

## Variation in Anastrozole Metabolism and Pharmacodynamics in Women with Early Breast Cancer

James N. Ingle<sup>1</sup>, Aman U. Buzdar<sup>4</sup>, Daniel J. Schaid<sup>2</sup>, Matthew P. Goetz<sup>1</sup>, Anthony Batzler<sup>2</sup>, Mark E. Robson<sup>5</sup>, Donald W. Northfelt<sup>6</sup>, Janet E. Olson<sup>2</sup>, Edith A. Perez<sup>7</sup>, Zeruesenay Desta<sup>8</sup>, Randy A. Weintraub<sup>9</sup>, Clark V. Williard<sup>9</sup>, David A. Flockhart<sup>8</sup>, and Richard M. Weinshilboum<sup>3</sup>

### Abstract

Aromatase inhibitors play a prominent role in the management of postmenopausal women with endocrine-sensitive breast cancer, but there is large variability in both efficacy and tolerability. The purpose of our study was to define interindividual variation in anastrozole metabolism and pharmacodynamics among patients treated with the approved daily dose of 1 mg in a standard practice setting as adjuvant therapy for resected early breast cancer. This study was performed in 191 women in whom pretreatment and during anastrozole plasma concentrations of estrone (E1), estradiol (E2), estrone conjugates, androstenedione, and testosterone were determined and correlated with plasma concentrations of anastrozole and anastrozole metabolites. There were large interindividual variations in plasma anastrozole and anastrozole metabolite concentrations, as well as pretreatment and postdrug plasma E1, E2, and E1 conjugate and estrogen precursor (androstenedione and testosterone) concentrations. E1 and E2 concentrations were below the lower limit of quantitation (LLQ) in most patients after anastrozole therapy (83% for both), but those with detectable concentrations had a broad range (1.58–45.2 and 0.635–97.0 pg/mL, respectively). E1 conjugates after anastrozole therapy were above the LLQ in most patients (93%), with wide interpatient variability (3.50–2,990 pg/mL). Two patients seemed to extensively metabolize anastrozole and failed to display substantial decreases in estrogens. Acknowledging the potential factor of variable compliance, our results showed large interindividual variation in anastrozole metabolism and its effect on circulating estrogens in postmenopausal patients. These findings may have implications with regard to efficacy and adverse events and may indicate the need to “individualize” therapy with this drug. *Cancer Res*; 70(8): 3278–86. ©2010 AACR.

### Introduction

The third generation aromatase inhibitors, anastrozole, exemestane, and letrozole, have become established therapy for postmenopausal women with breast cancer in the advanced disease (1) and adjuvant (2) settings and are a major focus of research in the prevention setting in women at high risk for developing breast cancer (3). An American Society of Clinical Oncology technology assessment panel concluded

that optimal adjuvant therapy for postmenopausal women with receptor-positive breast cancer includes an aromatase inhibitor, either as initial therapy or after treatment with tamoxifen (4).

Anastrozole is a nonsteroidal aromatase inhibitor that was reported to maximally suppress plasma estradiol concentrations at doses of 1 and 10 mg/d, with both doses suppressing estradiol “to the limits of detection used (5).” The drug had a plasma  $\beta$ -phase elimination half-life of 38 to 61 hours (5). Geisler and colleagues (6) reported equipotency of the 1-mg and 10-mg dose levels in terms of aromatase inhibition and suppression of plasma estrogen concentrations. Two clinical trials were performed in which the 1-mg and 10-mg doses were compared with megestrol acetate in patients with advanced breast cancer (7, 8), and a joint analysis of these two trials (9) supported equipotency of 1 and 10 mg, resulting in the endorsement of the 1-mg dose for clinical use. However, in this era of “individualized therapy,” it remains an open question as to whether the single dose currently used is appropriate for all patients. Therefore, in the present study, we set out to determine the nature and extent of anastrozole metabolism and its primary pharmacodynamic effect, i.e., alteration in estrogen precursors and product concentrations, in a large population of postmenopausal breast cancer patients.

**Authors' Affiliations:** <sup>1</sup>Division of Medical Oncology, <sup>2</sup>Department of Health Sciences Research, and <sup>3</sup>Division of Clinical Pharmacology, Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, Minnesota; <sup>4</sup>Department of Breast Oncology, M.D. Anderson Cancer Center, Houston, Texas; <sup>5</sup>Breast Medicine Service, Memorial Sloan Kettering Cancer Center, New York, New York; <sup>6</sup>Division of Hematology/Oncology, Mayo Clinic, Scottsdale, Arizona; <sup>7</sup>Division of Hematology/Oncology, Mayo Clinic, Jacksonville, Florida; <sup>8</sup>Division of Clinical Pharmacology, Indiana University School of Medicine, Indianapolis, Indiana; and <sup>9</sup>Taylor Technology, Inc., Princeton, New Jersey

**Corresponding Author:** James N. Ingle, Mayo Clinic, 200 First Street Southwest, Rochester, MN 55905. Phone: 507-284-8432; Fax: 507-284-1803; E-mail: [ingle.james@mayo.edu](mailto:ingle.james@mayo.edu).

doi: 10.1158/0008-5472.CAN-09-3024

©2010 American Association for Cancer Research.

Anastrozole was the first aromatase inhibitor to receive Food and Drug Administration approval for use in the adjuvant setting to treat women with early-stage breast cancer. It has shown value in the initial therapy setting (10), after 2 to 3 years of tamoxifen (11–13) and in the extended adjuvant setting after 5 years of tamoxifen (14). However, adherence to treatment seems to be an important issue with the use of anastrozole. Adherence to anastrozole therapy, defined as having drug available for >80% of days, decreased to 62% to 79% after 3 years (15). Thus, it seems that a substantial proportion of women may be suboptimally adherent to anastrozole therapy, a finding that would be expected to be associated with suboptimal efficacy.

Although anastrozole has shown clear efficacy and superiority relative to tamoxifen (10), many patients experience a recurrence of their cancer. In addition, there is substantial interindividual variability with respect to tolerability; and musculoskeletal complaints can be so severe that some patients withdraw from therapy. This variability is consistent with possible differences among patients in drug pharmacokinetics, especially metabolism, and/or pharmacodynamics, factors that, if understood, would offer the potential for individualizing therapy and ensuring that patients would receive optimal therapy.

This study describes changes in plasma concentrations of hormones and of anastrozole and its metabolites in a cohort of 191 patients taking anastrozole 1 mg/d. We observed striking interindividual variation in both plasma anastrozole and anastrozole metabolite concentrations with equally striking variation in changes in estrogen and estrogen precursor concentrations after anastrozole therapy.

## Materials and Methods

**Patients studied.** This clinical study enrolled postmenopausal women who were to receive anastrozole as adjuvant therapy for resected early-stage breast cancer at Mayo Clinic and the M.D. Anderson Cancer Center. Eligibility criteria included age of at least 18 y, postmenopausal status, breast cancer stage I, II, or III according to American Joint Committee on Cancer (AJCC) Staging Manual (Sixth Edition), a tumor that was estrogen receptor (ER) positive and/or progesterone receptor (PgR) positive and a planned treatment with anastrozole at the clinically approved dose of 1 mg/d. Patients could have received prior tamoxifen, but other prior endocrine therapy was not permitted. None of the patients were receiving hormone replacement therapy.

Two weeks or less before starting anastrozole, a blood sample was obtained for the acquisition of DNA and for pretreatment hormone measurements. A second blood draw for hormone measurements and anastrozole and anastrozole metabolite concentrations in plasma was scheduled for 4 wk to 6 mo after initiation of anastrozole, i.e., long enough to allow steady-state of anastrozole to be achieved. Patients were instructed not to take their dose of anastrozole for that day until after the blood was drawn. This trial was performed after approval by local institutional review boards in accor-

dance with assurances filed with and approved by the U.S. Department of Health and Human Services. Written informed consent was provided by each patient before entry on study.

**Anastrozole and metabolite assays.** Anastrozole and metabolite assays involved the extraction of plasma, followed by liquid chromatography/tandem mass spectrometry (LC/MS/MS) assay. To assess the potential role of conjugation in the metabolism of anastrozole and/or its metabolites, total concentrations (free + conjugated) of anastrozole and its hydroxylated metabolite were measured after incubation of plasma samples with  $\beta$ -glucuronidase ( $\beta$ -glucuronidase type H-5 from *Helix pomata*, Sigma-Aldrich). Specifically, 250  $\mu$ L of plasma were incubated for 18 h with 20  $\mu$ L of 1,000 units/mL  $\beta$ -glucuronidase, 200  $\mu$ L of 200 mmol/L acetate buffer, and 10  $\mu$ L of 600 mmol/L sodium azide. Because the  $\beta$ -glucuronidase type H-5 extracted from *Helix pomata* also contained sulfatase, we could not reliably distinguish between glucuronide and sulfate conjugates. Therefore, we report the data here as “conjugated” anastrozole or “conjugated” hydroxy-anastrozole. To obtain the free (unconjugated) concentrations of anastrozole and its hydroxylated metabolite, we used the same incubation approach, except that the samples were incubated without  $\beta$ -glucuronidase. The difference between the total and free is reported as conjugate drug or metabolite. After incubation, the internal standard (desmethyldiazepam) was added to 250  $\mu$ L of plasma, and the sample was extracted with ethyl acetate at alkaline pH (0.5 mL of 0.5 mol/L NaOH/glycine buffer, adjusted to pH 10). The sample was vortex-mixed and centrifuged for 15 min at 2,500 rpm in a Beckman GS-6R centrifuge. The organic layer was then removed and evaporated to dryness. The residue was reconstituted with 50  $\mu$ L of 0.1% formic acid in water, and 25  $\mu$ L were injected onto the LC/MS/MS system.

The LC/MS/MS assay system consisted of an LC-20AB pump with a SIL-20A HT autosampler (Shimadzu) and an API 2000 LC/MS/MS triple quadrupole system (Applied Biosystems) with an electrospray ion source. The separation system consisted of a 100  $\times$  2 mm Luna 3  $\mu$  C18 (2) 100A column (Phenomenex) with a mobile phase that was degassed in a sonicator for 15 min. The mobile phase was composed of 50% of 0.1% formic acid in water and 50% of acetonitrile. The mobile-phase flow rate was 0.15 mL/min. The multiple reaction monitoring (MRM) analysis was completed in positive mode for the entire run. The nitrogen nebulizer gas and curtain gas were both set at 20 p.s.i. Dwell time was set at 400 ms, and the internal voltage was set at 5,200 in positive mode with a temperature of 450°C. Two positive transitions were used for anastrozole (+294/225 and +294/115), hydroxy-anastrozole (+310.2/241.5 and +310.2/214.5), and the internal standard desmethyldiazepam (+271/140 and +271/208). Anastrozole and hydroxy-anastrozole were measured with the quantifier MRM and confirmed with the qualifier MRM transition.

Plasma concentrations of anastrozole were quantified using the ratio of area under the curve (AUC) of anastrozole to AUC of the internal standard and calibration curves that were constructed by spiking blank plasma with

known amounts of anastrozole. The limit of detection was 50 pg/mL, and the limit of quantification was 100 pg/mL. Because an authentic standard of hydroxy-anastrozole was not available, this metabolite was quantified using standard curves generated with anastrozole. The limitation of this approach is that the MS/MS properties of the metabolite and the parent compound may be different as a result of altered chromophore. Therefore, actual concentrations of hydroxy-anastrozole could not be established precisely, and concentrations of that metabolite presented in this paper should be viewed as “apparent” concentrations (arbitrary units/mL plasma). A detailed description of the LC/MS/MS assay and metabolite identification will be published separately.

**Hormone assays.** A validated bioanalytic method using gas chromatography negative ionization tandem mass spectrometry was used to measure physiologically relevant concentrations of the following steroids from 1.0 mL of human plasma, with lower limits of quantitation (LLQ): estrone (E1), 1.56 pg/mL; estradiol (E2), 0.625 pg/mL; testosterone, 25.0 pg/mL; androstenedione, 25.0 pg/mL; and estrone conjugates, 3.13 pg/mL sulfate and 4.15 pg/mL glucuronide. Standards and internal standards used were >98% pure and purchased from Steraloids, U.S. Pharmacopeia, Sigma-Aldrich, or CDN Isotopes. For each batch of samples analyzed, two standard curves for each analyte (front and back, eight concentration levels) were prepared in water and qualified with quality control samples (two replicates at low, mid, and high levels) prepared in charcoal-stripped plasma. Analytic runs were accepted when >75% of standards had back-calculated concentrations within  $\pm 15\%$  of nominal, except at the LLQ, wherein  $\pm 20\%$  of nominal concentrations was accepted. In addition, at least 67% of the quality control samples met accuracy requirements of being within  $\pm 15\%$  of their nominal concentrations. For some results, the LLQ was higher, based on the assay conditions. For this study, a mean LLQ was calculated as 1.64 pg/mL for E1, 0.66 pg/mL for E2, and 6.04 pg/mL for E1 conjugates.

Briefly, the analytes and their deuterated internal standards were extracted from 1 mL of plasma using Bond Elut Certify (Varian) solid-phase extraction cartridges. Estrone conjugates were eluted from the cartridges with water/ acetonitrile (75:25, v/v), dried down, and hydrolyzed to estrone using Glusulase ( $\beta$ -glucuronidase and sulfatase, NEN Research Products). The unconjugated analytes were then eluted with ethyl acetate. Estrogens were derivatized with pentafluorobenzoyl chloride and *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA); the androgens were derivatized with *O*-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine and MSTFA. All solvents and reagents were purchased from EMD Science or Sigma-Aldrich. The derivatized analytes were separated on a Varian 3400 gas chromatograph equipped with a DB-17 fused silica capillary column (15 m  $\times$  0.025 mm, J&W Scientific) and quantified using an interfaced Finnigan MAT TSQ-700 mass spectrometer operating in single-ion monitoring tandem mass spectrometry negative ion chemical ionization mode.

**Statistical methods.** To measure correlation of two quantitative variables, we used the Spearman rank correlation co-

**Table 1. Patient characteristics ( $n = 191$ )**

Age, y	
Median	60
Range	39–82
Race	
American Indian or Alaska Native	1 (1%)
Asian	5 (3%)
Black or African American	11 (6%)
Native Hawaiian or other Pacific Islander	0
White	162 (85%)
Unknown	12 (6%)
Ethnicity	
Hispanic or Latino	15 (8%)
Non-Hispanic or Latino	160 (84%)
Unknown	16 (8%)
BMI	
Median	27.5
Range	17.7–45.1
AJCC tumor stage*	
I	102 (54%)
II	60 (32%)
III	28 (15%)
ER/PgR status	
Positive/positive	158 (83%)
Positive/negative	30 (16%)
Negative/positive	3 (2%)
HER2 status	
Negative	149 (78%)
Positive	36 (19%)
Unknown	6 (3%)
Smoking status	
Never smoked	116 (61%)
Ever smoked	68 (36%)
Current smoker	14 (7%)
Missing data	7 (4%)
Prior chemotherapy	
Yes	81 (42%)
No	110 (58%)
Prior tamoxifen	
Yes	21 (11%)
No	170 (89%)
Time from starting anastrozole to second blood draw (wk)	
4–6	9 (5%)
>6–9	25 (13%)
>9–12	30 (16%)
>12–18	87 (46%)
>18–24	20 (10%)
>24	20 (10%)

\*AJCC Sixth Edition; one patient was T1NxM0.

efficient, a method robust to outliers. To evaluate whether clinical variables were statistically associated with baseline hormone concentrations and to evaluate whether anastrozole or any of its metabolites were statistically associated

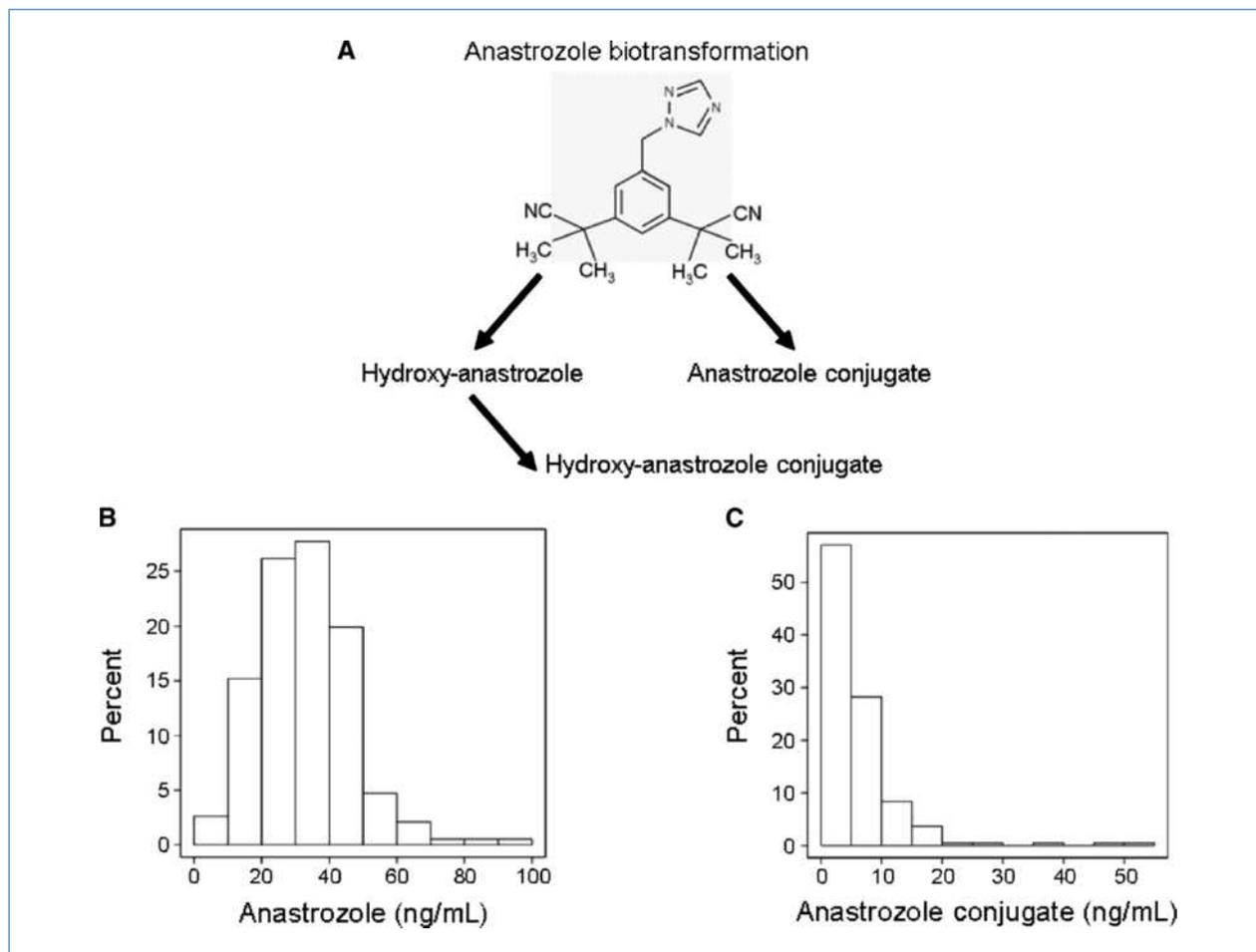
with changes in hormone concentrations as well as whether any clinical variables were associated with changes in hormone concentrations, we used linear regression methods. The hormone concentrations (pretreatment or change measured as posttreatment minus pretreatment) were regressed in a stepwise fashion on the following clinical/demographic variables [age at treatment, body mass index (BMI), smoking status, days from initial drug administration to blood draw, Mayo versus M.D. Anderson recruitment site, tumor T size, nodal status, ER status, PgR status, HER2 status, prior chemotherapy, and prior tamoxifen]. Changes in concentration were also regressed on pretreatment hormone level and drug levels (anastrozole, anastrozole conjugates, hydroxy-anastrozole, and hydroxy-anastrozole conjugates). Stepwise selection proceeded in a forward-backward manner, using a *P* value of 0.05 to retain a predictor variable in the model. Because some variables were highly skewed with outliers (e.g., hormone concentrations), we used Winsorized variables by replacing extreme values (>3 SD from the mean) with values exactly at 3 SD from the mean. This robust approach uses

more information than ranked data yet is less sensitive to outliers than the original data.

## Results

**Evaluable patients.** Steady-state plasma anastrozole and anastrozole metabolite trough concentrations were determined in 196 patients while chronically on a 1 mg/d dosage. Five patients were excluded from the analyses: one had no detectable plasma anastrozole or anastrozole metabolite (despite reporting that she was taking the drug), three patients had the second blood draw obtained <4 weeks after the initiation of therapy, and one patient was excluded because of technical problems with the comparison hormone assays. Thus, 191 patients were evaluable in these analyses, and their characteristics are listed in Table 1.

**Plasma anastrozole and anastrozole metabolite concentrations.** The three major metabolites detected in plasma were anastrozole conjugates, hydroxy-anastrozole and hydroxy-anastrozole conjugates (Fig. 1A). The median



**Figure 1.** Steady-state plasma anastrozole and anastrozole conjugate concentrations in breast cancer patients treated with 1 mg/d oral dose of anastrozole. A, observed anastrozole metabolism. Frequency distribution histograms for levels of anastrozole (B) and anastrozole conjugates (C).

plasma concentration of free anastrozole was 32.2 ng/mL, with a range from 0.0 to 98.8 ng/mL. Two patients without detectable anastrozole were included in the analysis, because they had measurable hydroxy-anastrozole and hydroxy-anastrozole conjugates. These patients will be discussed in more detail subsequently. The frequency distribution for anastrozole concentrations shown in Fig. 1B shows the wide variation among patients.

The median plasma concentration of anastrozole conjugates was 4.2 ng/mL (range, 0.0–54.4 ng/mL), and the frequency distribution shown in Fig. 1C shows wide variation in their plasma concentrations. Anastrozole and anastrozole conjugate concentrations were not statistically correlated (Spearman correlation, 0.10;  $P = 0.18$ ).

The majority (over 80%) of the hydroxy-anastrozole was recovered as conjugates. As noted previously, a lack of internal standards prevented absolute quantitation of these two metabolites, but there was a 29-fold range in hydroxy-anastrozole conjugate concentrations. Hydroxy-anastrozole and hydroxy-anastrozole conjugates were positively correlated (Spearman correlation, 0.63;  $P < 0.001$ ), implying that subjects with higher hydroxylated metabolites formed more conjugates. However, two outliers had high hydroxy-anastrozole but low hydroxy-anastrozole conjugates, indicating a possible deficiency in their ability to catalyze the conjugation reaction for the hydroxylated metabolite. Conversely—and more important clinically—two different patients had undetectable anastrozole concentrations, very high anastrozole conjugate concentrations, and very little drug response in terms of change in their estrogen hormone levels. These observations raised the possibility that these latter two patients might represent “ultrarapid” conjugators of the drug and, as a result, might fail to have the desired therapeutic response. These latter two patients will be discussed in greater detail subsequently.

There was no statistical association between time to second blood draw and anastrozole concentration (Spearman correlation,  $-0.004$ ;  $P = 0.95$ ) or for anastrozole conjugate concentration (Spearman correlation,  $0.07$ ;  $P = 0.36$ ). However, there were significant correlations between time to second blood draw and hydroxy-anastrozole (Spearman correlation,  $0.18$ ;  $P = 0.008$ ) and hydroxy-anastrozole conjugate (Spearman correlation,  $-0.20$ ;  $P = 0.005$ ) concentrations.

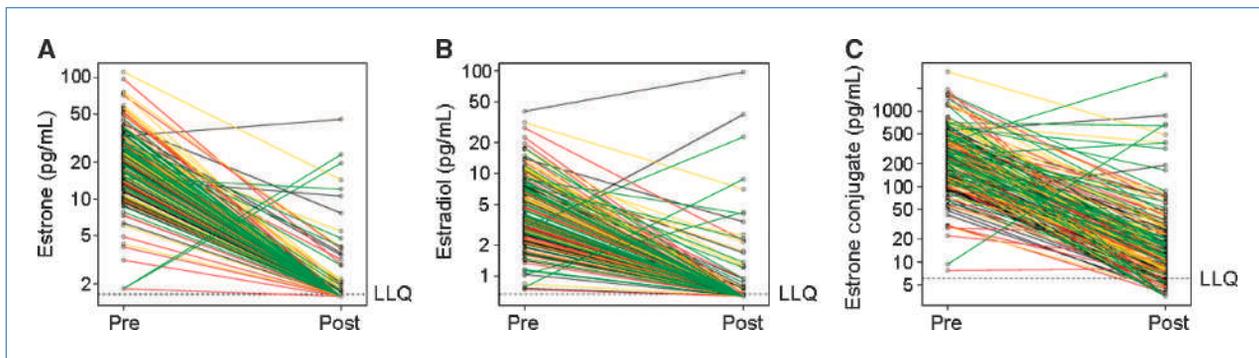
**Estrone, estradiol, and estrone conjugate concentrations pretreatment and after anastrozole therapy.** Pretreatment and posttreatment plasma levels of E1, E2, E1 conjugates, androstenedione, and testosterone are listed in Table 2. Pretreatment levels for all of these hormones showed substantial variability. Among patients considered to be clinically postmenopausal by their oncologist, 28 (15%) had E2 levels of  $>10$  pg/mL, the conventional concentration separating premenopausal from postmenopausal women, with a range of 10.2 to 40.3 pg/mL (median, 13.55 pg/mL). Sixteen of these patients had been entered from Mayo and 12 from M.D. Anderson. The median age of these 28 patients was 58.5 years, with a range from 47 to 80 years. Only 1 patient in this group had received prior tamoxifen, 9 (32%) had received prior chemotherapy, and 15 (54%) were active smokers. The median BMI for these 28 patients was 36.3 (range, 19.9–45.0), and

**Table 2.** Pretreatment hormone levels (pg/mL) and during anastrozole therapy

	Pretreatment (n = 191)	During anastrozole (n = 191)
<b>Estrone</b>		
No. patients	189	191
Missing	2	0
Mean (SD)	23.2 (15.64)	1.0 (4.28)
Median	19.9	0.0
Range	0.0–111.0	0.0–45.2
<b>Estradiol</b>		
No. patients	188	189
Missing	3	2
Mean (SD)	5.8 (5.41)	1.1 (7.75)
Median	3.9	0.0
Range	0.0–40.3	0.0–97.0
<b>Estrone conjugates</b>		
No. patients	189	190
Missing	2	1
Mean (SD)	340.5 (386.78)	53.4 (239.15)
Median	226.0	12.1
Range	7.7–3320.0	0.0–2990.0
<b>Androstenedione</b>		
No. patients	189	191
Missing	2	0
Mean (SD)	515.8 (310.85)	573.3 (303.73)
Median	451.0	486.0
Range	0.0–2470.0	0.0–1560.0
<b>Testosterone</b>		
No. patients	187	191
Missing	4	0
Mean (SD)	183.4 (134.53)	183.1 (116.92)
Median	151.0	160.0
Range	0.0–1120.0	0.0–678.0

9 (32%) had a BMI of  $>40.0$ . All but one of these patients had a decrease in their E2 levels after anastrozole therapy, with 18 (64%) dropping to undetectable levels.

**Relationship of anastrozole concentrations to estrone, estradiol, and estrone conjugate concentrations.** Figure 2A–C displays changes in E1, E2, and E1 conjugate concentrations, color-coded for quartile of anastrozole level. Figure 2A shows that only a small proportion of the patients (17%) had E1 concentrations above the LLQ while on anastrozole, with a median of 2.87 pg/mL but a very wide range (1.58–45.2 pg/mL). Likewise for E2, Fig. 2B shows that only a small proportion of patients (17%) had concentrations above the LLQ on anastrozole, with a median of 1.26 pg/mL but once again with a very wide range (0.65–97.0 pg/mL). The findings with respect to E1 conjugates were quite different, with the vast majority of patients (93%) having levels above the LLQ with a median of 12.95 pg/mL but an exceedingly wide range (3.50–2990 pg/mL).

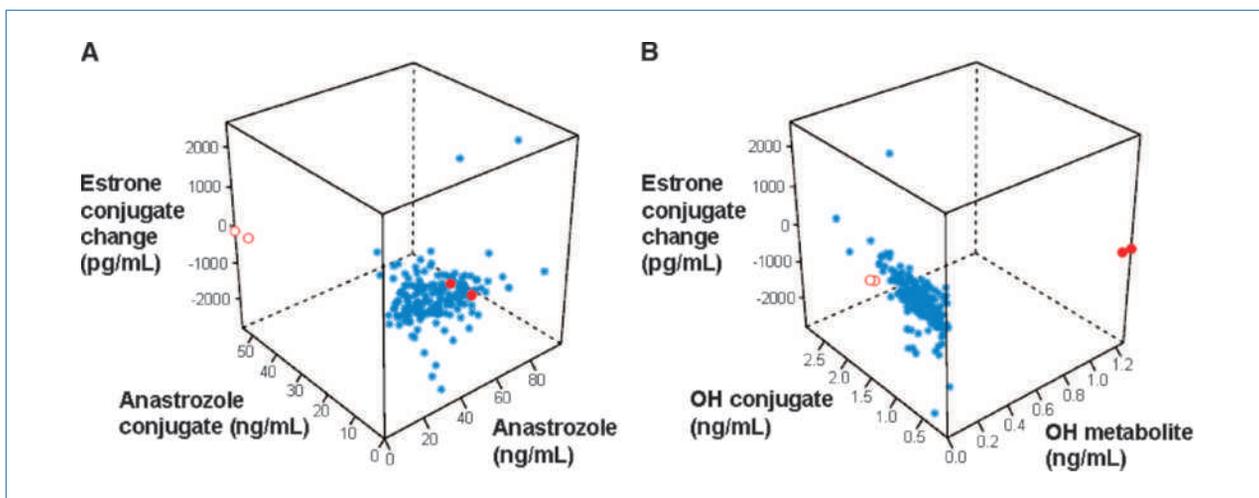


**Figure 2.** Plasma concentrations of estrone (A), estradiol (B), and estrone conjugates (C) according to quartile of anastrozole concentration in breast cancer patients before and after treatment with 1 mg/d oral dose of anastrozole. Key for line color: black, lowest quartile; red, second quartile; yellow, third quartile; green, highest quartile.

As anticipated, the majority of patients experienced a drop in E1, E2, and E1 conjugate concentrations after anastrozole therapy, but unexpected increases were identified in three patients (2%) for E1, in five patients (3%) for E2, and in six patients (3%) for E1 conjugates (Fig. 2A, B, and C). Eight patients (4%) had an increase in at least one of the estrogenic compounds (E1, E2, and E1 conjugates), and the ages of those patients were 47, 50, 52, 52, 57, 57, 58, and 66 years, indicating that the increases did not occur only in the younger postmenopausal women. All eight patients had follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels in the postmenopausal range at the time of the increase in one of the estrogenic compounds. The median BMI in these eight patients was 25.2, with a range of 18.3 to 38.7. The BMIs for these eight patients were not significantly different from the other 183 patients in this study (Wilcoxon  $P = 0.20$ ). As can be seen in Fig. 2, the majority of patients with an increase in the level of at least one of these estrogenic

compounds fell within the highest quartile for anastrozole concentrations.

The statistical association of clinical variables with pretreatment concentrations of E1, E2, and E1 conjugate, as evaluated by stepwise regression, showed that BMI was positively correlated with all three hormone concentrations (in addition, smoking status was associated with E1 and stage and age were associated with E1 conjugate). Hence, it was critical to adjust for clinical variables when evaluating the association of anastrozole concentrations with changes in E1, E2, and E1 conjugate concentrations. In addition to clinical variables, pretreatment hormone levels and anastrozole concentration were evaluated in stepwise regression. This allowed us to evaluate the contribution of each variable, adjusted for the others, in case variables might be correlated (such as pretreatment hormone level and BMI). In no instance was the level of anastrozole statistically associated with change in E1, E2, or E1 conjugates, although changes



**Figure 3.** Correlation of anastrozole and anastrozole conjugates and changes in estrone conjugates (A) and correlation of hydroxy-anastrozole and hydroxy-anastrozole conjugates and changes in estrone conjugates (B) in breast cancer patients treated with 1 mg/d oral dose of anastrozole. Red open and closed circles described in text.

were often associated with BMI and pretreatment hormone concentrations. However, whereas this was true of the entire group, there were two outliers in whom the lack of detectable anastrozole was associated with the lack of change in E1 conjugates (see subsequent section).

Similar to the regression analyses for change in hormone concentrations, we also evaluated actual posttreatment concentrations. In no instance was the concentration of anastrozole statistically associated with the final concentrations of E1, E2, or E1 conjugates.

**Relationships among anastrozole, anastrozole conjugate, and estrone conjugate concentrations.** Figure 3A displays the relationship among anastrozole, anastrozole conjugate, and E1 conjugate concentrations. Two patients (red open circles) had extremely low concentrations of anastrozole but very high levels of anastrozole conjugates. Those same two patients showed a relatively small change in plasma estrone conjugate levels after receiving anastrozole, i.e., a decrease by 58 and 124 pg/mL, which are substantially less than the median decrease of 208 pg/mL. These observations increase the possibility that these two patients might represent “ultrapid” conjugators of anastrozole, accounting for the low parent drug concentrations and relatively small changes in hormone levels after drug. Neither of these patients was a current smoker, and neither were being treated with drugs known to induce microsomal drug-metabolizing enzymes.

Figure 3B displays the relationship among hydroxy-anastrozole, hydroxy-anastrozole conjugate, and E1 conjugate concentrations. Two different patients (red closed circles) displayed high levels of hydroxy-anastrozole but low hydroxy-anastrozole conjugates, which increases the possibility of a relative decrease in the ability to conjugate the hydroxy metabolite. The E1 conjugates in these two patients decreased by 234 and 214 pg/mL, which are near the median change of -208 pg/mL, as anticipated, because they had measurable concentrations of the parent drug.

**Androstenedione and testosterone concentrations before treatment and after anastrozole therapy.** Figure 4A displays the relationship between pretreatment and post-

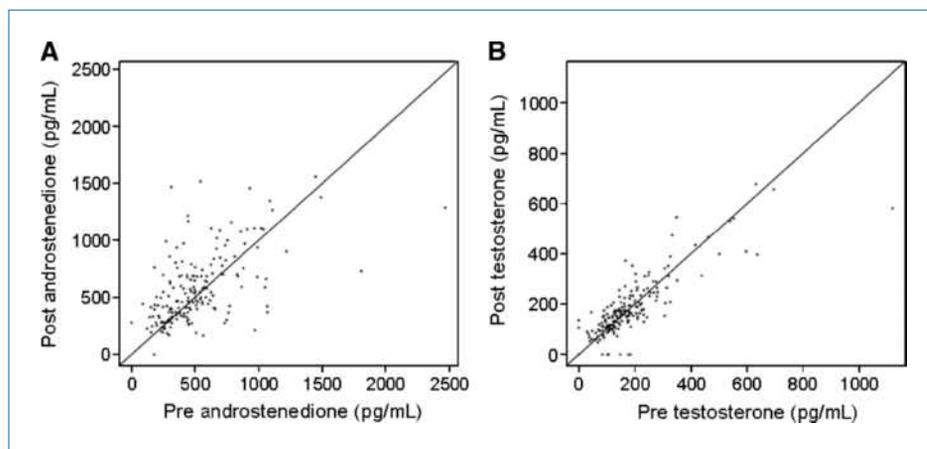
treatment androstenedione concentrations. Substantial variability is evident in pretreatment androstenedione concentrations but with no consistent change in androstenedione concentrations after treatment with anastrozole. In 187 patients, 55% showed an increase and 43% showed a drop in androstenedione after treatment with anastrozole.

Figure 4B displays the relationship between pretreatment and posttreatment testosterone concentrations. Again, there was substantial variability in pretreatment testosterone concentrations. In 189 patients, 61% showed an increase and 38% showed a drop in testosterone after treatment with anastrozole. Finally, there was not a consistent relationship between the changes in androstenedione and testosterone concentrations after anastrozole therapy (data not shown).

## Discussion

This study examined the metabolism and pharmacodynamics of anastrozole when given at the approved 1-mg daily dose as adjuvant endocrine therapy in a standard practice setting in two large oncology centers. The most striking observations were the degree of variation of pretreatment hormone levels; the degree of variation of change in concentrations of E1, E2, and E1 conjugates after anastrozole therapy; and the degree of variation of anastrozole and anastrozole metabolite concentrations in these women. To our knowledge, this is the largest study of this type, and it provides a “real-life” view into the use of anastrozole in women with early stage breast cancer.

Anastrozole and anastrozole metabolite concentrations, like hormone concentrations, also revealed substantial variability, with steady-state concentrations of anastrozole ranging from 0 in two patients with detectable anastrozole metabolites to 98.8 ng/mL. Three major metabolites were detected with wide variations in anastrozole conjugate, hydroxy-anastrozole, and hydroxy-anastrozole conjugate concentrations. The 29-fold range in the concentrations of hydroxy-anastrozole conjugates illustrates this wide variability. It is assumed that it is the parent drug (anastrozole) that has activity as an inhibitor of aromatase, but to our



**Figure 4.** Concentrations of androstenedione (A) and testosterone (B) in breast cancer patients before and during treatment with 1 mg/d oral dose of anastrozole.

knowledge, there are no data regarding anastrozole metabolites. The patterns of the histograms for the steady-state free anastrozole and conjugated anastrozole plasma concentrations (Fig. 1) are very different, suggesting marked variation in metabolism of anastrozole to its conjugates. The marked variability in anastrozole levels clearly indicates that the current "one size fits all" approach to anastrozole dosing may need to be reevaluated.

Pharmacodynamic studies also showed large variation. Most striking was the fact that eight patients (4%) had an increase in at least one of the estrogenic compounds (E1, E2, and E1 conjugates) after drug exposure. Although all of the patients in this group had detectable anastrozole concentrations, the majority of patients with an increase in estrogen concentrations were in the highest quartile for anastrozole concentration. The explanation for this observation is unclear. The FSH and LH levels were in the postmenopausal range in all eight patients at the time of the increase in one of the estrogenic compounds, and the ages of four of the patients were 57 to 66 years of age. Specimen mislabeling must always be considered a possibility, but the specimens were collected concurrently. It is of note that a recent report (16) found that 4 of 66 women treated with anastrozole, the aromatase inhibitor used in our study, had decreased E2 levels at 3 months but an increase in E2 at 6 months, and two additional patients with decreased E2 levels at 3 and 6 months had an increased E2 level at 9 months while receiving anastrozole.

The other finding of note with respect to the pharmacodynamic effects of anastrozole was the variability observed in decreases of the E1, E2, and E1 conjugates. Patients varied from those having profound reductions from relatively high pretreatment levels to undetectable concentrations to those who displayed more modest decreases, with post-anastrozole levels remaining in the detectable range. Given the increased appreciation of variation in the clinical tolerability of aromatase inhibitors, in general, and anastrozole, in particular, these observations raise the possibility that the degree of change in estrogen concentrations, rather than the final concentrations, may be related to a woman's tolerance and adherence to the drug and to toxicity, such as musculoskeletal adverse events (15, 17).

Examination of the relationship among anastrozole, anastrozole metabolites, and change in estrone conjugates revealed two patients with very low anastrozole and very high anastrozole conjugates but relatively small changes in estrone conjugates after drug treatment (Fig. 3A, red open circles). The relationship between low anastrozole with small decreases in estrone conjugates is consistent with the fact that anastrozole is the active inhibitor of aromatase. However, the very high levels of anastrozole conjugates in these patients raise the possibility that these two patients may have had elevated activity of phase II enzymes that conjugate, thereby inactivating anastrozole. If this possibility can be confirmed, it would indicate that the metabolism of anastrozole in some patients results in their being denied optimal therapy. These findings indicate that future studies should determine the drug metabolizing enzymes that cata-

lyze anastrozole hydroxylation and conjugation. Two other patients displayed high hydroxy-anastrozole concentrations, low hydroxy-anastrozole conjugates, and above average anastrozole levels (Fig. 3B and A, red closed circles). These two patients, as expected for subjects with adequate parent drug, displayed decreases in plasma estrogen, but they may have a relative deficiency in their ability to conjugate hydroxy-anastrozole. This latter observation would not be expected to have clinical management implications, as these metabolites are assumed to be pharmacologically inactive.

The sample of patients studied here showed variability in terms of age (range, 39–82 years), BMI (range, 17.7–45.1), smoking status, prior chemotherapy and, to a minor extent, prior tamoxifen, and ethnicity/race. Fifteen percent of the women had E2 levels of >10 pg/mL, whereas a level of <10 pg/mL is generally considered characteristic of postmenopausal women. The median age of these women was 58.5 years (range, 47–80); as a group, they were more overweight (median BMI, 36.3) than the remainder of the patients, and slightly over half were active smokers. Despite having E2 concentrations above the conventional postmenopausal level, all but one patient had a drop in E2 concentrations after anastrozole, with almost two thirds dropping to undetectable levels. These results suggest that the 10 pg/mL level may not be a definitive cutoff for defining postmenopausal women, especially because the age of these patients was up to 80 years. It can be speculated that the high levels of obesity seen in this group, with one third having a BMI of >40, may have contributed to these observations.

In summary, our study of anastrozole therapy at the approved daily dose of 1 mg has revealed substantial variability in both drug metabolism and drug effect in a large sample of women with early breast cancer. We acknowledge that variable compliance must be considered a potential factor, but it is clear that variability exists in both drug metabolism and drug effect. The variability observed suggests that this commonly used agent for the treatment of breast cancer is a prime candidate for pharmacogenomic studies aimed at identifying genetic variation in drug metabolism. The results of those studies might help to make it possible to move toward the goal of truly "individualized" anastrozole therapy.

## Disclosure of Potential Conflicts of Interest

D.A. Flockhart: commercial research grant, Pfizer and Novartis. J.N. Ingle: consultant/advisory board, Novartis and AstraZeneca.

## Grant Support

NIH grants U01 GM61388 and U01 GM61373 (Pharmacogenetics Research Network), K24RR020815, P50 CA166201 (Mayo Clinic Breast Cancer Specialized Program of Research Excellence), R01 GM28157, and a PhRMA Foundation "Center of Excellence in Clinical Pharmacology" Award. ClinicalTrials.gov study number NCT00283608.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 08/13/2009; revised 01/22/2010; accepted 01/27/2010; published OnlineFirst 04/06/2010.

## References

- Ingle JN, Suman VJ. Aromatase inhibitors for therapy of advanced breast cancer. *J Steroid Biochem Molec Biol* 2005;95:113–9.
- Ingle JN. Adjuvant endocrine therapy for postmenopausal women with early breast cancer. *Clin Cancer Res* 2006;12:1031–6S.
- Ingle JN. Endocrine therapy trials of aromatase inhibitors for breast cancer in the adjuvant and prevention settings. *Clin Cancer Res* 2005;11:900–5S.
- Winer EP, Hudis C, Burnstein HJ, et al. American Society of Clinical Oncology Technology assessment on the use of aromatase inhibitors as adjuvant therapy for postmenopausal women with hormone receptor-positive breast cancer: status report 2007. *J Clin Oncol* 2005;23:619–29.
- Plourde PV, Dyroff M, Dukes M. Arimidex®: a potent and selective fourth-generation aromatase inhibitor. *Breast Cancer Res Treat* 1994;30:103–11.
- Geisler J, King N, Dowsett M, et al. Influence of anastrozole (Arimidex), a selective, non-steroidal aromatase inhibitor, on *in vivo* aromatisation and plasma oestrogen levels in postmenopausal women with breast cancer. *Br J Cancer* 1996;74:1286–91.
- Jonat W, Howell A, Blomqvist C, et al. A randomized trial comparing two doses of the new selective aromatase inhibitor anastrozole (Arimidex) with megestrol acetate in postmenopausal patients with advanced breast cancer. *Eur J Cancer* 1996;32:404–12.
- Buzdar AU, Jones SE, Vogel CL, et al. A phase III trial comparing anastrozole (1 and 10 milligrams), a potent and selective aromatase inhibitor, with megestrol acetate in postmenopausal women with advanced breast carcinoma. *Cancer* 1997;79:730–9.
- Buzdar A, Jonat W, Howell A, et al. Anastrozole versus megestrol acetate in the treatment of postmenopausal women with advanced breast carcinoma. *Cancer* 1998;83:1142–52.
- The Arimidex, Tamoxifen, Alone or in Combination (ATAC) Trialists' Group. Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 100-month analysis of the ATAC trial. *Lancet Oncol* 2008;9:45–53.
- Kaufmann M, Jonat W, Hilfrich J, et al. Improved overall survival in postmenopausal women with early breast cancer after anastrozole initiated after treatment with tamoxifen compared with continued tamoxifen: the ARNO 95 study. *Clin Oncol* 2007;25:2664–70.
- Jakesz R, Jonat W, Gnant M, et al. Switching of postmenopausal women with endocrine-responsive early breast cancer to anastrozole after 2 years' adjuvant tamoxifen: combined results of ABCSG trial 8 and ARNO 95 trial. *Lancet* 2005;366:455–62.
- Boccardo F, Rubagotti A, Puntoni M, et al. Switching to anastrozole versus continued tamoxifen treatment of early breast cancer: preliminary results of the Italian Tamoxifen Anastrozole Trial. *J Clin Oncol* 2005;23:5138–47.
- Jakesz R, Greil R, Gnant M, et al. Extended adjuvant treatment with anastrozole: results from the Austrian Breast and Colorectal Cancer Study Group Trial 6a. *J Natl Cancer Inst* 2007;99:1845–53.
- Partridge AH, LaFountain A, Mayer E, Taylor BS, Winer E, Asnis-Alibozek A. Adherence to initial adjuvant anastrozole therapy among women with early-stage breast cancer. *J Clin Oncol* 2008;26:556–62.
- Nagao T, Kira M, Takahashi M, et al. Serum estradiol should be monitored not only during the peri-menopausal period but also the post-menopausal period at the time of aromatase inhibitor administration. *World J Surg Oncol*. Epub 2009 Nov 12.
- Burstein HJ. Aromatase inhibitor-associated arthralgia syndrome. *Breast* 2007;16:223–34.

# Cancer Research

The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

## Variation in Anastrozole Metabolism and Pharmacodynamics in Women with Early Breast Cancer

James N. Ingle, Aman U. Buzdar, Daniel J. Schaid, et al.

*Cancer Res* 2010;70:3278-3286. Published OnlineFirst March 30, 2010.

**Updated version** Access the most recent version of this article at:  
doi:[10.1158/0008-5472.CAN-09-3024](https://doi.org/10.1158/0008-5472.CAN-09-3024)

**Cited articles** This article cites 16 articles, 3 of which you can access for free at:  
<http://cancerres.aacrjournals.org/content/70/8/3278.full#ref-list-1>

**Citing articles** This article has been cited by 4 HighWire-hosted articles. Access the articles at:  
<http://cancerres.aacrjournals.org/content/70/8/3278.full#related-urls>

**E-mail alerts** [Sign up to receive free email-alerts](#) related to this article or journal.

**Reprints and Subscriptions** To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at [pubs@aacr.org](mailto:pubs@aacr.org).

**Permissions** To request permission to re-use all or part of this article, use this link  
<http://cancerres.aacrjournals.org/content/70/8/3278>.  
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.