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Authors

Hertz, Daniel L
Barlow, William E
Kidwell, Kelley M
et al.

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DRUG INTERACTIONS

Fulvestrant decreases anastrozole drug concentrations when taken concurrently by patients with metastatic breast cancer treated on SWOG study S0226

Correspondence Dr Daniel L Hertz, PharmD, PhD, Department of Clinical Pharmacy, University of Michigan College of Pharmacy, 428 Church St., Room 3054, Ann Arbor, MI 48109-1065, USA. Tel: +1 734 763 0015; Fax: +1 734 763 4480; E-mail: DLHertz@med.umich.edu

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Daniel L. Hertz[1], William E. Barlow[2], Kelley M. Kidwell[3], Kathy S. Albain[4], Ted A. Vandenberg[5], Shaker R. Dakhil[6], Nagendra R. Tirumali[7], Robert B. Livingston[8], Julie Gralow[9], Daniel F. Hayes[10], Gabriel N. Hortobagyi[11], Rita S. Mehta[12] and James M. Rae[10]

[1]College of Pharmacy, University of Michigan, Ann Arbor, MI, USA, [2]SWOG Statistical Center, Seattle, WA, USA, [3]University of Michigan, School of Public Health, Ann Arbor, MI, USA, [4]Loyola University Chicago Stritch School of Medicine, Maywood, IL, USA, [5]London Health Sciences Center/National Cancer Institute of Canada Clinical, Trials Group, London, ON, Canada, [6]Wichita NCORP, Wichita, KS, USA, [7]Kaiser Permanente NCORP, Portland, OR, USA, [8]Arizona Cancer Center, University of Arizona, Tucson, AZ, USA, [9]Seattle Cancer Care Alliance, Fred Hutchinson Cancer Research Center, University of Washington, Seattle, WA, USA, [10]Division of Hematology/Oncology, University of Michigan Comprehensive Cancer Center, Ann Arbor, MI, USA, [11]University of Texas, M.D. Anderson Cancer Center, Houston, TX, USA and [12]University of California Irvine Medical Center, Chao Family Comprehensive, Cancer Center, Orange, CA, USA

Keywords anastrozole, breast cancer, drug interaction, fulvestrant, pharmacokinetics

AIMS

In the SWOG S0226 trial the combination of anastrozole plus fulvestrant ($n = 349$) was superior to anastrozole alone ($n = 345$) in hormone receptor (HR)-positive metastatic breast cancer. Here we report a pharmacokinetic subset analysis investigating a possible drug interaction between anastrozole and fulvestrant.

METHODS

Post-menopausal patients with HR-positive metastatic breast cancer were randomized to anastrozole with or without concurrent fulvestrant. Blood samples were collected at 2, 4, 6 and 8 months, just prior to receiving the next dose of anastrozole and fulvestrant. Drug concentrations were measured via LC/MS-MS. Anastrozole concentration was compared in patients on anastrozole alone *vs.* patients on concomitant fulvestrant. Comparisons were made at each time point using parametric tests and over time using a linear mixed effects model.

RESULTS

A total of 483 anastrozole concentration measurements were included, 224 samples from 64 patients on the anastrozole alone arm and 259 from 73 patients on the combination arm. The mean anastrozole concentration in the combination arm was significantly lower than that in the anastrozole alone arm at each sample collection time (all $P < 0.01$) and in the mixed effects model (an estimated difference of 9.85 ng ml^{-1} (95% CI 5.69, 14.00 ng ml^{-1}), $P < 0.001$).

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CONCLUSION

A significant pharmacokinetic drug interaction was detected, in which the addition of fulvestrant to anastrozole treatment decreased the trough anastrozole concentration. Further research is needed to verify whether this interaction affects treatment efficacy and to determine the pharmacological mechanism by which this interaction occurs.

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- The addition of fulvestrant to anastrozole treatment improves overall survival in post-menopausal patients with HR-positive metastatic breast cancer.
- Drug interactions that decrease anastrozole and letrozole concentration have been previously reported with concomitant tamoxifen treatment.

WHAT THIS STUDY ADDS

- The addition of concomitant fulvestrant causes a similar drug interaction in which anastrozole concentration is decreased.
- We postulate that this interaction is mediated through fulvestrant-induced upregulation of hepatic drug metabolizing enzyme (s) involved in anastrozole elimination.
- There are likely to be drug–drug interactions between fulvestrant and other medications cleared by the hepatobiliary system.

Introduction

Breast cancer is the second most common cancer in women in the United States [1] and the majority of breast cancer is hormone (estrogen or progesterone) receptor positive (HR+). Endocrine (anti-estrogen) treatment is the primary weapon in the medical treatment of HR+ breast cancer. Aromatase inhibitors (AIs), which inhibit the production of estrogen by aromatase, are more effective than tamoxifen in the adjuvant setting in post-menopausal patients with HR+ breast cancer [2–4] and in pre-menopausal patients when combined with ovarian suppression [5]. AIs are also more effective than tamoxifen in patients with metastatic HR+ breast cancer [6] and are used as front line therapy for disease control and symptomatic improvement [7].

Despite the efficacy of AIs, nearly all patients with metastatic disease eventually progress during treatment, requiring alternative agents with differing mechanisms. Fulvestrant binds to and blocks the estrogen receptor and increases estrogen receptor degradation [8]. It is the only approved selective-estrogen receptor down-regulator (SERD) for patients with HR+ breast cancer [9]. Preclinical data suggest that the combination of fulvestrant and an AI (anastrozole) could be more effective than the AI alone [10]. SWOG protocol S0226 (ClinicalTrials.gov Identifier: NCT00075764) was designed to test whether the addition of fulvestrant to anastrozole improved progression-free survival in post-menopausal patients with HR+ metastatic breast cancer. The S0226 trial demonstrated a survival benefit from the addition of fulvestrant, especially in women not previously exposed to adjuvant endocrine therapy [11]. However, the fulvestrant dosage in S0226 was principally 250 mg month^{-1}, which has now been shown to be inferior to 500 mg month^{-1} [12]. In S0226, blood samples were collected for secondary analyses including a pre-specified pharmacokinetic analysis of trough drug concentrations of anastrozole and fulvestrant. The primary objective of this analysis was to determine whether a pharmacokinetic drug interaction occurs when anastrozole and fulvestrant are concomitantly administered to post-menopausal patients with HR+ metastatic breast cancer.

Methods

Patient/clinical study

S0226 was an unblinded, prospective, randomized phase III clinical trial comparing fulvestrant and anastrozole with anastrozole alone. Detailed study information and results have been previously published [11]. Briefly, post-menopausal patients with HR+ metastatic breast cancer who had not received prior chemotherapy, immunotherapy or endocrine therapy for metastatic disease were eligible to enrol. Prior adjuvant tamoxifen therapy was permissible. Adjuvant chemotherapy had to have been completed at least 1 year prior to enrolment Patients were ineligible if they were on long-term anticoagulant treatment or had another current malignancy. All enroled patients received anastrozole 1 mg oral daily and half of the patients were randomly assigned to receive concurrent fulvestrant intramuscular injection, starting with a 500 mg loading dose at day 0 and a 250 mg dose at day 14, 28 and then monthly. Treatment continued until disease progression, unacceptable toxicity, a delay of 4 or more weeks or patient withdrawal. Patients who progressed on the anastrozole alone arm were encouraged to crossover to fulvestrant alone (an amendment was approved in February 2011 to increase the monthly fulvestrant dose to 500 mg, based on superior efficacy from other clinical studies) [12].

The addition of fulvestrant significantly improved progression-free survival (PFS medians 13.5 *vs.* 15.0 months, HR = 0.80, $P = 0.007$) and overall survival (41.3 *vs.* 47.7 months, HR = 0.81, $P = 0.05$) with a non-significant increase in the proportion of patients experiencing any severe (grade 3+) toxicity (12.7% *vs.* 14.7%, $P = 0.44$). Improved overall survival was more pronounced in the subgroup of patients who had

not previously received tamoxifen treatment (HR = 0.74, 95% CI 0.56, 0.98, P = 0.04), though the formal statistical interaction between treatment arm and prior tamoxifen was not statistically significant (P = 0.22).

Pharmacokinetic sampling and analysis

The original protocol specified that the first 100 enroled patients would submit samples for PK analyses. However, accrual early in the trial was low and this requirement was removed since it was believed to impede accrual. Instead, clinics could enrol patients who volunteered and the clinic received support for the additional work. Consequently, only a small subset of patients enroled on the pharmacokinetic substudy, which was closed to enrolment in April 2009.

Blood samples were collected for measurement of trough drug concentrations during treatment. For patients on the anastrozole arm, samples were collected at baseline and at months 2, 4, 6 and 8. Samples for both drugs were collected from patients on the combination arm at baseline, day 14 and 28 and months 2, 4, 6 and 8. Importantly, these samples were all collected prior to the amendment that increased the monthly fulvestrant dose to 500 mg. Samples were collected just prior to dosing, approximately 24 h after the last anastrozole dose and 14 days or 1 month after the last fulvestrant dose, for estimation of trough concentration. For anastrozole analysis, 5 ml whole blood samples were collected in glass EDTA tubes and centrifuged at 3000 *g* for 15 min within 30 min of sampling. For fulvestrant 10 ml whole blood samples were collected in heparinized tubes, centrifuged at 1000 *g* for 10 min for plasma separation. Plasma samples for both drugs were transferred to polypropylene tubes, shipped to Quest Diagnostics and stored at –20°C until analysis.

Anastrozole analysis was performed by MDS Pharma Services (Blainville, Quebec, Canada) using an LC/MS-MS assay. Briefly, a 0.3 ml aliquot of EDTA plasma containing a trifluoperazine internal standard underwent protein precipitation extraction prior to injection into an HPLC with a Sciex API 3000 LC/MS-MS system. The range of quantitation was 1.00 to 60.0 ng ml^{-1} with >80% accuracy. Fulvestrant analysis was conducted by Analytico Medinet BV (Breda, The Netherlands). Deuterated fulvestrant was used as the internal standard in a validated LC/MS-MS assay that calculated fulvestrant concentration from peak area ratios and reference to a standard curve of known concentrations. All specimens were analyzed without knowledge of treatment assignment or clinical outcomes.

Statistical analysis

Anastrozole concentrations in months 2, 4, 6 and 8 were included in the analysis. Earlier concentrations (0.5 and 1 month) were excluded due to the use of a different fulvestrant (loading) dose at the time (500 mg). The primary analysis was conducted using a linear mixed effects model with a random intercept to account for natural heterogeneity between subjects and a random slope for time (time modeled continuously) to compare average anastrozole concentrations over time in patients on anastrozole alone with those on concurrent fulvestrant. Correlation between repeated measurements was assumed to vary specified by an unstructured variance covariance matrix. As a secondary analysis, the mean concentration of anastrozole was compared between patients on either treatment arm at each individual sample collection time point using *t*-tests that allowed different variances in the two treatment groups. Finally, in an exploratory analysis for those who received the combination and had concentration measurements for both drugs, the fulvestrant concentration for each patient was included in the model to assess whether the concentration of fulvestrant was significantly associated with the measured anastrozole level. A standard significance threshold of $P < 0.05$ was used for all statistical comparisons.

Results

Patients and samples

Of the 707 patients randomized on the S0226 clinical trial, 137 had anastrozole measurements at 2 months or beyond and were evaluable in this drug interaction substudy (Figure 1). Demographic and clinical information for these patients is shown in Table 1. PFS was higher in patients in the substudy compared with those not in the substudy since they would have to have survived to the measurement times, but the effect of treatment did not differ (interaction P = 0.65). A total of 483 anastrozole concentration measurements were included in this analysis, 259 from 73 patients in the combination arm and 224 from 64 patients in the anastrozole alone arm. There were an additional 24 assays for which a result was not obtained and three values below the limit of quantification. In the secondary analysis of fulvestrant concentrations 245 concentration measurements were included from 71 patients with fulvestrant and anastrozole concentrations measured at the same time point.

Anastrozole concentrations with and without concurrent fulvestrant

The mean anastrozole concentration for each treatment arm, at each time point, is reported in Table 2. At each of the four collection time points the mean anastrozole concentration in the combination arm was significantly lower than the concentration in the anastrozole alone arm (month 2 28.52 ng ml^{-1} *vs.* 38.22 ng ml^{-1}, P = 0.0002; month 4 30.20 ng ml^{-1} *vs.* 39.35 ng ml^{-1}, P = 0.0023; month 6 29.24 ng ml^{-1} *vs.* 40.12 ng ml^{-1}, $P < 0.0001$; month 8 30.79 ng ml^{-1} *vs.* 41.26 ng ml^{-1}, P = 0.0001). In the mixed effects model treatment arm was a significant predictor of anastrozole concentration during treatment (anastrozole only arm had increased concentration by 9.85 ng ml^{-1} (95% CI 5.69, 14.00 ng ml^{-1}), $P < 0.0001$, Figure 2). There was a small, but not statistically significant, increase in anastrozole concentration as treatment continued (P = 0.10) and no significant interaction of time with treatment arm (P = 0.47). Finally, for those in the combination arm who had measurements of both anastrozole and fulvestrant concentration (245 joint measures for n = 71 patients), when fulvestrant concentration was included in the model there was no significant effect of fulvestrant concentration on anastrozole concentration (P = 0.97).

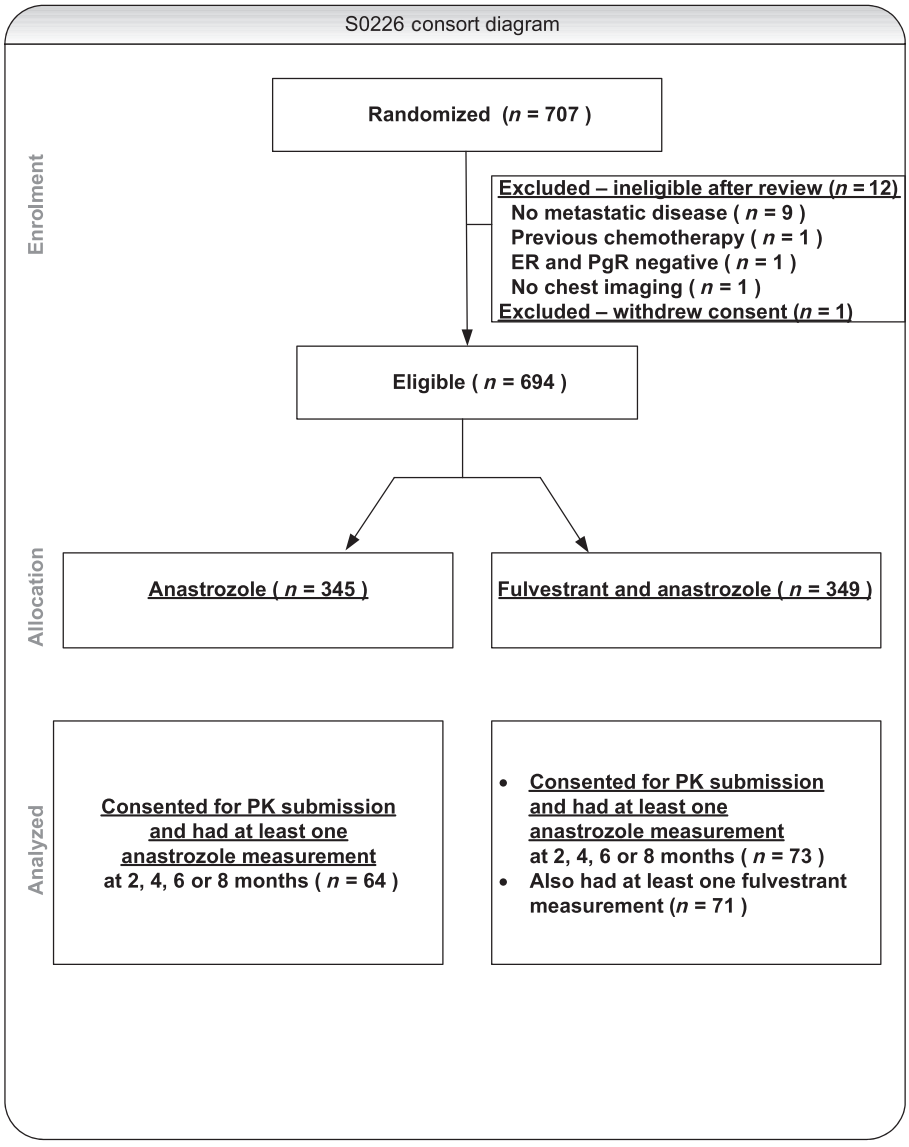

Figure 1
REMARK Diagram describing patient disposition from S0226 into the drug-interaction analysis

Discussion

The SWOG S0226 clinical trial demonstrated an improvement in overall survival from the addition of fulvestrant to anastrozole in post-menopausal patients with HR+ metastatic breast cancer, which was more pronounced in patients who had not previously received tamoxifen treatment [11]. In this pre-planned pharmacokinetic substudy a significant drug interaction between anastrozole and fulvestrant was discovered. Patients receiving the combination, as compared with those on single agent anastrozole, had significantly lower anastrozole trough concentrations at all sampled time points, beginning at 2 and continuing through 8 months of treatment.

Drug interactions between concomitantly administered endocrine treatments have been previously reported. In the Anastrozole and Tamoxifen Alone or in Combination (ATAC) trial, a similar decrease in anastrozole concentration was identified in patients concomitantly receiving tamoxifen treatment [13]. Similarly, in a small drug interaction study of the combination of tamoxifen and letrozole, an AI with pharmacological similarity to anastrozole, patients in the combination arm had decreased systemic letrozole concentrations [14]. Both of these reports hypothesized that these drug–drug interactions were mediated by tamoxifen inducing the cytochrome P450 (CYP) 3A4 enzyme, which is involved in the metabolism of both anastrozole and letrozole [15]. Other studies have reported modest *in vitro* induction of CYP3A4 by tamoxifen, but little effect on pharmacokinetics of the probe substrate midazolam in rats [16]. To our knowledge there have been no studies showing fulvestrant induces the expression of CYP3A4 [17]. Anastrozole is metabolized by other enzymes in addition to CYP3A4, including CYP2C8, followed by glucuronidation via UGT1A4 [18]. *In vitro* anastrozole glucuronidation is highly sensitive to

Table 1

Demographic information for patients Included in analysis

Characteristic	Anastrozole only (*n* = 64)	Anastrozole and fulvestrant (*n* = 73)*
Race		
White	59 (92%)	68 (93%)
Black	3 (5%)	4 (5%)
Other/Unknown	2 (3%)	1 (2%)
Age (years) (range)		
Prior adjuvant treatment		
Tamoxifen	31 (48%)	42 (58%)
At enrolment	65.2 (46–87)	64.3 (48–89)
Chemotherapy	17 (27%)	28 (38%)
None	29 (45%)	26 (37%)
HER2 status		
Positive	2 (3%)	5 (7%)
Negative	51 (80%)	56 (78%)
Missing	11 (17%)	12 (16%)
Anastrozole concentrations collected at each time point		
2 h	52 (81%)	60 (82%)
4 h	51 (80%)	68 (93%)
6 h	59 (92%)	62 (85%)
8 h	62 (97%)	69 (95%)

*There were no significant differences in the baseline characteristics of the two arms in this secondary analysis.

Table 2

Comparison of anastrozole concentration between treatment arms

	Anastrozole and fulvestrant			Anastrozole alone					
Month	*n*	Mean	SD	*n*	Mean	SD	Difference	95% CI	*P* value
2	60	28.52	10.92	52	38.22	14.68	9.71	4.90, 14.51	0.0002
4	68	30.20	14.56	51	39.35	16.67	9.15	3.47, 14.84	0.0023
6	62	29.24	11.35	59	40.12	16.86	10.88	5.73, 16.03	<0.0001
8	69	30.79	10.29	62	41.26	17.90	10.47	5.49, 15.45	0.0001

95% CI, 95% confidence interval

UGT1A4 activity [19] and fulvestrant is known to induce UGT1A4 [20]. Interestingly, estradiol metabolites including 17β-estradiol are also known to upregulate UGT1A4 [21], providing an alternative mechanistic explanation for previously reported drug interactions between concomitantly administered endocrine treatments.

The clinical relevance of the drug–drug interaction between anastrozole and fulvestrant is unclear. The superior efficacy in the combination arm suggests that the decrease in anastrozole concentration did not diminish efficacy. However, it has now been shown that fulvestrant 500 mg month^{-1} alone is superior to anastrozole [22] so it is possible that the superior efficacy in the combination arm of S0226 was primarily due to the effect of fulvestrant. This is also intriguing considering that the addition of tamoxifen to anastrozole, which resulted in a similar drug–drug

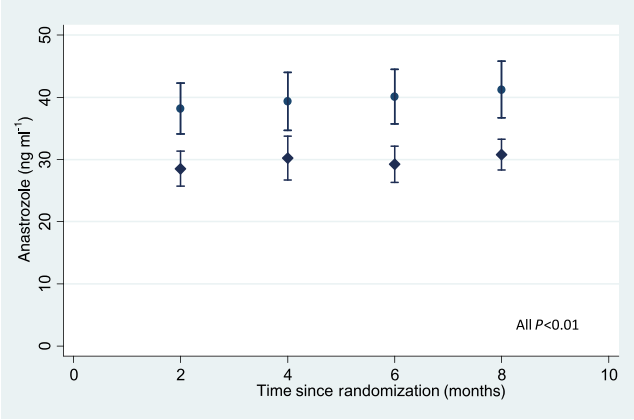

Figure 2

Anastrozole concentration in single agent (●) and combination (◆) arms. The mean anastrozole concentration was greater in the single agent arm at each sample collection time point. In the repeated measures regression model treatment arm was the only significant predictor of anastrozole concentration ($P < 0.0001$)

interaction [13], was not superior to anastrozole alone in ATAC [23]. It may be possible to improve efficacy of combination regimens further by increasing the anastrozole dose to achieve typical drug concentrations. In an analysis by Ingle *et al.* the subgroup (8.9%) of patients whose estradiol did not decrease during anastrozole treatment had lower on-treatment anastrozole concentration [24], suggesting the possibility of an exposure/response relationship. However, clinical trials have not identified any benefit of increasing the anastrozole dose from 1 to 10 mg in the general population [25]. Furthermore, the superior estradiol lowering effect of letrozole [26] does not seem to confer any efficacy benefit, suggesting that efficacy of aromatase inhibitors is relatively insensitive to variability in drug or estradiol exposure.

While this study was being conducted it was found that a fulvestrant dose of 500 mg month^{-1} has superior efficacy to 250 mg [12], leading to a new standard of care. It is possible that high dose fulvestrant is non-inferior to the combination of fulvestrant and anastrozole; this hypothesis will be tested in the ongoing ALTERNATE study (NCT01953588). It is also plausible that this higher fulvestrant dose would have a greater effect on anastrozole concentration, though we did not find a significant association between fulvestrant concentration and the magnitude of the drug interaction in a secondary analysis. More research is needed to understand the mechanism of this drug interaction and develop appropriate strategies for dosing both drugs to maximize treatment effectiveness.

While S0226 detected improved survival from the addition of fulvestrant to anastrozole, the FACT and SoFEA studies did not confirm these results [27, 28]. This discrepancy may be attributed to the high proportion of endocrine and chemotherapy naive patients in S0226. Patients likely develop acquired resistance during prior rounds of treatment, which diminishes the benefit from optimized endocrine treatment. This is further supported by cross-study comparison of SoFEA with PALOMA3 [29]. The median progression-free survival of 3.8 months for high dose fulvestrant in PALOMA3 was similar to that reported for either low dose fulvestrant alone or the combination of fulvestrant and anastrozole in SoFEA.

Some additional limitations of this analysis should be considered. The single anastrozole trough concentration measurement every 2 months does not provide the rich pharmacokinetic data necessary to characterize the effect of fulvestrant on anastrozole exposure, or area under the curve (AUC). AUC may be more sensitive to the drug–drug interactions and more predictive of treatment effectiveness. Additionally, this analysis did not assess the effect of decreased anastrozole concentration on estradiol depletion or treatment efficacy. An analysis of anastrozole concentration and estradiol level is currently underway in this dataset to ascertain whether this drug interaction may have clinical importance. Unfortunately, formal assessment of the effect of estradiol level on treatment efficacy is not possible in S0226 due to the limited size of the anastrozole alone arm and the confounding introduced by concomitant administration of fulvestrant on the combination arm.

In conclusion, while the addition of fulvestrant to anastrozole improves overall survival in post-menopausal patients with HR+ metastatic breast cancer, a pharmacokinetic interaction occurs that decreases anastrozole concentrations by 2 months and persists throughout treatment. This interaction is of unclear clinical relevance, but the addition of fulvestrant may compromise the efficacy of anastrozole. Ongoing analyses in this rich dataset, including analyses of the relationship between anastrozole concentration and effective depletion of estradiol, will provide additional information regarding the clinical relevance of this drug–drug interaction. More importantly, this is the first report of fulvestrant causing a drug–drug interaction that could be clinically relevant for other drugs, particularly those with narrow therapeutic indices. Additional research is needed to verify the mechanism by which this interaction, and other interactions between endocrine treatments, is occurring so that appropriate strategies can be developed to predict and prevent unsafe treatment combinations in patients with breast cancer.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare RSM had support from AstraZeneca for the submitted work and DFH has received unrelated research funding from with AstraZeneca in the previous 3 years. There are no other relationships or activities that could appear to have influenced the submitted work.

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Contributors

D. L. Hertz: correlative analysis PI, manuscript preparation and final approval. W. E. Barlow: correlative analysis lead

statistician, manuscript review. K. M. Kidwell: correlative analysis statistician, manuscript review. K. S. Albain: clinical trial enrolment, manuscript final approval. T. A. Vandenberg: clinical trial enrolment, manuscript final approval. S. R. Dakhil: clinical trial enrolment, manuscript final approval. N. R. Tirumali: clinical trial enrolment, manuscript final approval.

R. B. Livingston: clinical trial enrolment, manuscript final approval. J. Gralow: clinical trial enrolment, manuscript final approval. D. F. Hayes: clinical trial design and enrolment, correlative analysis design, manuscript review. G. N. Hortobagyi: clinical trial design and enrolment, manuscript review. R. S. Mehta: clinical trial PI, manuscript review. J. M. Rae: correlative analysis design, manuscript review and final approval.

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