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Effects of exemestane and letrozole therapy on plasma concentrations of estrogens in a randomized trial of postmenopausal women with breast cancer

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Abstract

Purpose—Inter-individual differences in estrogen concentrations during treatment with aromatase inhibitors (AI) may contribute to therapeutic response and toxicity. The aim of this study was to determine plasma concentrations of estradiol (E2), estrone (E1), and estrone sulfate (E1S) in a large cohort of AI-treated breast cancer patients.

Methods—In a randomized, multicenter trial of postmenopausal women with early-stage breast cancer starting treatment with letrozole (n = 241) or exemestane (n = 228), plasma estrogen concentrations at baseline and after 3 months were quantitated using a sensitive mass spectrometry-based assay. Concentrations and suppression below the lower limit of quantification (LLOQ) were compared between estrogens and between drugs.

Results—The ranges of baseline estrogen concentrations were <LLOQ-361 pg/mL for E2, <LLOQ-190 pg/mL for E1, and 8.3–4060 pg/mL for E1S. For E2, the frequency of suppression

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Conflicts of interest

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below the LLOQ was not statistically significantly different between AIs (exemestane: 89.0%, letrozole: 86.9%, p=0.51). However, patients on letrozole were more likely to achieve suppression below the LLOQ of both E1 (exemestane: 80.1%, letrozole: 90.1%, p=0.005) and E1S (exemestane: 17.4%, letrozole: 54.9%, p=4.34e-15). After 3 months of AI therapy, the ranges of estrogen concentrations were <LLOQ-63.8 pg/mL, <LLOQ-36.7 pg/mL, and <LLOQ-1090 pg/mL for E2, E1, and E1S, respectively. During treatment, 16 patients had an increased concentration compared to baseline of at least one estrogen.

Conclusions—Letrozole had greater suppression of plasma E1 and E1S than exemestane, though response was highly variable among patients. Additional research is required to examine the clinical relevance of differential estrogen suppression.

Keywords

letrozole; exemestane; estradiol; estrone; estrone-sulfate; breast cancer

Introduction

The aromatase inhibitors (AIs) anastrozole, exemestane, and letrozole, are recommended as first line anti-estrogen therapy in postmenopausal women with hormone receptor (HR)-positive, early stage breast cancer [1]. Anastrozole and letrozole, which are both azoles, are competitive inhibitors of aromatase. In contrast, the steroidal exemestane is an inactivator of aromatase. Clinical response to these drugs varies widely among patients. Adjuvant AI therapy significantly reduces breast cancer mortality compared to the selective estrogen receptor modulator taxmoxifen, and therefore by extension compared to no endocrine therapy [2]. However, a substantial proportion of patients with HR-positive breast cancer will nonetheless develop recurrent disease despite receiving adjuvant AI therapy [2]. In addition, many patients develop adverse effects during AI therapy that may lead to treatment discontinuation [3].

Taken together, these data suggest heterogeneity in response to and toxicity from AI therapy. Currently, only standard factors such as pathologic stage are used to identify those patients at high risk of disease recurrence, and there are no validated biomarkers of increased risk of toxicity. We hypothesize that multiple mechanisms account for variable response to AIs including inter-patient differences in residual estrogen concentrations achieved during AI treatment [4]. The goal of many previous studies measuring AI-induced changes in blood estrogens and whole body aromatization of androgens was to estimate and compare potency among AIs, with less attention to variability in their effects [4, 5]. Even so, many studies suggest heterogeneity in the pharmacologic effect of AIs [5–7]. However, it is unlikely that the heterogeneity observed in these early smaller trials, which were designed to conduct intensive monitoring and measurements, accurately reflects effects of AIs on estrogen concentrations in the larger breast cancer population.

On average, third generation AIs cause aromatase inhibition of at least 97.9% [8]. As a result, the ability to measure variability in residual estrogens during AI therapy requires analytical methods that are selective and sensitive [9–11]. Routine immunoassays for estradiol used in most clinical laboratories are not sufficiently sensitive to measure low

concentrations of estradiol during AI therapy [12–14]. Furthermore, immune-based routine clinical assays cross-react with exemestane, the steroidal AI, which may result in underestimation of the degree of estrogen suppression in patients receiving treatment with the medication [6]. In contrast, mass spectrometry-based methods are highly sensitive and more accurate for measurement of low levels of estradiol [13, 15].

We conducted a prospective, randomized clinical trial of postmenopausal women with HR-positive breast cancer who were randomly assigned to letrozole or exemestane, and had serial plasma concentrations of E2, E1, and E1S measured using a selective and ultra-sensitive gas chromatography – tandem mass spectrometry assay (GC/MS/MS). In these analyses we examine the inter-patient heterogeneity in the reduction of circulating concentrations of estrogens during treatment with the two AI medications.

Methods

Patients

This analysis of plasma estrogens was conducted as one component of a prospective, open-label clinical trial, the Exemestane vs. Letrozole Pharmacogenomics (ELPh) study (ClinicalTrials.gov identifier: NCT00228956) conducted by the Consortium on Breast Cancer Pharmacogenomics (COBRA), a team of investigators from the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University, the University of Michigan Comprehensive Cancer Center, and the Indiana University Melvin and Bren Simon Cancer Center. The study design and inclusion and exclusion criteria have been previously described in detail [16, 17]. Briefly, eligible participants were postmenopausal women diagnosed with stage 0–III hormone receptor-positive breast cancer initiating AI therapy either as upfront adjuvant therapy or following tamoxifen therapy. Patients were screened and recruited at the study sites from August 2005 through July 2009. Recommended surgery, chemotherapy, and radiation for breast cancer were completed prior to study enrollment. The study was approved by the institutional review board at each study site and reviewed biannually by an independent Data and Safety Monitoring Committee. Written informed consent was obtained from each patient prior to undergoing protocol-directed procedures.

Study design

Eligible patients were stratified based on prior chemotherapy, prior tamoxifen therapy, and prior bisphosphonate use and then randomized to receive 25 mg exemestane or 2.5 mg letrozole orally once per day for 2 years. Venous blood samples were drawn in heparinized green top tubes prior to starting the study drug (baseline) and after 3 months of AI therapy. Patients who discontinued AI therapy before the 3-month time point, which occurred primarily because of drug toxicity, had plasma estrogens measured at baseline only [3]. Plasma was isolated after centrifugation at 1600 x g for 10 min at 4°C. Patients were requested to take the AI approximately two hours before the estimated time of blood draw.

Analysis of exemestane and letrozole plasma concentrations

A liquid chromatography – tandem mass spectrometry (LC/MS/MS) method was developed to quantify steady state plasma exemestane concentrations, whereas steady state plasma

letrozole concentrations were quantified using high performance liquid chromatography with fluorescence detection, as previously described by Desta et al [18] (described in Online Resource 1).

Analysis of estrogens in plasma

Plasma E2, E1, and E1S were measured by inVentiv Health (Princeton, NJ) using an established gas chromatography – tandem mass spectrometry (GC/MS/MS) assay [15]. Briefly, the analytes and deuterated internal standards were extracted from 0.4 mL of human plasma using BondElut Certify® solid-phase cartridges. Compounds were eluted from the cartridges with ethyl acetate and then underwent three separate derivatizations: reaction with pentafluorobenzoyl chloride, reaction with O-(2,3,4,5,6-pentaflurorobenzyl)-hydroxylamine hydrochloride, and reaction with N-methyl-N-(trimethylsilyl) trifluoroacetamide. The derivatized analytes and standards were separated by gas chromatography and detected by tandem mass spectrometry using negative-ion chemical ionization. Calibration curves were obtained by performing weighted linear regression (weighted $1/x^2$) on the calibration standards. Lower and upper limits of quantification for each estrogen were dependent on the calibration curve for the corresponding analytical run. As a result, two lower limits of quantification (LLOQ) were observed for E2 and E1, which reflect slight variability in the assay between analytical runs. LLOQs for E2 were 0.625 or 1.25 pg/mL, LLOQs for E1 were 1.56 or 3.12 pg/mL, and the LLOQ for E1S was 3.13 pg/mL. Upper limits of quantification (ULOQ) for E2, E1, and E1S were 80, 200, and 800 pg/mL, respectively.

Characteristics of plasma estrogen measurements

Not all patients had samples available at both baseline and 3 months (Figure 1). Reasons for missing data included: patient withdrawal, insufficient sample volume, inability to draw blood, or un-assayed samples. Two patients with month-3 estrogen samples were excluded from the analysis because insufficient plasma was available for baseline measurements.

Based on an initial review of the plasma estrogen concentrations, the following exclusion criteria were applied prior to data analysis. Nine patients had baseline plasma concentrations of one or more estrogens above the respective assay ULOQ. Due to uncertainty in the true concentrations in these samples, these specific measurements were excluded from data analysis. None of the estrogen concentrations were above the ULOQ after 3 months of AI therapy. In addition, the concentration of estrogens in some plasma samples could not be reliably determined and these specific measurements were also excluded from analysis (baseline: n=3, month-3: n=8). Excluding one estrogen metabolite from analysis did not influence inclusion of other successfully measured estrogens in a given plasma sample (Online Resource 2).

Change in one or more estrogens could not be assessed in fifteen patients because both the baseline and month-3 concentrations were at or below the LLOQ. These pairs of estrogen measurements were excluded from the analysis of change due to the inability to detect a drug effect; however, the baseline and month-3 concentrations were included in the analysis at the respective time points. In total, concentration change from baseline to month-3 was not calculated for 18 pairs of estrogen measurements from 15 individual patients: E2 only

(N=10), E1 only (N=2), E2 and E1 (N=2), and E2 and E1S (N=1). Ten of these fifteen patients were randomized to receive exemestane and five to receive letrozole.

Statistical analysis

The primary objective of the ELPh trial was to examine the correlation between changes in breast density and genetic variants in *CYP19A1* [17]. In the pre-planned subanalysis reported in this manuscript, we describe the inter-patient heterogeneity in plasma estrogens before and during AI therapy. Preliminary descriptive analysis suggested plasma estrogen concentrations were well described by a lognormal distribution, therefore measurements were $\log_{10}^{-}$ transformed for data presentation and correlation analysis. Unless otherwise specified, summary statistics are given in the original scale as their median values (first quartile (Q_1), third quartile (Q_3)), where Q_1 and Q_3 are the median of the bottom and top half of ranked values, respectively. Variability in plasma concentrations is described as standard deviation (SD) for $\log_{10}$-transformed concentrations and percent coefficient of variation (CV%) in the original scale, where CV% is calculated from $\log_{10}$-transformed concentrations ($CV\%=100\times\sqrt{e^{(SD\times\ln 10)^2}-1}$). Estrogen concentrations below the respective assay LLOQs were fixed at the LLOQ to approximate the actual sample concentration for percent-change and fold-change calculations. The frequency of suppression below the assay LLOQ at baseline and month-3 was compared between drugs using chi-square tests, or Fisher's exact test when appropriate. The drug effect on estrogen suppression below the assay LLOQs was determined using McNemar's test. Estrogen concentrations and change from baseline were compared between patients randomized to exemestane or letrozole using the Wilcoxon rank sum test and the drug-induced change within patients was analyzed by Wilcoxon signed rank test. Pearson's correlation coefficients (r) and p-values were determined for pairwise correlation between concentrations of plasma estrogens at baseline. Statistical analysis and plotting was performed using R (version 2.15.2, Vienna, Austria). Due to limits of the statistical package's numerical precision, p-values smaller than 2.2e-16 are reported as "<2.2e-16".

Results

Characteristics of patients and plasma estrogen measurements

Baseline demographics and clinical characteristics of patients enrolled in this study have been described in detail previously [3]. Briefly, median age was 59 years, 88.2% of patients were white, and mean BMI was 29.9 kg/m^2. Baseline plasma estrogen concentrations were measured from 241 of 252 patients randomized to letrozole (96%) and 228 of 248 patients randomized to exemestane (92%) (Figure 1). Following 3 months of AI therapy, plasma estrogens were measured from 204 patients receiving letrozole (85%) and 201 patients (88%) receiving exemestane. Reasons for missing data are described in the methods section.

Estradiol, estrone, and estrone sulfate concentrations in plasma prior to and during exemestane or letrozole therapy

Estrogen Concentrations at Baseline—To qualitatively describe the effect of AI treatment on plasma estrogens, we classified patients based on whether baseline and

month-3 estrogen concentrations were greater or less than the respective assay LLOQs. At baseline, 2.8%, 0.9%, and 0.2% of patients had E2, E1, and E1S concentrations below the assay LLOQ, respectively (Table 1). However, although all participants were considered postmenopausal at entry based on clinical assessment, baseline concentrations of quantifiable estrogens were highly variable, ranging from the LLOQ to 361.00 pg/mL (CV_{E2}(%) = 176) for E2, from the LLOQ to 190.00 pg/mL (CV_{E1}(%) = 106) for E1, and from the LLOQ to 4060.00 pg/mL (CV_{E1S}(%) = 181) for E1S (Figure 2, Table 2).

Estrogen Concentrations during AI Therapy—Month-3 concentrations of E2, E1, and E1S fell below assay LLOQ in 87.9%, 85.1%, and 36.3% of patients, respectively (Table 1). The frequency of E2 suppression below LLOQ was not statistically significantly different between AIs (exemestane: 89.0%, letrozole: 86.9%, p=0.51); however, significant differences were observed for both E1 and E1S. E1 concentrations were reduced below the assay LLOQ in 90.1% of patients taking letrozole, compared to 80.1% of patients taking exemestane (p=0.005). Similarly, although 54.9% of patients taking letrozole had month-3 E1S concentrations below the assay LLOQ, this reduction was only observed in 17.4% of patients taking exemestane (p=4.34e-15).

Comparing analyte levels during treatment with the two drugs by fixing concentrations below the assay LLOQ at the LLOQ yielded similar findings to the analysis of the proportion of patients with estrogen levels below the LLOQ during AI therapy. Mean $\log_{10}$ plasma E2 concentrations during therapy were not different between the two drugs (p=0.60, Figure 2, Table 2). In contrast, mean $\log_{10}$ concentrations of both E1 and E1S at month-3 were significantly higher in patients receiving exemestane versus letrozole (E1: exemestane 0.29 (SD 0.24), letrozole 0.24 (SD 0.18), p=0.007; E1S: exemestane 1.07 (SD 0.43), letrozole 0.78 (SD 0.49), p<0.0001). Despite a significant reduction of plasma concentrations in response to AI therapy, estrogen concentrations remaining above the respective assay LLOQs during therapy exhibited large inter-patient variability (Figure 2).

Inter-individual variability in drug-induced change in plasma estrogens during letrozole and exemestane therapy

The majority of patients had reduced plasma estrogen concentrations after 3 months of AI treatment compared to baseline (Figure 2). However, the effect of AI treatment on plasma estrogens calculated as intra-individual change from baseline concentrations showed a large variability in response to both AIs (Figure 3). In the group of 185 patients receiving exemestane with paired baseline and month-3 E2 measurements, 167 (90.2%) achieved 90% reduction from baseline or had a month-3 value below the LLOQ. For E1 and E1S, the number of exemestane-treated patients achieving that degree of suppression was 153 (82.7%) and 144 (77.8%), respectively. Similarly, of the 158 letrozole-treated patients with paired baseline and month-3 samples, the number of patients who achieved a 90% reduction from baseline or had a month-3 value below the LLOQ was 138 (87.3%) for E2, 145 (91.8%) for E1, and 142 (89.9%) for E1S.

Some of the observed variability in month-3 concentrations and percent change from baseline could be attributed to patients with increased concentrations of one or more

estrogen metabolites from baseline. Of the patients with E2 (n=384), E1 (n=398), or E1S (n=402) measured at baseline and month-3, we observed increased concentrations of one or more estrogens in 16 patients. Estrogen concentrations, drug concentrations, and clinical characteristics of these patients are presented in Online Resource 3. Five of the 16 patients had increased concentrations of at least two estrogens, and three of these five patients had increased concentrations of all 3 estrogens. The remaining 11 patients had increased concentration of only one estrogen, with corresponding decreases in the other measured estrogens. Nine of these 11 patients had increased E2 concentrations from baseline. Interestingly, exemestane or letrozole was detected in the plasma of all 16 patients that exhibited increases in 1 or more estrogens, confirming that the patients were adhering to their therapy.

Relationships among plasma E2, E1, and E1S concentrations prior to AI therapy

Prior to treatment initiation, we observed statistically significant, strongly positive pairwise correlations between plasma concentrations of all estrogen pairs. The Pearson's correlation coefficient for the correlation between E2 and E1 concentrations was r=0.74 ($p<2.2e-16$), for E1 and E1S concentrations it was r=0.69 ($p<2.2e-16$), and for E2 and E1S concentrations it was r=0.63 ($p<2.2e-16$) (Online Resource 4).

Discussion

In this prospective, randomized study examining the pharmacokinetic and pharmacodynamic effects of two third generation AIs in postmenopausal women with HR-positive breast cancer, we compared the relative suppression of plasma estrogens between the non-steroidal AI letrozole and the steroidal AI exemestane, with specific attention to inter-patient variability in the pharmacodynamic effects. Baseline estrogen concentrations were highly variable among patients, and were consistent with those previously reported for postmenopausal women when measured with highly sensitive assays [19–21]. During treatment with the AIs, there was significant inter-patient heterogeneity in the degree of estrogen suppression, which may have potential clinical relevance.

Our observations are consistent with a previous study demonstrating variability in conjugated plasma estrogen changes in breast cancer patients receiving a different non-steroidal AI, anastrozole. In that study, plasma estrogens were analyzed using the same highly sensitive methodology employed in our analysis [19, 22]. Taken together, these studies demonstrate that circulating conjugated estrogens persist in a substantial number of patients receiving AI therapy, while the concentrations of unconjugated estrogens above the assays' LLOQ are uncommon in AI-treated patients.

Data from our study are in agreement with previous work suggesting letrozole is a more potent suppresser of estrogen production than exemestane and anastrozole [5, 6, 23, 24]. Notably, these differences in average potency have not translated to differences in clinical outcomes in large randomized trials comparing AIs.[25] However, it is possible that the continued presence of detectable systemic levels of conjugated estrogens could have clinical significance for the minority of patients with incomplete suppression of estrogens and lead to AI resistance. Although circulating E2 is suppressed below the LLOQ in the vast majority

of patients, inter-conversion of E1 and E2 or desulfation of E1S through the action of tissue-specific sulfatases may significantly increase the tissue content of unconjugated estrogens in the breast and thereby influence local estrogen-dependent processes [26–29]. The association between concentrations of estrogens during AI therapy and disease outcomes has not been directly examined in the previously conducted large prospective trials. In addition, despite the use of a highly sensitive assay, we were unable to quantify plasma concentrations of the unconjugated estrogens, E2 and E1, for the majority of patients during AI treatment. A more complete characterization of the effects of the absolute degree of estrogen suppression on disease and toxicity outcomes relationships may require an assay to measure unconjugated estrogens with an LLOQ several fold lower than the assay used in this study.

There are a number of factors that could mediate the variability in plasma estrogen concentrations during AI exposure identified in our study, including germline genetic variation in genes mediating estrogen biosynthesis and metabolism, adiposity, noncompliance with therapy, and variable drug exposure [22, 30–32]. However, not all studies have demonstrated positive associations with these factors, potentially due to differences in the genetic variants analyzed or differences in estrogen measurement methods [33, 34]. The 16 patients in our study that had an increase in one or more estrogens after 3 months of therapy compared to baseline had detectable drug concentrations, although they did not have suppression of all circulating estrogens. It is possible that a subset of patients had experienced recovery of ovarian production of estrogen, thereby accounting for lack of suppression of estrogen [14, 35]. However, this is a plausible reason in only a minority of the patients considering that 11 of the 16 patients were above age 50 and at least two of the younger patients had undergone bilateral oophorectomy. Alternative explanations include rebound or incomplete suppression of plasma estrogens resulting from intermittent usage of therapy, or pharmacologic mechanisms, such as incomplete aromatase inhibition.

In summary, in these data derived from a large prospective randomized trial of postmenopausal women starting AI therapy, we demonstrated considerable variability in circulating estrogen concentrations. For each of the estrogens, more than 80% of patients had suppression to at least 90% of the baseline level or to below the level of quantification, although a minority of patients failed to achieve that goal. Failure to adequately suppress estrogen production is a potential mechanism of resistance for AI therapy. However, because of methodologic issues related to measurement of estrogens, investigation of this mechanism remains challenging. Studies that examine associations between the effect of AI therapy on circulating estrogens and the effects of AI therapy on disease outcomes and secondary effects are essential for tailoring therapy for individual patients in order to optimize the benefits and risks of endocrine therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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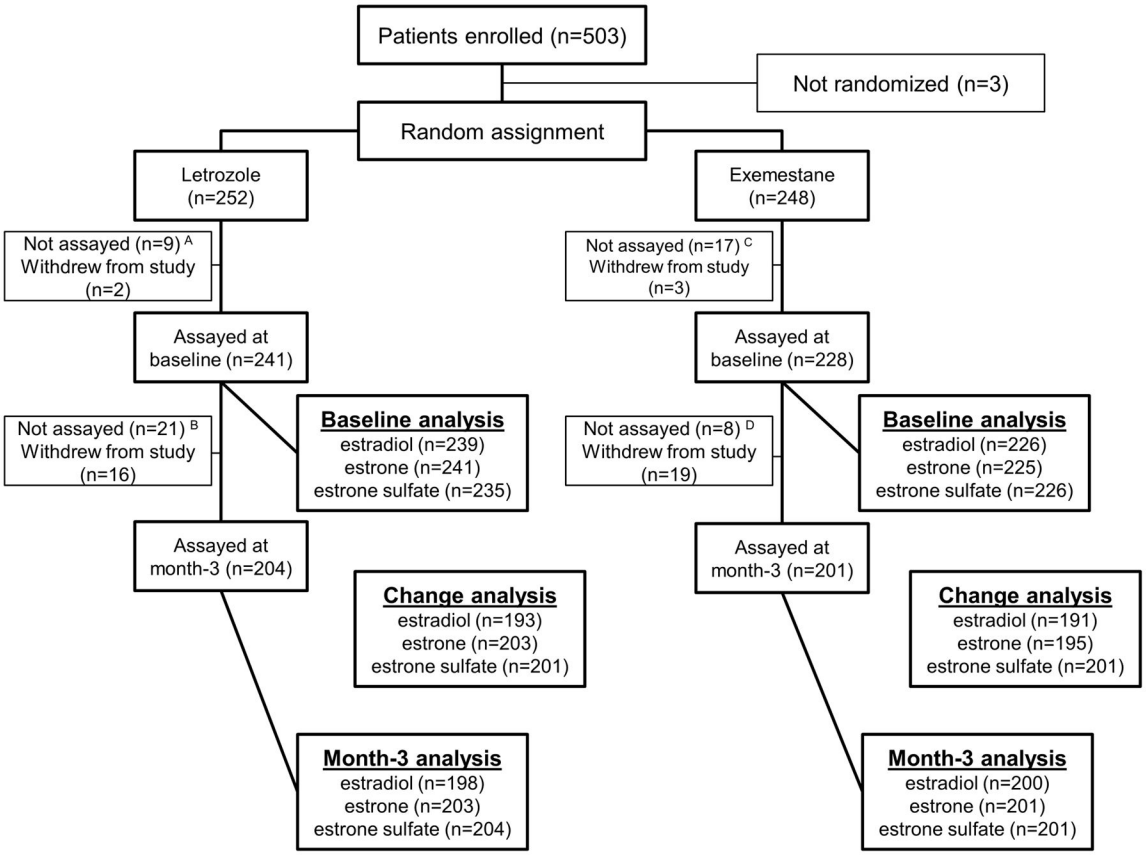

Figure 1. Patient flow diagram

Reasons for patient plasma samples not being assayed included the following: (A) No sample (N=2); not enough sample (N=1); sample not assayed (N=6). (B) Unable to draw blood (N=1); not enough sample (N=8); sample not assayed (N=12). (C) Unable to draw blood (N=2); not enough sample (N=4); sample not assayed (N=11). (D) Not enough sample (N=3); sample not assayed (N=5).

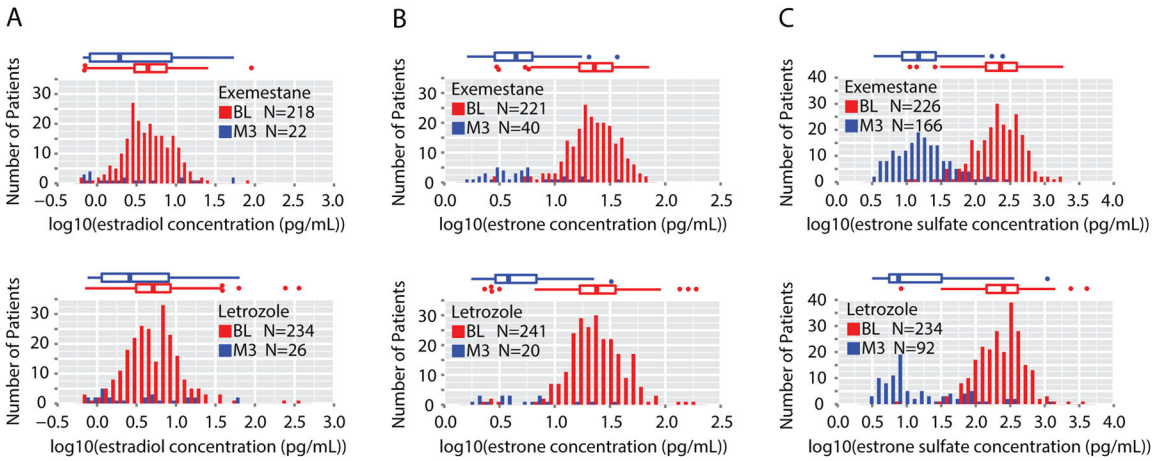

Figure 2. Distribution of plasma estrogen concentrations at baseline and during exemestane or letrozole therapy

The frequency distribution of log-transformed concentrations of estradiol (A), estrone (B), and estrone sulfate (C) are represented as red bars at baseline (BL), while month-3 (M3) concentrations are represented as blue bars. Bin widths are 1/30th of the log-transformed concentration of each estrogen. Boxplots plotted above each histogram provide additional distributional detail of log-transformed concentrations at baseline (red) and M3 (blue). Boxplots depict five-number summaries as horizontal lines representing (from left to right): 75th percentile + (1.5 x interquartile range) (end of upper whisker), 75th percentile, median, 25th percentile, 25th percentile - (1.5 × interquartile range) (end of lower whisker).

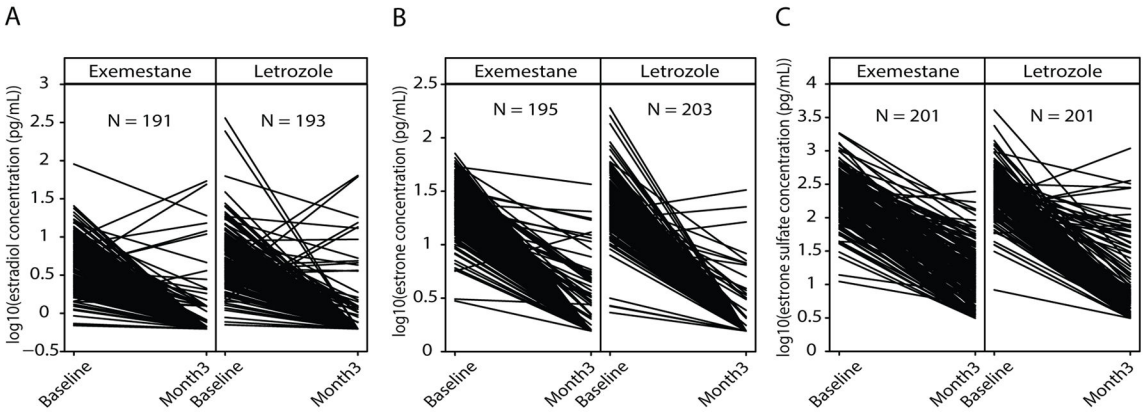

Figure 3. Intra-individual change in plasma estrogen concentrations during exemestane or letrozole therapy

Lines join log-transformed plasma concentrations of estradiol (A), estrone (B), or estrone sulfate (C) prior to and following 3 months of exemestane or letrozole therapy. Each line represents a subject. Month-3 estrogen concentrations determined to be below the respective assay LLOQ were fixed at the assay LLOQ.

Table 1

Percentage of patients whose estradiol, estrone, and estrone sulfate concentrations were below the respective assay lower limit of quantification (<LLOQ) at baseline and after three months of exemestane or letrozole therapy.

	Baseline			Month-3			Baseline vs. Month-3 p-value [d]	
	Exemestane	Letrozole	p-value [b]	Exemestane	Letrozole	p-value [c]	Exemestane	Letrozole
Estradiol [a]	3.5% (8/226)	2.1% (5/239)	0.407	89.0% (178/200)	86.9% (172/198)	0.514	< 2.20e-16	< 2.20e-16
Estrone	1.8% (4/225)	0% (0/241)	0.054	80.1% (161/201)	90.1% (183/203)	0.005	< 2.20e-16	< 2.20e-16
Estrone Sulfate	0% (0/226)	0.4% (1/235)	1.00	17.4% (35/201)	54.9% (112/204)	4.34e-15	9.08e-09	< 2.20e-16

[a] Each cell reports the percentage of patients in whom the estrogen analyte concentration was <LLOQ.

[b] Fisher's exact test.

[c] Pearson's chi-square test.

[d] McNemar's test.

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Table 2

Estrogen concentrations at baseline and after 3 months of exemestane or letrozole therapy

For each estrogen, concentrations below the LLOQ are fixed at the respective assay LLOQ. N: number of patients. SD: standard deviation.

		Baseline			Month-3			Baseline vs. Month-3 p-value[c]	
		Exemestane	Letrozole	p-value[b]	Exemestane	Letrozole	p-value[b]	Exemestane	Letrozole
Estradiol[a]	n	226	239		200	198			
	Median (range)	4.19 (0.63, 90.10)	5.08 (0.63, 361.00)		0.625 (0.63, 54.00)	0.625 (0.63, 63.80)			
	Log10: mean (SD)	0.64 (0.35)	0.72 (0.39)	0.045	−0.12 (0.29)	−0.09 (0.32)	0.603	< 2.20e-16	< 2.20e-16
Estrone[a]	n	225	241		201	203			
	Median (range)	22.4 (1.56, 71.40)	23.9 (2.32, 190.00)		1.56 (1.56, 36.70)	1.56 (1.56, 32.50)			
	Log10:mean (SD)	1.34 (0.29)	1.39 (0.26)	0.182	0.29 (0.24)	0.24 (0.18)	0.007	< 2.20e-16	< 2.20e-16
Estrone Sulfate[a]	n	226	235		201	204			
	Median (range)	227.50 (11.10, 1850.00)	253.00 (3.13, 4060.00)		11.80 (3.13, 246.00)	3.13 (3.13, 1090.00)			
	Log10: mean (SD)	2.35 (0.35)	2.40 (0.36)	0.286	1.07 (0.43)	0.78 (0.49)	3.29e-16	< 2.20e-16	< 2.20e-16

[a] Median and range in pg/mL

[b] Wilcoxon rank sum test.

[c] Wilcoxon signed rank test.