UC Irvine
UC Irvine Previously Published Works

Title

Effects of CYP3A4 and CYP2C9 genotype on systemic anastrozole and fulvestrant concentrations in SWOG S0226

Permalink

https://escholarship.org/uc/item/0qz9q2k2

Journal

Pharmacogenomics, 24(12)

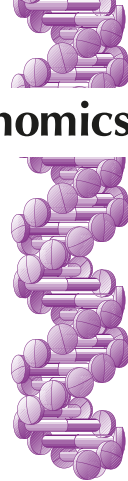**ISSN**

1462-2416

Authors

Rutherford, Delaney V

Medley, Sarah

Henderson, Nicholas C

et al.

Publication Date

2023-08-01

DOI

10.2217/pgs-2023-0097

Peer reviewed

Effects of *CYP3A4* and *CYP2C9* genotype on systemic anastrozole and fulvestrant concentrations in SWOG S0226

Delaney V Rutherford[1], Sarah Medley[2], Nicholas C Henderson[2], Christina L Gersch[1], Ted A Vandenberg[3], Kathy S Albain[4], Shaker R Dakhil[5], Nagendra R Tirumali[6], Julie R Gralow[7], Gabriel N Hortobagyi[8], Lajos Pusztai[9], Rita S Mehta[10], Daniel F Hayes[1], Kelley M Kidwell[2], N Lynn Henry[1], William E Barlow[11], James M Rae[1] & Daniel L Hertz*,[12]

[1]Department of Internal Medicine, Division of Hematology/Oncology, University of Michigan Medical School, Ann Arbor, MI 48109, USA
[2]Department of Biostatistics, University of Michigan School of Public Health, Ann Arbor, MI 48109, USA
[3]Western University/Canadian Cancer Trials Group, London, ON, N5X 3K8, Canada
[4]Loyola University Chicago Stritch School of Medicine, Maywood, IL 60153, USA
[5]Cancer Center of Kansas, Wichita, KS 67214, USA
[6]Kaiser Permanente, Beaverton, OR 97005, USA
[7]American Society of Clinical Oncology, Alexandria, 22314, Virginia
[8]University of Texas, M.D. Anderson Cancer Center, Houston, TX 77030, USA
[9]Yale School of Medicine, New Haven, CT 06510, USA
[10]University of California Irvine Medical Center, Chao Family Comprehensive Cancer Center, Orange, CA 92868, USA
[11]SWOG Statistical Center, Seattle, WA 98109, USA
[12]Department of Clinical Pharmacy, University of Michigan College of Pharmacy, Ann Arbor, MI 48109, USA
*Author for correspondence: Tel.: +1 734 763 0015; dlhertz@med.umich.edu

Objective & methods: This study tested associations of genotype-predicted activity of CYP3A4, other pharmacogenes, *SLC28A7* (rs11648166) and *ALPPL2* (rs28845026) with systemic concentrations of the endocrine therapies anastrozole and fulvestrant in SWOG S0226 trial participants. **Results:** Participants in the anastrozole-only arm with low CYP3A4 activity (i.e. *CYP3A4*22* carriers) had higher systemic anastrozole concentrations than patients with high CYP3A4 activity (β-coefficient = 10.03; 95% CI: 1.42, 18.6; p = 0.025). In an exploratory analysis, participants with low CYP2C9 activity had lower anastrozole concentrations and higher fulvestrant concentrations than participants with high CYP2C9 activity. **Conclusion:** Inherited genetic variation in *CYP3A4* and *CYP2C9* may affect concentrations of endocrine therapy and may be useful to personalize dosing and improve treatment outcomes.

Nearly 70% of breast cancers express the estrogen receptors and/or progesterone receptors and are together referred to as hormone receptor-positive (HR+) [1]. Endogenous estrogenic hormones bind to estrogen receptors, resulting in transcriptional activation, leading to cellular proliferation. Aromatase inhibitors are a class of anti-estrogen drugs that decrease estrogen levels by inhibiting the aromatase-mediated conversion of androgens to estrogens, thereby blocking the growth of HR+ breast cancers. Anastrozole is a third-generation nonsteroidal aromatase inhibitor [2,3]. Fulvestrant is an anti-estrogen drug in a different drug class that binds to the estrogen receptor and accelerates its degradation [4,5].

Results from SWOG S0226, a randomized, open-label clinical trial comparing the efficacy of anastrozole alone to anastrozole plus fulvestrant, showed that adding fulvestrant to anastrozole standard of care improved overall survival in postmenopausal patients with metastatic HR+ breast cancer [5]. A secondary analysis of systemic drug concentrations measured in a subset of S0226 participants detected a drug–drug interaction, in which patients receiving

fulvestrant had lower anastrozole concentrations [6]. The authors of the present study hypothesized that, in addition to drug–drug interactions, a possible genetic variation in the enzymes responsible for anastrozole and fulvestrant metabolism could affect systemic drug concentrations. Both drugs are eliminated primarily by hepatic metabolism mediated by CYP3A4, with additional contributions from several other drug-metabolizing enzymes, including CYP2B6, CYP2D6, CYP2C8 and UGT1A4 for anastrozole [2,3], SULT1A1 and SULT1E1 for fulvestrant [4]. The only study, to the authors' knowledge, that has investigated pharmacogenetic associations of inherited variation in these genes with systemic concentrations of anastrozole or fulvestrant, is a recent genome-wide association study that reported associations for polymorphisms in *SLC28A7* (rs16960359) and *ALPPL2* (rs883013) with systemic anastrozole concentrations, which they attributed to altered systemic and cellular anastrozole uptake [7].

This retrospective exploratory analysis aimed to investigate whether genetic variation in major pharmacogenes, including drug-metabolizing enzymes and transporters, is associated with systemic concentrations of anastrozole or fulvestrant using data and samples collected on the S0226 clinical trial. The authors hypothesized that patients with genetically-predicted reduced CYP3A4 phenotype would have higher systemic concentrations of fulvestrant and anastrozole. Associations for an additional 35 pharmacogenes, and the two polymorphisms previously reported to be associated with anastrozole, were also investigated.

Methods

Enrollment in S0226

Patients with HR+ metastatic breast cancer were eligible to enroll in the SWOG S0226 trial if they had not received prior chemotherapy, immunotherapy or endocrine therapy for metastatic disease [5]. Adjuvant chemotherapy treatment, if given, had to be completed at least 1 year prior to enrollment. Prior tamoxifen therapy was acceptable. Patients were excluded from the trial if they were receiving treatment with an anticoagulant or had another malignancy. Patients were randomly assigned 1:1 to either anastrozole 1 mg orally daily alone, or with fulvestrant 500 mg intramuscular loading dose and 250 mg at day 14, day 28 and then monthly. The S0226 protocol was amended during the study to increase the fulvestrant maintenance dose from 250 to 500 mg monthly. Treatment continued until disease progression, unacceptable toxicity, a treatment delay of 4 or more weeks or patient withdrawal.

Measurement of systemic drug concentrations

All participants on the S0226 pharmacokinetic substudy were enrolled and treated with 250 mg fulvestrant maintenance dose before the amendment increasing the dose to 500 mg. The details of sample collection and systemic drug concentration measurement have been previously reported [6]. Briefly, 5 or 10 ml blood 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 on days 14 and 28 on the combination arm and at months 2, 4, 6 and 8 on both arms. Samples were processed to isolate plasma and stored at -20°C. Anastrozole and fulvestrant concentrations were measured using previously described liquid chromatography with tandem mass spectrometry assays [6].

Pharmacogene & candidate variant genotyping

Whole blood was collected pretreatment from S0226 trial participants for isolation of germline DNA for pharmacogenetic analysis. Genotyping of candidate pharmacogenes was conducted on the iPLEX ADME PGx Pro Panel by Agena Biosciences (CA, USA). The panel evaluated >150 polymorphisms in 36 genes, including *CYP3A4*2*, *CYP3A4*6*, *CYP3A4*20*, *CYP3A4*22*, *CYP2C9*2*, *CYP2C9*3* (or *18*), *CYP2C9*4*, *CYP2C9*5*, *CYP2C9*6*, *CYP2C9*8*, *CYP2C9*9*, *CYP2C9*10*, *CYP2C9*11*, *CYP2C9*12*, *CYP2C9*13*, *CYP2C9*15*, *CYP2C9*25* and *CYP2C9*27*. Noncarriers of any of these variants were assigned the wild-type (*CYP3A4*1* or *CYP2C9*1*) genotype. Raw genotype calls for each polymorphism were translated into haplotypes and metabolic activity phenotypes, as previously described [8]. This translation was modeled after the Clinical Pharmacogenetics Implementation Consortium process [9], with necessary variations to accommodate genes and variants that are not included in Clinical Pharmacogenetics Implementation Consortium guidelines. For *CYP3A4* and *CYP2C9*, alleles considered to be reduced activity included *CYP3A4*20*, *CYP3A4*22*, *CYP2C9*2*, *CYP2C9*3*, *CYP2C9*5*, *CYP2C9*6*, *CYP2C9*8*, *CYP2C9*11* and *CYP2C9*12*; all other alleles were considered normal activity. Each patient was characterized as a poor (PM), intermediate (IM), normal (NM) or ultra-rapid (UM) metabolizer. For *CYP3A4* and *CYP2C9*, a PM is a patient carrying two reduced activity alleles, an IM is a patient carrying one reduced activity allele and an NM is a

patient carrying no reduced activity alleles. Before analysis, patients were classified into 'low' and 'high' phenotypes for each gene by grouping phenotypes into two groups to maximize the number of patients in the smaller group (e.g., PM vs IM/NM/UM or PM/IM vs NM/UM).

In addition, genome-wide genotyping of S0226 patients was conducted using the Illumina Infinium Global Screening Array by the University of Michigan Advanced Genomics Core. Genotyping and genetic data quality control were conducted as previously described [10]. Candidate variants in *SLC28A7* (rs11648166) and *ALPPL2* (rs28845026) that were previously reported to be associated with anastrozole systemic concentrations [7] were obtained from genome-wide genotyping data. The rs11648166 and rs28845026 single nucleotide variants were not incorporated onto the Illumina genotyping array used; therefore, the strongly linked variants *SLC28A7* rs16960359 (r^2 = 0.99; D′ = 1.0) and *ALPPL2* rs883013 (r^2 = 0.92; D′ = 0.97), respectively, were used in the analysis. There were insufficient numbers of patients in this pharmacokinetic substudy for genome-wide association testing of noncandidate variants.

Statistical analysis

Anastrozole and fulvestrant concentrations were compared between low and high metabolic phenotypic activity groups in the candidate pharmacogene analysis. The *a priori* selected primary hypothesis was that patients with low CYP3A4 activity would have higher anastrozole concentrations. The primary analysis included anastrozole measurements at months 2, 4, 6 and 8 using a linear mixed effects model with a random intercept, a random slope for time and an unstructured variance-covariance matrix. Analyses of anastrozole concentration were adjusted for the treatment arm due to the known effect of fulvestrant on anastrozole systemic concentrations [6]. *Post hoc* analyses were conducted stratified by treatment arm to explore whether there was an effect of metabolic activity in either arm. Exploratory pharmacogenetic analyses of all other genes with anastrozole and fulvestrant were conducted similarly to the primary analysis. Candidate variant analyses of *SLC28A7* rs16960359 and *ALPPL2* rs883013 assumed a dominant genetic effect comparing patients who carried at least one variant allele with patients homozygous for the wild-type allele. All analyses were conducted using two-sided α = 0.05 without multiplicity adjustment. The *a priori* selected primary analysis of CYP3A4 metabolic activity with anastrozole concentration should be considered hypothesis-directed and all other analyses should be considered exploratory. All analyses were conducted using R statistical software.

Results
Patients, genetics & systemic drug concentrations

Of the 707 patients enrolled on S0226, 92 had anastrozole and/or fulvestrant concentrations measured at least once during treatment, 40 in the anastrozole alone arm and 52 in the anastrozole–fulvestrant combination arm. The number of patients with measured drug concentrations who also had genetic data and were included in the analyses of anastrozole was 79; for fulvestrant, 52. The median age of the patients included in the analysis was 63, 91% were Caucasian and 39% had received prior chemotherapy treatment (Table 1).

The numbers of patients included in the analyses with low and high phenotype activity for each gene and the wild-type and variant carrier for each of the candidate polymorphisms are reported in Supplementary Table 1. For *CYP3A4*, only the *CYP3A4*22* variant was identified, and all carriers were heterozygous (*CYP3A4*1/*22*) and were assigned IM phenotype and CYP3A4 low activity (n = 12 in anastrozole analysis, n = 4 in fulvestrant analysis). Variant alleles of *CYP2C9* that were detected included *CYP2C9*2, CYP2C9*3, CYP2C9*8, CYP2C9*9, CYP2C9*11* and *CYP2C9*12*, which were translated into PM (n = 2 anastrozole, n = 1 fulvestrant) and IM (n = 20 anastrozole, n = 12 fulvestrant) phenotypes as described in the methods section, all of whom were included in the CYP2C9 low activity group. The median systemic concentration of anastrozole was 32 ng/ml (range: 21–67) and fulvestrant was 8 ng/ml (range: 1–18).

Association of systemic drug concentrations with pharmacogene activity or candidate variants

In the primary analysis, there was no difference in systemic anastrozole concentrations between low and high CYP3A4 activity phenotype groups (p = 0.13; Table 2 & Figure 1A). However, the *post hoc* analysis revealed the expected association of low CYP3A4 activity with higher anastrozole concentration in the anastrozole-only arm (β-coefficient = 10.03; 95% CI: 1.42, 18.6; p = 0.025; Figure 1B). This association was not found in the anastrozole and fulvestrant combination arm (p = 0.50; Figure 1C).

Table 1. Clinical information for patients included in the analysis (numbers are mean [standard deviation] or n [%]).		
Characteristic	Anastrozole measurement[†], n = 79 (%)	Fulvestrant measurement, n = 52 (%)
Age	63.7 (9.2)	63.8 (9.3)
Race		
White	72 (91)	47 (90)
Black	5 (6)	4 (8)
Other/unknown	2 (3)	1 (2)
S0226 treatment arm		
Anastrozole alone	40 (51)[†]	0
Anastrozole–fulvestrant combination	39 (49)[†]	52 (100)
Prior adjuvant endocrine treatment		
Tamoxifen	31 (39)	21 (40)
None	48 (61)	31 (60)
HER2 status		
Positive	3 (4)	4 (8)
Negative	62 (78)	40 (77)
Missing	14 (18)	8 (15)
Anastrozole concentrations at each time point		
2 months	72 (91)	44 (85)
4 months	74 (94)	48 (92)
6 months	76 (96)	48 (92)
8 months	79 (100)	51 (98)
Fulvestrant concentrations at each time point		
2 months		44 (85)
4 months		48 (92)
6 months		48 (92)
8 months		51 (98)

[†]Anastrozole was measured in 79 participants, 40 from the anastrozole arm and 39 from the anastrozole and fulvestrant arm.

In the exploratory analyses of the remaining genes with anastrozole and fulvestrant, patients with low CYP2C9 metabolic activity had lower anastrozole (β-coefficient = -8.1; 95% CI: -13.7, -2.5; p = 0.006; Table 2 & Figure 2A) and higher fulvestrant (β-coefficient = 1.1; 95% CI: 0.08, 2.2; p = 0.041; Table 2 & Figure 2B) systemic concentrations than patients with high CYP2C9 activity. Metabolic phenotypic activity for other tested pharmacogenes was not associated with anastrozole or fulvestrant systemic concentrations (all p > 0.05; Table 2). Similarly, neither of the candidate polymorphisms, *SLC28A7* rs16960359 nor *ALPPL2* rs883013, were associated with systemic anastrozole concentration (both p > 0.05; Table 2 & Supplementary Figure 1).

Discussion

Variability in systemic concentrations of anastrozole and fulvestrant may contribute to variable treatment response and toxicity, and it may be caused by inherited genetic variation and drug–drug interactions [11]. This study aimed to identify pharmacogenetic associations with systemic concentrations of anastrozole and fulvestrant. As hypothesized, patients with reduced CYP3A4 activity had higher concentrations of anastrozole when used alone, though this association was not seen in patients also receiving fulvestrant, which is not a standard-of-care regimen used in most patients. The authors also found evidence that lower CYP2C9 activity may be associated with lower concentrations of anastrozole and higher concentrations of fulvestrant.

The main finding supported the primary hypothesis that patients with lower CYP3A4 activity have higher steady-state systemic anastrozole concentrations. In this analysis, only the reduced activity *CYP3A4*22* [12] variant was detected, so it is not possible to investigate whether any other *CYP3A4* variants, such as the inactive *CYP3A4*20* [13] allele, affect anastrozole metabolism. The association of reduced activity *CYP3A4* polymorphisms with anastrozole pharmacokinetics has not been investigated, to the authors' knowledge, and prior studies have not found associations of *CYP3A4* genotype with downstream phenotypes such as changes in bone mineral density [14].

Gene	Comparison	Anastrozole systemic concentration† (n = 79)		Fulvestrant systemic concentration (n = 52)	
		β (95% CI)	p-value	β (95% CI)	p-value
CYP3A4	PM/IM vs NM	5.8 (91.6, 13.2)	0.13	0.1 (-1.6, 1.9)	0.88
CYP3A5	PM vs IM/NM	1.1 (-1.6, 13.2)	0.77	0.1 (-1.3, 1.5)	0.84
ABCB1	PM vs IM/NM	1.2 (-4.9, 7.3)	0.70	0.4 (-0.6, 1.5)	0.43
ABCC2	PM/IM vs NM/UM	1.6 (-4.2, 7.3)	0.60	-1.0 (-2.1, -0.001)	0.06
SULT1A1	PM/IM vs NM	0.6 (-5.0, 6.3)	0.83	0.9 (-0.09, 1.9)	0.08
UGT1A1	PM/IM vs NM	3.1 (-2.4, 8.6)	0.27	0.3 (-0.9, 1.4)	0.66
CYP2B6	PM/IM vs NM	-0.3 (-6.2, 5.6)	0.93	0.4 (-0.6, 1.4)	0.45
SLCO1B1	PM/IM vs NM	0.4 (-5.1, 5.9)	0.89	0.01 (-0.99, 1.0)	0.99
CYP2C8	PM/IM vs NM	-3.4 (-9.6, 2.8)	0.29	-0.01 (-1.1, 1.1)	0.98
CYP2C9	PM/IM vs NM	-8.1 (-13.7, -2.5)	**0.006**	1.1 (0.08, 2.2)	**0.041**
CYP1A1	PM/IM vs NM	0.7 (-6.2, 7.5)	0.84	-0.6 (-1.9, 0.62)	0.33
CYP1A2	PM/IM/NM vs UM	2.5 (-6.6, 11.6)	0.59	-0.7 (-2.3, 0.9)	0.40
UGT2B17	PM/IM vs NM	-4.7 (-9.8, 0.4)	0.07	-0.4 (-1.4, 0.5)	0.41
CYP2C19	PM/IM vs NM/UM	-3.7 (-8.9, 1.6)	0.18	0.01 (-0.97, 0.99)	0.99
CYP2A6	PM/IM vs NM	0.5 (-5.9, 6.9)	0.87	-0.7 (-1.9, 0.5)	0.29
VKORC1	PM/IM vs NM	0.8 (-4.9, 6.5)	0.78	-0.2 (-1.2, 0.8)	0.71
ABCG2	PM/IM vs NM	-4.6 (-10.5, 1.4)	0.14	0.4 (-0.7, 1.5)	0.47
UGT2B7	PM vs IM/NM	-0.5 (-6.4, 5.5)	0.88	-0.3 (-1.3, 0.7)	0.53
SLC15A2	PM vs IM/NM	-1.2 (-7.2, 4.7)	0.69	0.4 (-0.7, 1.5)	0.45
SLC22A2	PM/IM vs NM	-0.18 (-6.5, 6.1)	0.96	-0.7 (-1.7, 0.4)	0.23
SLC22A6	PM/IM vs NM	-5.7 (-28.9, 17.6)	0.63	NA	NA
SLCO1B3	PM vs IM/NM	-4.9 (-10.4, 0.69)	0.09	-0.2 (-1.2, 0.8)	0.68
TPMT	PM/IM vs NM	0.02 (-8.4, 8.5)	0.97	-0.4 (-2.2, 1.4)	0.65
CYP2E1	IM vs NM/UM	0.84 (-4.7, 6.4)	0.77	-0.1 (-1.2, 0.9)	0.79
NAT1	PM/IM vs NM	-2.9 (-13.8, 7.9)	0.60	-0.7 (-2.3, 0.9)	0.39
NAT2	PM vs IM/NM	1.3 (-4.1, 6.7)	0.63	-0.1 (-1.1, 0.9)	0.86
UGT2B15	PM vs IM/NM	-5.2 (-10.9, 0.5)	0.08	0.05 (-1.0, 1.1)	0.93
SLCO2B1	PM/IM vs NM	-2.4 (-16.0, 11.2)	0.73	0.7 (-1.7, 3.2)	0.57
SLC22A1	PM/IM vs NM	-0.06 (-5.4, 5.3)	0.98	-0.5 (-1.5, 0.4)	0.29
GSTT2B	PM/IM vs NM	-3.4 (-8.8, 2.0)	0.22	-0.3 (-1.3, 0.7)	0.55
GSTT1	PM/IM vs NM	-2.2 (-9.4, 4.9)	0.54	-0.3 (-1.4, 1.0)	0.71
GSTP1	PM/IM vs NM	0.5 (-5.4, 6.3)	0.87	0.2 (-0.8, 1.2)	0.74
GSTM1	PM/IM vs NM	-3.6 (-8.8, 1.6)	0.18	-0.03 (-1.0, 0.9)	0.95
DPYD	PM/IM vs NM	NA	NA	0.2 (-3.2, 3.7)	0.89
CYP2D6	PM/IM vs NM/UM	1.2 (-4.1, 6.6)	0.65	0.08 (-0.9, 1.1)	0.88
COMT	PM vs IM/NM	0.24 (-5.2, 5.7)	0.93	-0.6 (-1.6, 0.3)	0.20
SLC28A7 rs16960359	Variant carrier vs wild type	-2.1 (-4.8, 9.0)	0.55	–	–
ALPPL2 rs883013	Variant carrier vs wild-type	3.1 (-9.1, 2.9)	0.31	–	–

–: Analysis not attempted because prior literature indicates the association is with anastrozole systemic concentrations.
†Anastrozole analysis conducted in all patients with measured anastrozole systemic concentration.
Bold indicates p < 0.05.
IM: Intermediate metabolizer; NA: Analysis could not be conducted, since all patients had the same metabolizer phenotype; NM: Normal metabolizer; PM: Poor metabolizer; UM: Ultra-rapid metabolizer.

The authors of the present study were unable to replicate associations with anastrozole systemic concentrations for two polymorphisms (*SLC28A7* rs16960359 and *ALPPL2* rs883013) recently identified within a genome-wide association study, although further attempted validation in a larger cohort is needed to determine their clinical relevance [7].

Exploratory analyses of other pharmacogenes indicate that patients with lower CYP2C9 activity, primarily due to carrying the low activity *CYP2C9*2* or *CYP2C9*3* variants, had, surprisingly, lower anastrozole concentrations
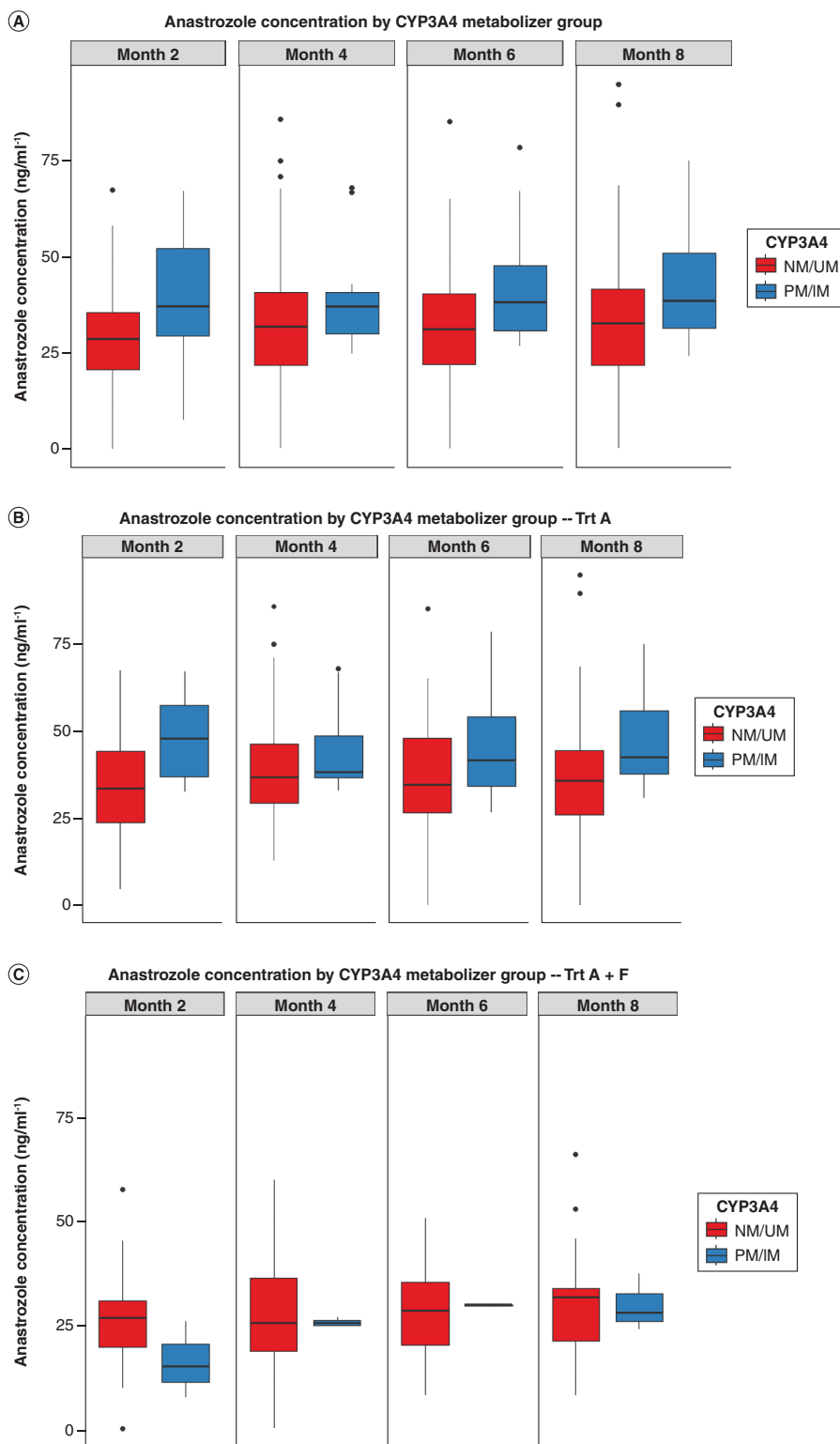
Figure 1.   Systemic anastrozole concentration by CYP3A4 metabolic activity. (A) Systemic anastrozole concentrations in all patients with measured anastrozole systemic concentration by CYP3A4 metabolic activity phenotype over time (p = 0.13). **(B)** Systemic anastrozole concentrations in the anastrozole-only treatment arm (p = 0.025). **(C)** Systemic anastrozole concentrations in the anastrozole and fulvestrant combination arm (p = 0.50). Boxplots present the median and interquartile range of anastrozole concentrations for each CYP3A4 metabolic activity phenotype group. IM: Intermediate metabolizer; NM: Normal metabolizer; PM: Poor metabolizer; UM: Ultra-rapid metabolizer.
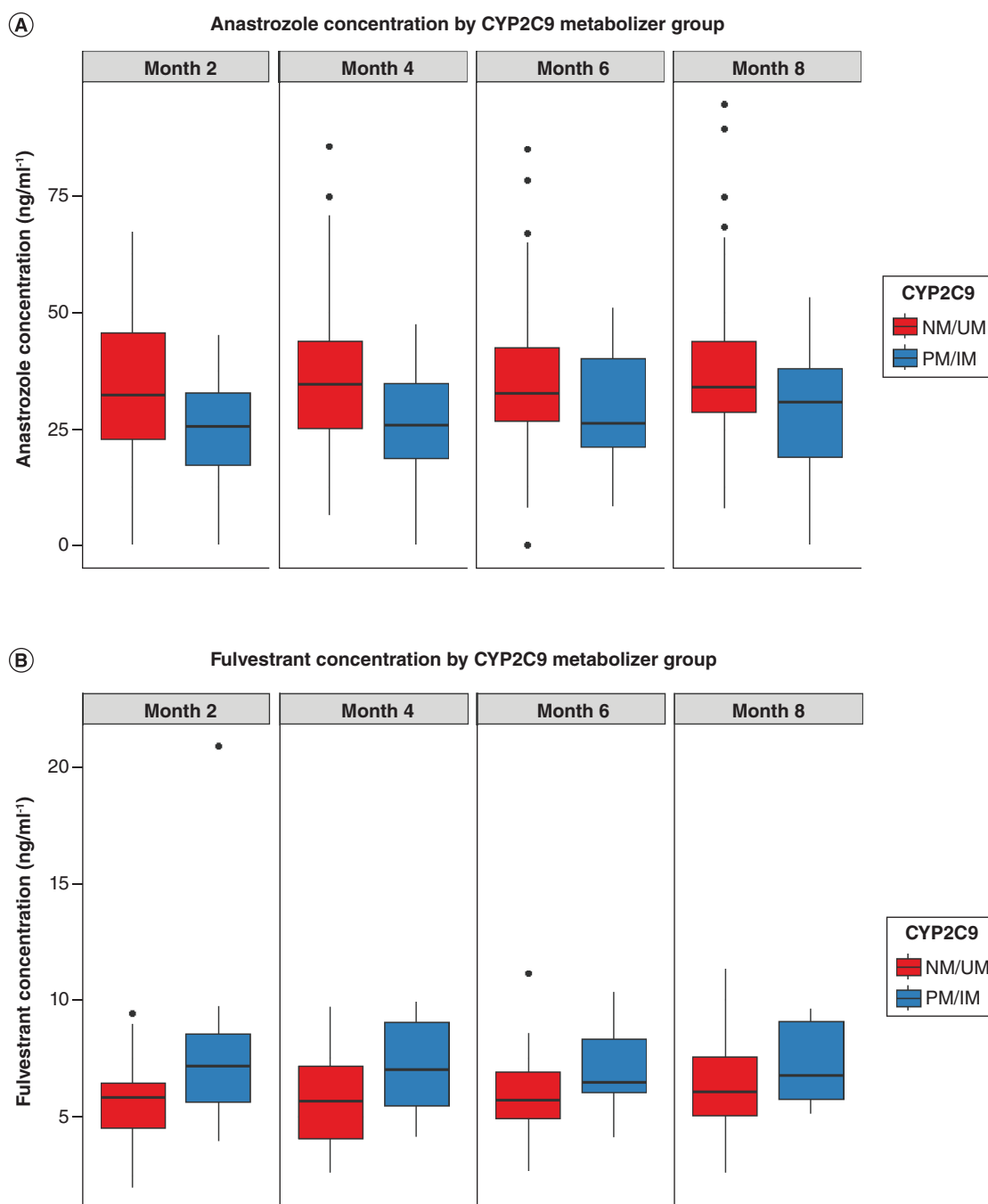
Figure 2.   Systemic anastrozole concentration by CYP2C9 metabolic activity. (A) Systemic anastrozole concentrations in all patients by CYP2C9 metabolic activity phenotype over time (p = 0.006). **(B)** Systemic fulvestrant concentrations in the anastrozole–fulvestrant combination arm (p = 0.041). The boxplots present the median and interquartile ranges of anastrozole and fulvestrant concentrations for each CYP2C9 metabolic activity phenotype group over time.
IM: Intermediate metabolizer; NM: Normal metabolizer; PM: Poor metabolizer; UM: Ultra-rapid metabolizer.

and higher fulvestrant concentrations. Low activity CYP2C9 phenotype is associated with reduced metabolism, and therefore higher systemic concentrations, of various drugs, including tamoxifen and warfarin [8,15]. Previous studies have speculated that lower CYP2C9 activity may be associated with higher anastrozole concentrations [16], but CYP2C9 is not known to contribute to fulvestrant metabolism [4]. The explanation for these unexpected findings is not known and may be a false association caused by the lack of correction for multiple comparison testing. However, it is also possible that patients with low CYP2C9 activity have higher fulvestrant concentrations,

which caused a greater interaction between these drugs [6] and resulted in lower anastrozole concentrations, though there were insufficient numbers of patients to investigate this hypothesis. Additional studies in larger cohorts of patients will be needed to confirm the effects of CYP2C9 activity on fulvestrant concentrations and perhaps the downstream effects on anastrozole concentrations.

Although this study identified pharmacogenetic predictors of systemic concentrations of anastrozole and fulvestrant, the analyses did not investigate downstream effects on clinical outcomes, including estrogenic response, efficacy or toxicity. Prior studies have not identified evidence that systemic concentrations of anastrozole, fulvestrant or other aromatase inhibitors are associated with estrogenic suppression, efficacy or toxicity [14,16–18], although the genome-wide association study that identified the associations of *SLC28A7* rs16960359 and *ALPPL2* rs883013 with anastrozole systemic concentration did report higher anastrozole systemic concentration in patients with undetectable plasma estrone and estradiol [7]. Future studies will need to validate associations between systemic anti-estrogen concentrations and clinically relevant treatment outcomes before investigating and implementing personalized dosing based on inherited genetics.

This study has some limitations that should be considered. Of the 707 patients enrolled in S0226, only 92 patients with measured systemic drug concentrations and genotyped germline DNA could be included in this analysis, limiting power to detect weaker associations or associations for lower-frequency variants of activity phenotypes and precluding genome-wide association testing. Additionally, the genotyping strategy was limited to 36 pharmacogenes and two additional candidate polymorphisms. Finally, this small, exploratory study was unable to account for multiple comparisons.

Conclusion

In conclusion, patients carrying *CYP3A4*22*, or with low CYP3A4 activity phenotype, have higher concentrations of anastrozole compared with those with high CYP3A4 activity, when it is administered without fulvestrant. In addition, patients with low CYP2C9 activity may have higher concentrations of fulvestrant and lower concentrations of anastrozole, perhaps due to a drug–drug interaction. Future studies are needed to assess the indirect effects of *CYP3A4* and *CYP2C9* genetics, or the direct effects of anastrozole and fulvestrant systemic concentrations, on clinical outcomes, which could lead to the personalization of anti-estrogen dosing based on genetics and subsequently to improved treatment outcomes.

Summary points

- Anastrozole and fulvestrant are endocrine agents commonly used to treat hormone receptor-positive breast cancer.
- Anastrozole and fulvestrant are primarily eliminated via hepatic metabolism mediated by CYP3A4.
- Interpatient variability in systemic concentrations of anastrozole and fulvestrant may be caused by genetic variation in *CYP3A4* and other drug-metabolizing enzymes and transporters.
- The authors genotyped 92 participants from the SWOG S0226 clinical trial of anastrozole alone or anastrozole plus fulvestrant using a pharmacogenetic panel that included >150 variants in 36 pharmacogenes.
- Patients were also genotyped for two variants in *SLC28A7* (rs11648166) and *ALPPL2* (rs28845026), which have been reported to be associated with systemic concentrations of anastrozole.
- The primary analysis found, as hypothesized, that patients who had low CYP3A4 activity, due to the *CYP3A4*22* variant, had higher systemic anastrozole concentrations than wild-type patients with high CYP3A4 activity.
- The association of *CYP3A4* genotype with anastrozole concentrations was detected only in the patients receiving anastrozole alone.
- In an exploratory analysis, patients with low CYP2C9 activity had lower anastrozole concentrations and higher fulvestrant concentrations than patients with high CYP2C9 activity.
- The results suggest that inherited genetic variation in *CYP3A4*, and perhaps *CYP2C9*, may affect concentrations of these endocrine agents.
- There is limited evidence that systemic concentrations of these endocrine agents determine treatment efficacy and toxicity, limiting the clinical relevance of these findings.
- If systemic concentrations of these agents are associated with clinically important treatment outcomes, genetics may be useful to personalize the dosing of these agents.

References

1. Kohler BA, Sherman RL, Howlader N *et al.* Annual report to the nation on the status of cancer, 1975–2011, featuring incidence of breast cancer subtypes by race/ethnicity, poverty, and state. *J. Natl Cancer Inst.* 107(6), djv048 (2015).

2. Hamadeh IS, Patel JN, Rusin S *et al.* Personalizing aromatase inhibitor therapy in patients with breast cancer. *Cancer Treat. Rev.* 70, 47–55 (2018).

3. Kamdem LK, Liu Y, Stearns V *et al. In vitro* and *in vivo* oxidative metabolism and glucuronidation of anastrozole. *Br. J. Clin. Pharmacol.* 70(6), 854–869 (2010).

4. Edavana VK, Yu X, Dhakal IB *et al.* Sulfation of fulvestrant by human liver cytosols and recombinant SULT1A1 and SULT1E1. *Pharmgenomics Pers. Med.* 4, 137–145 (2011).

5. Mehta RS, Barlow WE, Albain KS *et al.* Overall survival with fulvestrant plus anastrozole in metastatic breast cancer. *N. Engl. J. Med.* 380(13), 1226–1234 (2019).

6. Hertz DL, Barlow WE, Kidwell KM *et al.* Fulvestrant decreases anastrozole drug concentrations when taken concurrently by patients with metastatic breast cancer treated on SWOG study S0226. *Br. J. Clin. Pharmacol.* 81(6), 1134–1141 (2016).

7. Dudenkov TM, Liu D, Cairns J *et al.* Anastrozole aromatase inhibitor plasma drug concentration genome-wide association study: functional epistatic interaction between SLC38A7 and ALPPL2. *Clin. Pharmacol. Ther.* 106(1), 219–227 (2019).

8. Chen Y, Marcath LA, Eliassen FM *et al.* Effect of genetic variability in 20 pharmacogenes on concentrations of tamoxifen and its metabolites. *J. Pers. Med.* 11(507), 1–12 (2021).

9. Relling MV, Klein TE, Gammal RS *et al.* The Clinical Pharmacogenetics Implementation Consortium: 10 years later. *Clin. Pharmacol. Ther.* 107(1), 171–175 (2020).

10. Hertz DL, Douglas JA, Kidwell KM *et al.* Genome-wide association study of letrozole plasma concentrations identifies non-exonic variants that may affect CYP2A6 metabolic activity. *Pharmacogenet. Genomics* 31(5), 116–123 (2021).

11. Hertz DL, Henry NL, Rae JM. Germline genetic predictors of aromatase inhibitor concentrations, estrogen suppression and drug efficacy and toxicity in breast cancer patients. *Pharmacogenomics* 18(5), 481–499 (2017).

12. Elens L, van Gelder T, Hesselink DA *et al.* CYP3A4*22: promising newly identified CYP3A4 variant allele for personalizing pharmacotherapy. *Pharmacogenomics* 14(1), 47–62 (2013).

13. Westlind-Johnsson A, Hermann R, Huennemeyer A *et al.* Identification and characterization of CYP3A4*20, a novel rare CYP3A4 allele without functional activity. *Clin. Pharmacol. Ther.* 79(4), 339–349 (2006).

14. Bojanic K, Kuna L, Bilic Curcic I *et al.* Representation of CYP3A4, CYP3A5 and UGT1A4 polymorphisms within Croatian breast cancer patients' population. *Int. J. Environ. Res. Public Health* 17(10), 3692 (2020).

15. Daly AK, Rettie AE, Fowler DM *et al.* Pharmacogenomics of CYP2C9: functional and clinical considerations. *J. Pers. Med.* 8(1), 1 (2017).

16. Robertson JF, Harrison M. Fulvestrant: pharmacokinetics and pharmacology. *Br. J. Cancer* 90(Suppl. 1), S7–S10 (2004).

17. Hertz DL, Speth KA, Kidwell KM *et al.* Variable aromatase inhibitor plasma concentrations do not correlate with circulating estrogen concentrations in post-menopausal breast cancer patients. *Breast Cancer Res. Treat.* 165(3), 659–668 (2017).

18. Kadakia KC, Snyder CF, Kidwell KM *et al.* Patient-reported outcomes and early discontinuation in aromatase inhibitor-treated postmenopausal women with early stage breast cancer. *Oncologist* 21(5), 539–546 (2016).