td>0.0172</td>
<td>n.s.</td>
</tr>
<tr>
<td>Responders</td>
<td>89.8</td>
<td>86.2</td>
<td></td>
<td>-4.1%</td>
<td></td>
<td></td>
<td>n.s.</td>
</tr>
<tr>
<td>Nonresponders</td>
<td>53.4</td>
<td>57.0</td>
<td></td>
<td>+2.0%</td>
<td></td>
<td></td>
<td>n.s.</td>
</tr>
<tr>
<td>PgR (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>42.7</td>
<td>27.3</td>
<td>5.3</td>
<td>-63.9%</td>
<td>0.0119</td>
<td>0.0625</td>
<td>0.0117</td>
</tr>
<tr>
<td>Responders</td>
<td>48.8</td>
<td>0.0</td>
<td></td>
<td>-66.7%</td>
<td>0.0679</td>
<td>0.0679</td>
<td>n.s.</td>
</tr>
<tr>
<td>Nonresponders</td>
<td>35.4</td>
<td>10.6</td>
<td></td>
<td>-60.7%</td>
<td>0.0679</td>
<td>0.0679</td>
<td>n.s.</td>
</tr>
<tr>
<td>Ki67</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>17.3</td>
<td>11.3</td>
<td>7.7</td>
<td>-63.1%</td>
<td>0.0007</td>
<td>0.0322</td>
<td>0.0033</td>
</tr>
<tr>
<td>Responders</td>
<td>16.9</td>
<td>3.3</td>
<td></td>
<td>-82.8%</td>
<td>0.0277</td>
<td>0.0277</td>
<td>n.s. (0.0679)</td>
</tr>
<tr>
<td>Nonresponders</td>
<td>17.8</td>
<td>12.2</td>
<td></td>
<td>-39.5%</td>
<td>0.0431</td>
<td>0.0431</td>
<td>n.s.</td>
</tr>
<tr>
<td>pS2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>43.6</td>
<td>3.2</td>
<td>16.1</td>
<td>-71.7%</td>
<td>0.0002</td>
<td>0.0051</td>
<td>0.0033</td>
</tr>
<tr>
<td>Responders</td>
<td>50.2</td>
<td>12.5</td>
<td></td>
<td>-77.6%</td>
<td>0.0277</td>
<td>0.0277</td>
<td>n.s.</td>
</tr>
<tr>
<td>Nonresponders</td>
<td>53.8</td>
<td>19.6</td>
<td></td>
<td>-64.7%</td>
<td>0.0431</td>
<td>0.0431</td>
<td>n.s.</td>
</tr>
<tr>
<td>Apoptotic cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>1.3</td>
<td>1.2</td>
<td>1.5</td>
<td>-23.2%</td>
<td>0.4966</td>
<td>0.3863</td>
<td>n.s.</td>
</tr>
<tr>
<td>Responders</td>
<td>1.1</td>
<td>0.7</td>
<td></td>
<td>-42.9%</td>
<td>n.s. (0.0747)</td>
<td>n.s.</td>
<td>0.0446</td>
</tr>
<tr>
<td>Nonresponders</td>
<td>1.6</td>
<td>2.3</td>
<td></td>
<td>+0.5%</td>
<td></td>
<td></td>
<td>n.s.</td>
</tr>
</tbody>
</table>

a ER (%), percentage of tumor cells staining positive.
b n.s., not significant.
among responders but an increase in nonresponders to treatment during anastrozole treatment may seem contradictory. However, a high mitotic activity has been associated with a high apoptotic activity in invasive breast cancer (43). Thus, our finding may indicate that anastrozole decreases the cell turnover in tumors responding to therapy. Our observation is in contrast to the findings made with neoadjuvant chemotherapy in which, responses are in general associated with an induction of apoptosis (19) and with the minor increase in apoptosis seen with antiestrogens after 1–2 weeks treatment (44). Recent data from the use of vorozole, another aromatase inhibitor, also showed a decreased apoptotic index after 2 weeks treatment (45). Thus, effects on apoptosis may be differential between antiestrogens and aromatase inhibitors.

In conclusion, we found that anastrozole given as neoadjuvant therapy causes a profound suppression of tumor levels of E2, E1, and E1S, and that this is associated with a reduction of tumor cell proliferation and of the estrogen-regulated proteins PgR and pS2.

ACKNOWLEDGMENTS

We thank the skillful technical assistance of Dagfinn Ekse in determining plasma hormone levels.

REFERENCES

Influence of Neoadjuvant Anastrozole (Arimidex) on Intratumoral Estrogen Levels and Proliferation Markers in Patients with Locally Advanced Breast Cancer

Jürgen Geisler, Simone Detre, Hildegunn Berntsen, et al.

Clin Cancer Res 2001;7:1230-1236.

Access the most recent version of this article at: http://clincancerres.aacrjournals.org/content/7/5/1230

This article cites 41 articles, 12 of which you can access for free at: http://clincancerres.aacrjournals.org/content/7/5/1230.full.html#ref-list-1

This article has been cited by 21 HighWire-hosted articles. Access the articles at: /content/7/5/1230.full.html#related-urls

Sign up to receive free email-alerts related to this article or journal.

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.