Nonlinear Mixed-Effects Model of Z-Endoxifen Concentrations in Tamoxifen-Treated Patients from the CEPAM Cohort

Anna M. Mc Laughlin[1,2,†], Thomas Helland[3,4,5,†], Fenja Klima[1,2], Stijn L.W. Koolen[6,7], Ron H.N. van Schaik[8], Ron H.J. Mathijssen[6], Patrick Neven[9], Jesse J. Swen[10], Henk-Jan Guchelaar[10], Florence Dalenc[11,12], Melanie White-Koning[12], Robin Michelet[1], Gerd Mikus[1,13], Werner Schroth[14,15], Thomas Mürdter[14,15], Hiltrud Brauch[14,15,16,17], Matthias Schwab[15,16,17,18], Håvard Søiland[5,19], Gunnar Mellgren[4,5], Fabienne Thomas[11,12], Charlotte Kloft[1,‡], and Daniel L. Hertz[3,*,‡] on behalf of the CYP2D6 Endoxifen Percentage Activity Model (CEPAM) Consortium

Tamoxifen is widely used in patients with hormone receptor-positive breast cancer. The polymorphic enzyme CYP2D6 is primarily responsible for metabolic activation of tamoxifen, resulting in substantial interindividual variability of plasma concentrations of its most important metabolite, Z-endoxifen. The Z-endoxifen concentration thresholds below which tamoxifen treatment is less efficacious have been proposed but not validated, and prospective trials of individualized tamoxifen treatment to achieve Z-endoxifen concentration thresholds are considered infeasible. Therefore, we aim to validate the association between Z-endoxifen concentration and tamoxifen treatment outcomes, and identify a Z-endoxifen concentration threshold of tamoxifen efficacy, using pharmacometric modeling and simulation. As a first step, the CYP2D6 Endoxifen Percentage Activity Model (CEPAM) cohort was created by pooling data from 28 clinical studies (> 7,000 patients) with measured endoxifen plasma concentrations. After cleaning, data from 6,083 patients were used to develop a nonlinear mixed-effect (NLME) model for tamoxifen and Z-endoxifen pharmacokinetics that includes a conversion factor to allow inclusion of studies that measured total endoxifen but not Z-endoxifen. The final parent-metabolite NLME model confirmed the primary role of CYP2D6, and contributions from body weight, CYP2C9 phenotype, and co-medication with CYP2D6 inhibitors, on Z-endoxifen pharmacokinetics. Future work will use the model to simulate Z-endoxifen concentrations in patients receiving single agent tamoxifen treatment within large prospective clinical trials with long-term survival to identify the Z-endoxifen concentration threshold below which tamoxifen is less efficacious. Identification of this concentration threshold would allow personalized tamoxifen treatment to improve outcomes in patients with hormone receptor-positive breast cancer.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Efficacy of tamoxifen treatment may be compromised in patients with hormone-receptor positive breast cancer whose systemic Z-endoxifen is below a threshold concentration. Systemic concentrations of Z-endoxifen are determined by *CYP2D6* genotype and potentially other clinical and genetic factors.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ This study uses nonlinear mixed-effects modeling of a large (> 6,000) pooled cohort of tamoxifen-treated patients with measured systemic endoxifen concentrations to determine the contribution of genetic and clinical variables to systemic Z-endoxifen concentrations.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ The final parent-metabolite model can predict Z-endoxifen concentrations for patients based on their *CYP2D6* and *CYP2C9* genotype, body weight, and co-medication with CYP2D6 inhibitors.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ This model can be used to simulate Z-endoxifen concentrations in patients who received tamoxifen within large prospective clinical trials with long-term survival data to identify the Z-endoxifen systemic concentration threshold below which tamoxifen treatment is less efficacious.

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[1]Department of Clinical Pharmacy and Biochemistry, Institute of Pharmacy, Freie Universitaet Berlin, Berlin, Germany; [2]PharMetrX Graduate Research Training Program, Berlin/Potsdam, Germany; [3]Department of Clinical Pharmacy, University of Michigan College of Pharmacy, Ann Arbor, Michigan, USA; [4]Hormone Laboratory, Department of Medical Biochemistry and Pharmacology, Haukeland University Hospital, Bergen, Norway; [5]Department of Clinical Science, University of Bergen, Bergen, Norway; [6]Department of Medical Oncology, Erasmus MC Cancer Institute, Erasmus University Medical Center, Rotterdam, The Netherlands; [7]Department of Hospital Pharmacy, Erasmus University Medical Center, Rotterdam, The Netherlands; [8]Department of Clinical Chemistry, Erasmus University Medical Center, Rotterdam, The Netherlands; [9]Department of Gynecological Oncology and Multidisciplinary Breast Center, University Hospitals Leuven, Leuven, Belgium; [10]Department Clinical Pharmacy & Toxicology, Leiden University Medical Center, Leiden, the Netherlands; [11]Institut Claudius Regaud, Institut Universitaire du Cancer de Toulouse – Oncopole, Toulouse, France; [12]Cancer Research Center of Toulouse (CRCT), Inserm U1037, Université Paul Sabatier, Toulouse, France; [13]Department of Clinical Pharmacology and Pharmacoepidemiology, Heidelberg University Hospital, Heidelberg, Germany; [14]Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany; [15]University Tübingen, Tübingen, Germany; [16]iFIT Cluster of Excellence, University of Tübingen, Tübingen, Germany; [17]German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ), Partner Site Tübingen, Tübingen, Germany; [18]Departments of Clinical Pharmacology, and of Biochemistry and Pharmacy, University of Tübingen, Tübingen, Germany; [19]Department of Research, Stavanger University Hospital, Stavanger, Norway. *Correspondence: Daniel L. Hertz (dlhertz@med.umich.edu)

[†]Shared First Authorship.
[‡]Shared Senior Authorship.
See CEPAM Consortium Members: Appendix.

Tamoxifen is a selective estrogen receptor modulator approved for use in the prevention, adjuvant, and metastatic treatment of hormone receptor positive breast cancer and its use has resulted in large gains in survival when used in the adjuvant treatment of pre- and postmenopausal women.[1] Currently, the drug is most commonly used in the adjuvant setting for premenopausal patients either as monotherapy or with ovarian function suppression or for postmenopausal patients who cannot tolerate aromatase inhibitors, with a recommended treatment time of 5–10 years.[2,3] Despite its effectiveness, ~25% of patients with early-stage breast cancer experience recurrence within 10 years of diagnosis.

Endocrine resistance caused by aberrations in the ER/PgR signaling pathways or conversion to estrogen receptor (ER)-independent pathways[4] are established mechanisms of resistance to tamoxifen therapy. Another mechanism is metabolic resistance, which is the inability to generate adequate concentrations of tamoxifen active metabolites. Tamoxifen is a weak anti-estrogen that is metabolically activated to more potent anti-estrogens, specifically Z-endoxifen and Z-4-hydroxy-tamoxifen (4OHtam). Z-endoxifen is regarded as the most important active metabolite due to its plasma concentrations exceeding 4OHtam by 5–10 times.[5] The major route of Z-endoxifen formation is via CYP2D6, an enzyme encoded by the highly polymorphic *CYP2D6* gene. Patients carrying allelic variants that impair CYP2D6 activity have lower systemic Z-endoxifen concentrations, which may predict worse tamoxifen efficacy.[6,7] Secondary analyses of prospective studies have identified threshold Z-endoxifen concentrations below which breast cancer recurrence risk may be increased[8–11]; however, prospective studies have failed to validate a direct association between recurrence and Z-endoxifen pharmacokinetics[12–14] or *CYP2D6* genotype.[15] This has resulted in conflicting clinical guidelines on whether to use drug concentration measurements or *CYP2D6* genotype to personalize tamoxifen treatment.[16–18]

Validation of a therapeutic Z-endoxifen threshold in large, prospective tamoxifen clinical trials with long-term efficacy follow-up has not been possible because these trials did not collect samples for endoxifen measurement.[19,20] Conducting such a trial is also infeasible, as a recent analysis concluded that 1,500–4,500 patients with ER-positive breast cancer would have to be followed for many years for a sufficiently powered trial.[21] Previous attempts to use retrospectively genotyped *CYP2D6* as a surrogate marker for predicting Z-endoxifen concentrations in these trials have been unsuccessful,[19,20] possibly because *CYP2D6* genotype explains only about half of the variability in Z-endoxifen concentration[6] and potentially due to technical challenges with genotyping archived tumor tissue,[22,23] limitations with translating genotype to activity phenotype,[24] or inclusion of patients currently receiving other anti-cancer treatment.[25] Clinical factors, such as age, weight, and concomitant medications, also contribute to tamoxifen/Z-endoxifen pharmacokinetics.[5,6,26–28] The generation of endoxifen from tamoxifen requires multiple metabolic steps and genetic alterations in enzymes responsible for upstream metabolism have also been associated with endoxifen concentrations. Reduced activity variants (*2 and *3) in CYP2C9 have been associated with lower endoxifen concentrations in several studies, whereas CYP3A4 *22, a reduced activity variant, demonstrated the opposite effect by increasing endoxifen levels in tamoxifen-treated patients.[6,27] In addition, genetic alterations in phase II metabolic enzymes, such as UGTs and SULTs, responsible for endoxifen elimination have been demonstrated to affect endoxifen concentration.[6]

The primary objective of the CYP2D6 Endoxifen Percentage Activity Model (CEPAM) consortium is to apply pharmacometric modeling and simulation to existing data to determine whether there is an association between Z-endoxifen pharmacokinetics and tamoxifen treatment outcomes. The first critical step, reported here, is the generation of an endoxifen concentration prediction algorithm by pharmacometric modeling of existing datasets of tamoxifen-treated patients in whom systemic endoxifen concentrations were also measured, and pharmacogenetic and clinical data are available.

METHODS

Clinical study database

The raw CEPAM database collected clinical study cohorts of patients receiving tamoxifen treatment with endoxifen concentration measurement and *CYP2D6* genetic data. All studies had been conducted in accordance with the Declaration of Helsinki and approved by the respective institutional review board. Tamoxifen and Z-endoxifen or total endoxifen (measurements obtained using assays not separating Z- and Z-4′-endoxifen) concentrations had been quantified in plasma or serum using various analytical methods (detailed information in Table S1). For each patient, one measurement was available for inclusion in the CEPAM database. Genetic variants (Table S2) and enzyme inhibitors and inducers for *CYP2D6* and other relevant pharmacogenes were collected from each study that had such data available.

In the data cleaning step, each pharmacokinetic study was checked for representation of the target population, completeness, meeting the inclusion criteria, and missing data values. Graphical and numerical methods

were used to assess dose-normalized endoxifen and tamoxifen concentrations, and studies with concentration ranges that deviated substantially with no explanation were excluded. Missing data in the analysis dataset was addressed using imputation. Details on the data cleaning process, including the studies/patients that were excluded and the rationale, and a detailed list of the imputation strategy are provided in **Supplementary Text S1**.

Tamoxifen adherence, based on patient self-report, medication diaries, or prescription records, was reported for < 50% of the patients in the database. Therefore, tamoxifen concentrations ≤ 60 ng/mL[29] were used to assign nonadherence status in patients receiving ≥ 20 mg tamoxifen daily.

Development of the Z-endoxifen concentration prediction algorithm

The development of the endoxifen concentration prediction algorithm was to be based on a previously published joint parent-metabolite nonlinear mixed-effects pharmacokinetic model of tamoxifen and Z-endoxifen.[26] The model was selected following the principle of parsimony and taking the patient population and the available analyte measurements into consideration. From recently published models,[30,31] the most recent and parsimonious model built on the broadest patient cohort compared with other available models of the two analytes tamoxifen and Z-endoxifen was selected.[26] In short, this pharmacokinetic model consisted of a gut compartment, a tamoxifen compartment, and a Z-endoxifen compartment (**Figure 1a**) and postulated linear absorption, metabolism, and elimination processes. Detailed information on this model and its development are available in our previous publications.[26,32]

Using the previous pharmacokinetic model as a starting point, the development of the endoxifen concentration prediction algorithm using NONMEM consisted of several steps:

1. External evaluation of the previous model using the CEPAM analysis dataset.
2. Exploratory graphical analysis of the CEPAM analysis dataset to identify potential additional covariates for testing.
3. Covariate analysis to refine already included covariate relationships and test for the inclusion of new covariates.
4. Model extension to allow inclusion of patients with total endoxifen measurements.

External model evaluation. The external evaluation aimed to identify the overall predictive performance of the previously published pharmacokinetic model for the CEPAM analysis dataset. For this, the model was used to predict tamoxifen and Z-endoxifen concentrations in the CEPAM analysis dataset (excluding patients with co-medication, nonadherent patients, patients with only total endoxifen concentrations, and patients included in 2 studies (18 and 19), which had already been used for model development). The predictions were then compared with the measured concentrations, and bias and precision were assessed using mean prediction errors (MPEs) and mean absolute prediction errors (MAPEs), respectively.[33]

Exploratory graphical analysis of the CEPAM analysis dataset. An exploratory graphical analysis (EGA) was performed to identify trends in tamoxifen and/or Z-endoxifen concentrations across patient and/or treatment characteristics and to guide the selection of potential patient/treatment characteristics to be included for the covariate analysis. A list of tested patient and treatment characteristics included in the EGA is provided in **Supplementary Text S1**.

Covariate analysis. Patient and treatment characteristics that had shown a trend in the exploratory graphical analysis and whose impact on a model parameter was considered physiologically plausible were selected for testing on model parameters, that is, tamoxifen clearance (CL20/F), Z-endoxifen formation (CL23/F) or tamoxifen bioavailability (F). Available genotype data were translated to predicted activity phenotype, as previously described.[27,34] To reduce the number of parameters to estimate, the previously applied categorical implementation of CYP2D6 activity score (AS) on CL23/F[26] was replaced with an exponential ordinal CYP2D6 AS covariate relationship,

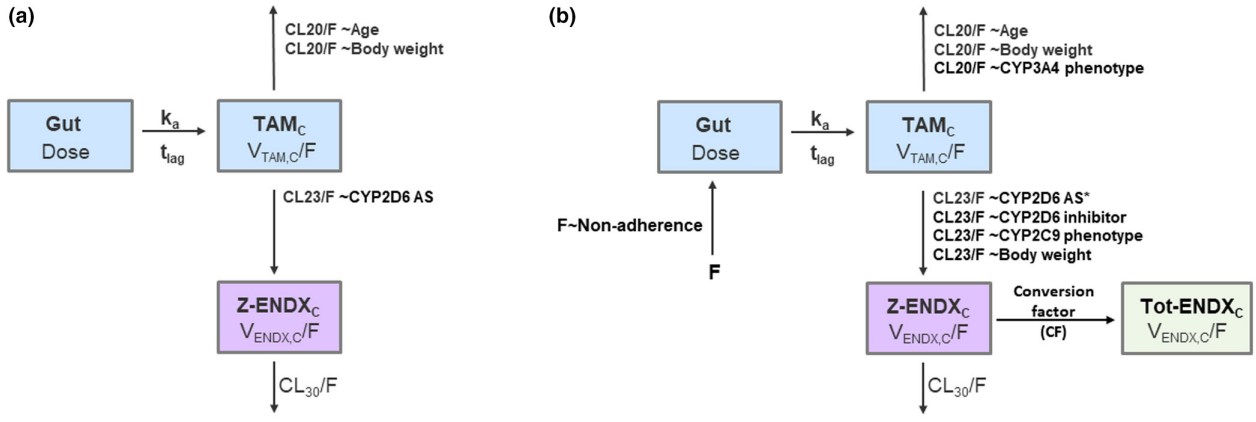

Figure 1 Schematic representation of the joint tamoxifen (TAM) and endoxifen (END) pharmacokinetic model. The model and implemented covariate relationships for the starting model were generated from a previous publication[26] (**a**) and extended in this updated analysis (**b**). (**a**) Tamoxifen was modeled to be absorbed from the gut compartment by a first-order process (k_a) with lag time (t_{lag}). Once absorbed and distributed in the central compartment (TAM_C) with the apparent volume of distribution $V_{TAM,C}/F$, it could either be metabolized to Z-endoxifen (apparent formation C23/F) or to other metabolites (apparent clearance CL20/F); both metabolism pathways were implemented as first-order processes. The apparent elimination of Z-endoxifen from its central compartment (Z-$ENDX_C$) with apparent volume of distribution $V_{ENDX,C}/F$ was modeled using a first-order process (CL30/F). Covariate-parameter relationships on CL20/F and CL23/F which had been included in the starting model are shown using tildes (~). (**b**) In the extended model, total endoxifen (TOT-$ENDX_C$) was included using a conversion factor (CF) from the central Z-endoxifen compartment. Covariate-parameter relationships on CL20/F, CL23/F, or on the apparent bioavailability (F) which had been included in the starting model or were additionally identified during model development are shown using tildes (~). Covariate-parameter relationships with a star (*) had been included in the starting model but were now refined in the extended model.

requiring the estimation of a single parameter only. The same approach was applied for the implementation of CYP2C9 and CYP3A4 metabolic phenotypes and the implementation of co-medication with CYP enzyme inhibitors and inducers, provided that the strength of the inhibitor/inducer was reported. Continuous covariates were normalized to the median value in the CEPAM dataset and implemented using power functions. Nonadherence was implemented on F using a fractional change model. Concomitant medication information was translated into strength of inhibition or induction for each relevant enzyme system using the Drug Interactions Flockhart Table[35] and tested as an ordinal covariate in the model. Stepwise covariate modeling[36] using significance criteria of $\alpha = 0.05$ for the forward inclusion followed by $\alpha = 0.001$ for the backward exclusion was performed to assess the significance of the potential covariate impact and account for multivariate analysis. Covariates included in the previous model were re-tested in the backward exclusion step to determine whether they still provided significant contributions. For covariates of interest, if the percentage of missing data was high (fractions > 32%), a sensitivity analysis regarding the impact of the imputation was performed by excluding patients with missing covariate information and re-estimating model parameters.

Model extension to allow for inclusion of patients with total endoxifen measurements

Total endoxifen is the sum of the active Z-endoxifen and other inactive endoxifen isomers, of which Z-4′-endoxifen is the most abundant.[37] Z-endoxifen represents approximately half of the total, but this can vary

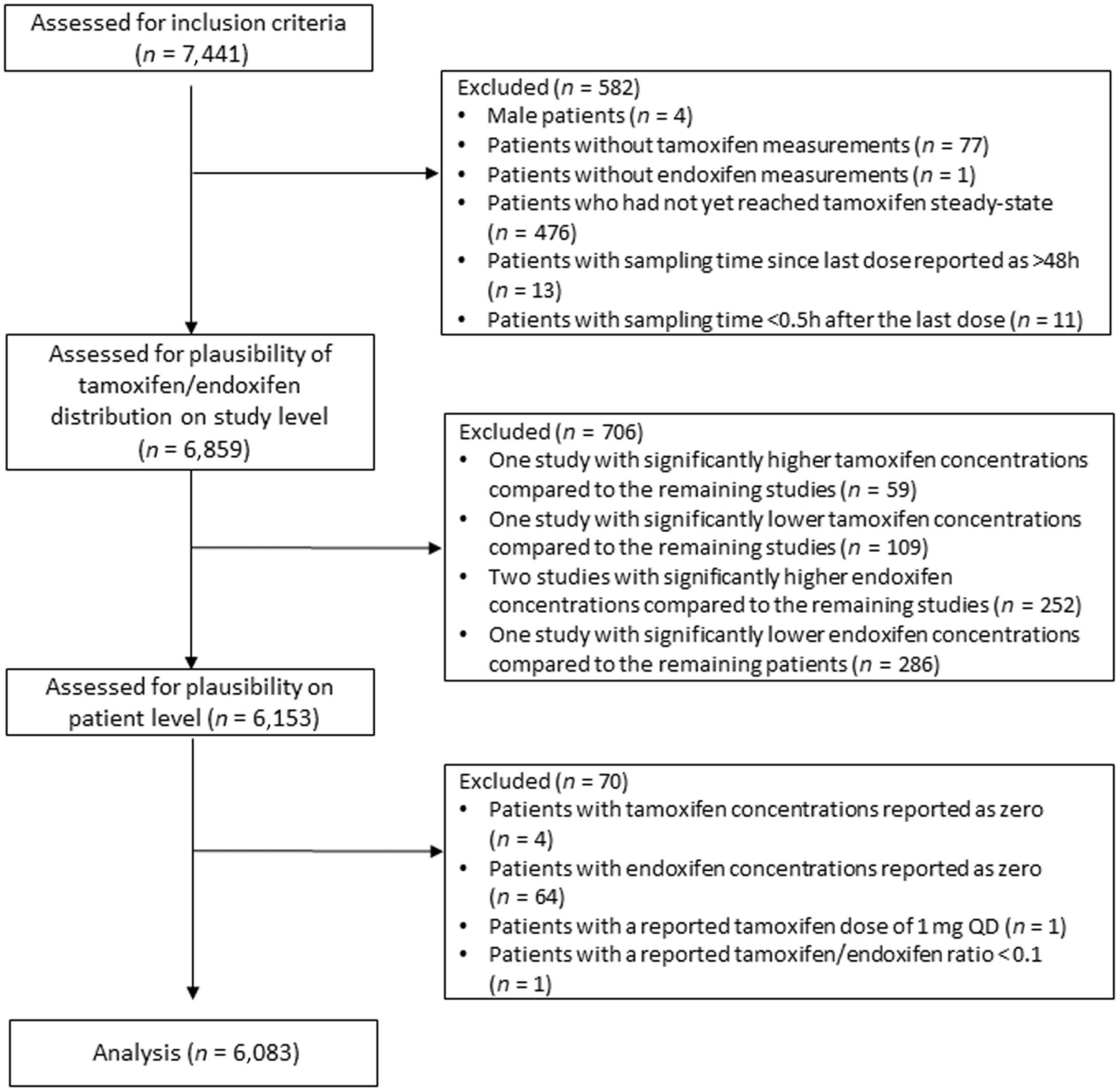

Figure 2 CONSORT diagram. Patient and data exclusion steps from the complete CEPAM database to the CEPAM analysis dataset are depicted. CEPAM, CYP2D6 Endoxifen Percentage Activity Model.

dramatically between patients[38] and may be related to genetic and clinical variables. The CEPAM database includes endoxifen concentrations measured by various bioanalytical assays, some of which did not separate the isomeric forms of endoxifen. To allow the inclusion of studies with measured total endoxifen concentrations into the analysis, the model was investigated for extension. The formations of Z-endoxifen and Z-4′-endoxifen are assumed to be competing processes[27] and the conversion factor is therefore assumed to vary based on the individual Z-endoxifen formation.[37] Consequently, the conversion factor was implemented in the model as a function of the individual Z-endoxifen formation (CL23/F). Details on the model extension process are given in **Supplementary Text S1**.

Assessment of model performance

Information on the parameter estimate precision was obtained using sampling importance resampling.[39] Model performance was assessed using the objective function value (OFV) criterion, goodness-of-fit plots, plots of conditional weighted residuals vs. typical predictions and time, and stratified prediction-corrected visual predictive checks (pcVPCs)[40] using 200 simulations for accurate predictions.

RESULTS

CEPAM analysis dataset

The raw CEPAM database included 7,441 patients from 28 study cohorts, from which studies and patients were eliminated for a variety of reasons (**Figure 2**): 582 (7.8%) patients were removed due to not meeting the analysis inclusion criteria (**Supplementary Text S2**). Five studies (9.5% of the patients) were removed due to substantial deviations from tamoxifen or endoxifen ranges as compared with the rest of the database (**Supplementary Text S2**). Finally, 70 (0.94%) patients were removed due to implausible concentration measurements or dosing as specified in **Figure 2**. The final CEPAM analysis dataset comprised 6,083 patients from 23 study cohorts (**Table 1**, **Table S3**).

Estimation of Z- and total endoxifen

The measured endoxifen type was reported as total and Z-endoxifen for 21.8% and 78.2% of patients, respectively. Four studies (5.6% of patients in the raw database) were re-assigned from total to Z-endoxifen, and one study (2.4% of patients in the raw database) was re-assigned from Z-endoxifen to total endoxifen based on inspection of concentration distributions and after review of the analytical chemistry methods (**Supplementary Text S2**). To test the impact of the study re-assignment on the final parameter estimates, the re-assigned studies were removed and the model parameters re-estimated. No substantial changes in the parameter estimates were observed. After re-assignment, 86% of endoxifen concentration measurements were assumed to be Z-endoxifen measurements. For 26% of patients with Z-endoxifen measurements, additional Z-4′-endoxifen measurements were available.

Development of the endoxifen concentration prediction algorithm

External model evaluation. The model predictions, especially for the relevant lower concentration range, for the external

Table 1 Clinical study and population characteristics in the CEPAM analysis dataset

Characteristic	Total (n = 6,083) number of patients or PK measurements (%), except for age and body weight: median, range
Number of PK measurements (after re-classification)	
Tamoxifen	6,083 (100)
Z-endoxifen	5,231 (86.0)
Z-4′-endoxifen	1,384 (22.8)
Total endoxifen	852 (14.0)
Tamoxifen dose	
5 mg q.d.	154 (2.53)
10 mg q.d.	4 (0.0658)
20 mg q.d.	5,919 (97.3)
40 mg q.d.	2 (0.0329)
Missing data[a]	4 (0.0658)
Age, years	
Median	51
Range	22–95
Missing data[a]	472 (7.76)
Body weight [kg]	
Median	67
Range	32–190
Missing data[a]	2,022 (33.2)
CYP2D6 activity score	
0	324 5.33)
0.25	97 (1.59)
0.5	497 (8.17)
0.75	33 (0.542)
1	111 (26.5)
1.25	427 (7.02)
1.5	707 (11.6)
2	2,168 (35.6)
2.25	3 (0.0493)
2.5	17 (0.279)
3	125 (2.05)
Missing data[a]	74 (1.22)
CYP2D6 inhibitor co-medication[b]	
None	3,832 (63.0)
Weak	203 (3.34)
Moderate	7 (0.115)
Strong	64 (1.05)
Missing data[a]	1,977 (32.5)
CYP3A4 genotype-predicted phenotypes	
Poor metabolizer	3 (0.0493)
Intermediate metabolizer	230 (3.78)
Normal metabolizer	2,208 (36.3)
Missing data[a]	3,642 (59.9)

(Continued)

Table 1 (Continued)

Characteristic	Total ($n = 6,083$) number of patients or PK measurements (%), except for age and body weight: median, range
CYP2C9 genotype-predicted phenotypes	
Poor metabolizer	28 (0.460)
Intermediate metabolizer	446 (7.33)
Normal metabolizer	1,100 (18.1)
Missing data[a]	4,509 (74.1)
Adherence (PK threshold > 60 ng/mL)[c]	
Yes	5,410 (88.9)
No	663 (10.9)
Missing data[a]	10 (0.164)

Further clinical study and population characteristics in the CEPAM analysis dataset are provided in **Table S3**.
CEPAM, CYP2D6 Endoxifen Percentage Activity Model; PK, pharmacokinetic(s); q.d., once daily.
[a]Missing data was imputed for analysis as described in **Supplementary Text S1**. [b]Inhibitor strength as defined by Flockhart Table available at: https://medicine.iu.edu/internal-medicine/specialties/clinical-pharmacology/drug-interaction-flockhart-table. [c]For patients receiving tamoxifen doses <20 mg, adherence was assigned as reported in the database.

evaluation dataset ($n = 3,615$ patients) were accurate and precise (**Figure 3**), as indicated by small MPEs of 1.91 ng/mL and −0.983 ng/mL, and small/moderate MAPEs of 7.75 ng/mL and 7.05 ng/mL for tamoxifen and Z-endoxifen, respectively. Thus, the previously published joint parent-metabolite model[26] was considered appropriate for use in the further model development.

Exploratory graphical analysis of the CEPAM analysis dataset. Several associations between patient/treatment characteristics and the two main model parameters were identified: Z-endoxifen formation (CL23/F) showed an inverse relation with body weight and a direct relation with increasing CYP2C9 activity; tamoxifen clearance (CL20/F) was positively related with increasing CYP3A4 activity. Co-medication with CYP2D6 inhibitors was associated with lower Z-endoxifen formation whereas co-medication with CYP3A4 inducers was associated with higher tamoxifen clearance. For co-medication with CYP2C9 inducers or inhibitors, a weak association was observed with tamoxifen clearance. All other potential covariates tested did not show any trends (**Supplementary Text S2**).

Covariate analysis. **Table S4** and **Supplementary Text S2** show all tested covariate-parameter relationships, their result during stepwise covariate modeling and the final covariate-parameter equations; **Table 2** shows their estimates. The magnitude of the covariate impact was high on Z-endoxifen formation (CL23/F) for CYP2D6 AS[34] (62% decrease from AS = 2 to AS = 0) and CYP2D6 inhibitor co-medication (60% decrease for strong inhibitors; **Figure 4**) and on tamoxifen F for nonadherence (66% lower). The magnitude of the covariate impact was moderate-to-low for other covariates: Z-endoxifen formation was impacted by CYP2C9 phenotype (27% decrease from normal metabolizer (NM) to poor metabolizer (PM)) and body weight (53% decrease from lightest to heaviest; **Figure S2**) and tamoxifen clearance by age (20% increase from oldest to youngest), body weight (47% increase from lightest to heaviest), and CYP3A4 phenotype (30% increase from PM to NM). In total, a patient with "worst case" combination of the dataset characteristics for Z-endoxifen formation being at highest risk of subtherapeutic Z-endoxifen (5th or 95th percentiles of patient characteristics: young (37 years), heavy (100 kg) with CYP2D6 AS 0, CYP3A4 nNM and CYP2C9 intermediate metabolizer (IM) phenotype and weak CYP2D6 inhibitor co-medication) revealed a 80% lower Z-endoxifen formation (without CYP2D6 inhibitor co-medication: 72% lower Z-endoxifen formation) and 30% higher tamoxifen clearance

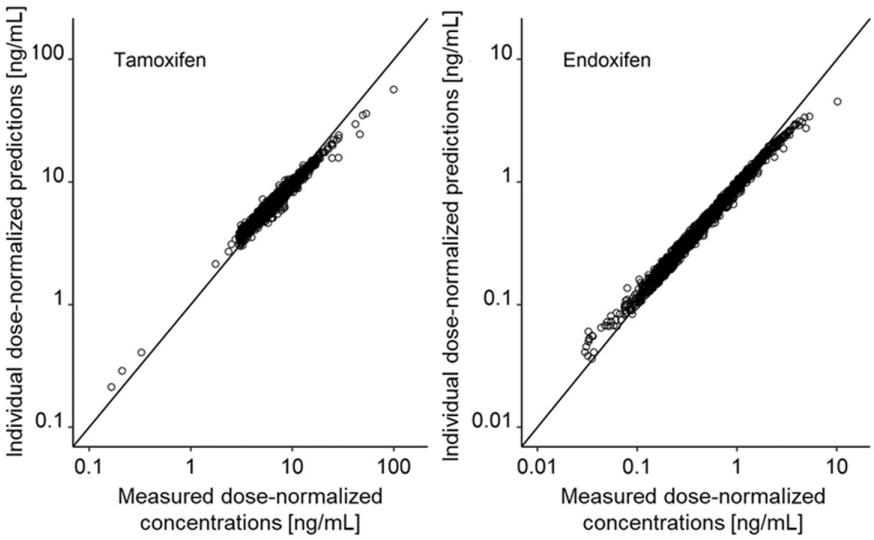

Figure 3 Goodness-of-fit plots for external evaluation. Goodness-of-fit plots showing the external evaluation results for the previous model for the measured tamoxifen (left) and Z-endoxifen (right) concentrations in the CEPAM analysis dataset. The diagonal black line marks the line of identity. CEPAM, CYP2D6 Endoxifen Percentage Activity Model.

Table 2 Final parameter estimates of the extended joint parent-metabolite tamoxifen and endoxifen model and their imprecision

	Parameter [unit]	Parameter description	Estimate	RSE, %
Fixed effects	k_a [1/h][a]	Absorption rate constant	1.08	Fixed
	t_{lag} [h][a]	Absorption lag time	0.442	Fixed
	V_{TAM}/F [L][a]	Tamoxifen volume of distribution	912	Fixed
	CL30/F [L/h][a]	Z-endoxifen clearance	5.10	Fixed
	V_{ENDX}/F [L][a]	Z-endoxifen volume of distribution	400	Fixed
	CL20/F [L/h]	Tamoxifen clearance for the reference patient	5.36	1
	CL20/F_Age[b]	Exponent for the covariate effect of age on the tamoxifen clearance	−0.126	23
	CL20/F_Body weight[b]	Exponent for the covariate effect of body weight on the tamoxifen clearance	0.213	15
	CL20/F_CYP3A4 phenotype[c]	Fractional change in the tamoxifen clearance for CYP3A4 phenotype ≠ IM	0.129	9
	CL23/F [L/h]	Z-endoxifen formation for the reference patient	0.569	1
	CL23/F_CYP2D6 AS[c]	Fractional change in the Z-endoxifen formation for CYP2D6 AS≠2	0.480	2
	CL23/F_CYP2D6 inhibitor[d]	Fractional change in the Z-endoxifen formation for CYP2D6 inhibitors	−0.302	7
	CL23/F_CYP2C9 phenotype[c]	Fractional change in the Z-endoxifen formation for CYP2C9 phenotype ≠ NM	0.158	16
	CL23/F_Body weight[b]	Exponent for the covariate effect of body weight on the Z-endoxifen formation	−0.419	11
	F_Nonadherence	Fractional change in the apparent bioavailability due to non-adherence	−0.661	1
	Conversion Factor	Conversion factor intercept (power function)	1.44	1
	Conversion Factor_Exponent	Conversion factor exponent (power function)	−0.333	3
Random effects	IIV CL20/F	Interindividual variability in the tamoxifen clearance	43.7% CV	1
	IIV CL23/F	Interindividual variability in the Z-endoxifen formation	60.5% CV	1
	IIV CF	Interindividual variability in the conversion factor	12.3% CV	10
	RUV tamoxifen	Residual unexplained variability in the observed tamoxifen concentrations	0.0295 (17.3% CV)	Fixed
	$COV_{RUVtam\text{-}RUVendx}$	Correlation between RUV tamoxifen and RUV endoxifen	0.0228	Fixed
	RUV endoxifen[e]	Residual unexplained variability in the observed endoxifen concentrations	0.037 (19.4% CV)	Fixed

The reference patient had a body weight of 67 kg, an age of 51 years, CYP2D6 AS 2, CYP3A4 IM phenotype, CYP2C9 NM phenotype, was considered adherent and received no CYP2D6 inhibitor co-medication. Final parameter equations including reference covariate values are provided in **Supplementary Text S2**.
[a]Model parameters were fixed to the estimates from the previously published pharmacokinetic model as the dataset comprised mainly minimal concentrations not containing sufficient informativeness to estimate them. [b]Implemented as power covariate model. [c]Fractional change using an ordinal scale. [d]Fractional change using an ordinal scale (0: no inhibitor, 1: weak inhibitor, 2: moderate inhibitor, 3: strong inhibitor). [e]RUV parameters were fixed to previously published values,[26] as the availability of only one sample per patient did not allow the simultaneous identification of IIV and RUV parameters.

compared with the reference patient (51 years, 67 kg, CYP2D6 AS 2 with CYP3A4 IM and CYP2C9 NM phenotype and no CYP2D6 inhibitor co-medication; see **Table 2**). Comparable parameter estimates were observed for covariates impacting the endoxifen formation rate or tamoxifen clearance in the sensitivity analysis when excluding the imputed missing values for CYP2D6 inhibitor information, CYP2D6 phenotype, CYP2C9 phenotype, and CYP3A4 phenotype, demonstrating imputation to be justified (see **Table S5**). In addition, the individual parameter estimates for Z-endoxifen formation and tamoxifen clearance stratified by CYP2D6 phenotype, CYP2D6 inhibitor, CYP2C9 phenotype, or CYP3A4 phenotype, respectively, showed an incremental increase among the groups, supporting the implementation of these covariates using ordinal scales.

Model extension to allow for inclusion of patients with total endoxifen measurements. The model was successfully extended with a total endoxifen compartment linked to the Z-endoxifen compartment via a conversion factor from Z-endoxifen to

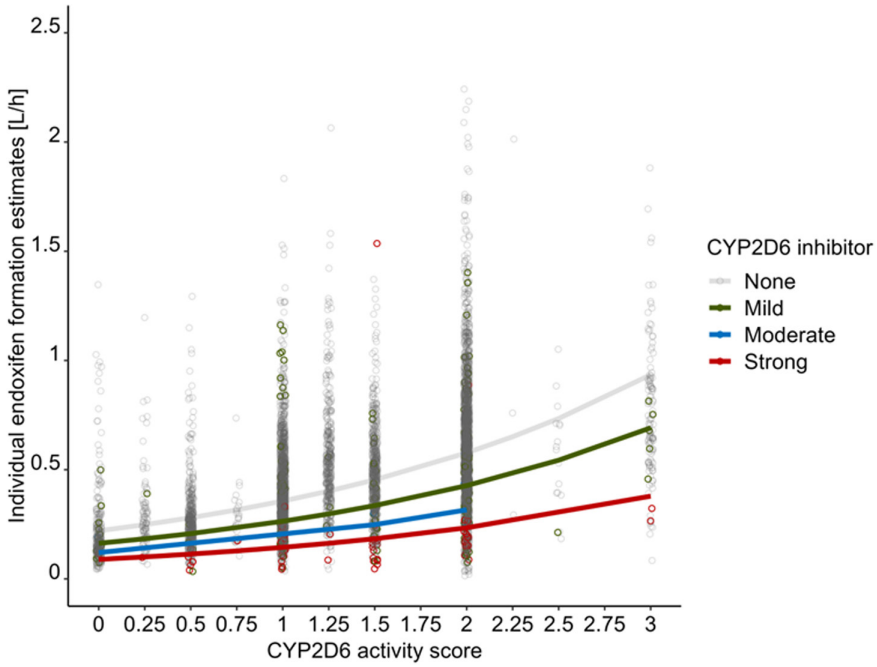

Figure 4 Impact of CYP2D6 activity and CYP2D6 inhibitors on individual endoxifen formation. Dots are the individual endoxifen formation estimates. Colored lines: typical endoxifen formation relation to CYP2D6 activity score stratified by co-medication with no, mild, moderate, or strong CYP2D6 inhibitor.

total endoxifen estimated based on the CEPAM analysis dataset (**Figure 1b**, **Supplementary Text S1**: Derivation of the Z-endoxifen – total endoxifen conversion factor). The median estimated conversion factor was 1.94 (range: 1.19–3.92) allowing the inclusion of both Z-endoxifen only and total endoxifen only studies in the analysis. The final model structure is shown in **Figure 1b** and the final model parameters are presented in **Table 2**.

Assessment of model performance

All model parameters were estimated with sufficient precision. The model predictions captured the observed concentrations of tamoxifen (**Figure S3**), and both total and Z-endoxifen (**Figure 5**, **Figure S5**). The conditional weighted residuals vs. typical predictions were randomly spread around zero and thus did not indicate systematic bias in the model predictions. Finally, the pcVPCs for both tamoxifen and endoxifen confirmed the high predictivity of the developed model demonstrated by the high overlap of the observed concentrations and the model predictions along all percentiles (**Figure S4**).

DISCUSSION

An association between Z-endoxifen concentrations and tamoxifen efficacy has been observed[8–11]; however, it has been challenging to validate in prospective trials and is unlikely to be directly validated in existing datasets or future prospective clinical trials.[21] Indirect validation via *CYP2D6* genotype as a surrogate of Z-endoxifen concentration has also been unsuccessful, perhaps because CYP2D6 only partially explains the variability in Z-endoxifen concentrations[6] and other factors, including concurrent anti-cancer treatment.[25] As such, we conducted a pooled analysis of data from >6,000 tamoxifen-treated patients with measured endoxifen concentration to develop a precision endoxifen concentration prediction algorithm based on a previously published parent-metabolite pharmacokinetic model.[26] Importantly, the resulting extended model confirmed the primary role of *CYP2D6* genotype on Z-endoxifen concentration and identified significant contributions from additional clinical and genetic variables. This final joint parent-metabolite model could be used to simulate Z-endoxifen concentrations in patients who participated in large prospective clinical trials of tamoxifen to test the association between (simulated) Z-endoxifen pharmacokinetics and tamoxifen treatment survival.

CYP2D6 genotype-predicted phenotype has been reported to explain up to 50% of Z-endoxifen concentration variability.[6] Although our model-based results cannot be directly compared with R^2 measures from linear regression, our results confirm the predominant effect of *CYP2D6* genotype-predicted phenotype. In our model, 15% of the total 18% of variability in Z-endoxifen formation explained by the model was due to *CYP2D6* (15%/18% = 84%; see **Supplementary Text S2**). Numeric modeling diagnostic parameters such as OFV and Akaike information criterion (AIC) also confirm this, as removing *CYP2D6* AS causes the largest reduction in model performance of any variable influencing Z-endoxifen formation (ΔOVF = +1,398 and ΔAIC = +1,395). The model confirmed, and has now quantified, the previously reported contributions of additional variables including age,[41] body weight,[9] and CYP2D6 inhibitor use[5,6,28] to Z-endoxifen concentrations. Of note, patients who are younger, have higher body weight, and are co-administered CYP2D6 inhibitors have lower Z-endoxifen concentrations, and may be at higher risk of tamoxifen treatment failure.[26]

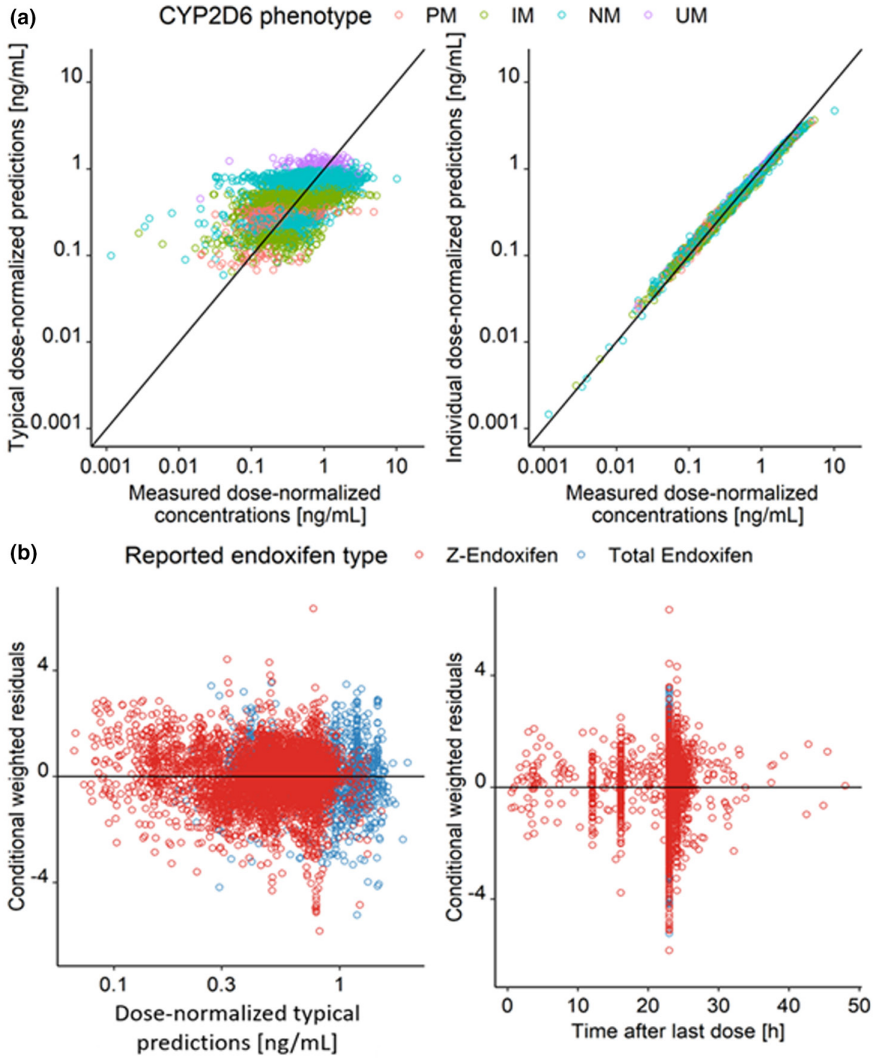

Figure 5 Goodness-of-fit plots for the final model. (**a**) Goodness-of-fit plots for the final extended joint parent-metabolite model comparing typical (left) and individual (right) dose-normalized endoxifen predictions with the measured dose-normalized concentrations, colored by CYP2D6 genotype-predicted phenotype. Measured and predicted total endoxifen concentrations were converted to Z-endoxifen predictions using the individual model-estimated conversion factors. The diagonal black line marks the line of identity. (**b**) Diagnostic plots for the endoxifen predictions using the final extended joint parent-metabolite model. Left: conditional weighted residual vs. typical endoxifen predictions, colored by endoxifen type. Right: Conditional weighted residual vs. time after last dose. The black horizontal lines mark the zero-line. IM, intermediate metabolizer; NM, normal metabolizer; PM, poor metabolizer; UM, ultrarapid metabolizer.

Inherited variants in genes encoding several other tamoxifen-metabolizing enzymes (e.g., CYP3A4/5, CYP2C9/19, SULTs, and UGTs) have been reported to contribute to concentrations of Z-endoxifen or other metabolites.[6] Our pooled analysis confirmed and quantified contributions for *CYP3A4* and *CYP2C9* genotype-predicted phenotype, but did not confirm roles for other variables reported to be associated with tamoxifen metabolism or benefit including *CYP2C19* phenotype[42] or CYP3A4 inhibitor co-administration.[43] CYP3A4 metabolizes tamoxifen to N-desmethyltamoxifen and 4OHtam to Z-endoxifen. *CYP3A4*22* is a variant that is associated with reduced CYP3A4 activity. Patients homozygous for *CYP3A4*22* (PM) and patients with *CYP3A4*1/*22* (IM) had a 23% and 12% lower predicted tamoxifen clearance than NMs, respectively, consistent with other studies reporting higher tamoxifen concentrations among these patients,[27,30] which may be related to the role of CYP3A4 in first-pass metabolism. We also observed increased tamoxifen clearance to be associated with the use of CYP3A4 inducers, further indicating a role for CYP3A4 in first-pass metabolism. Carriers of common, diminished activity *CYP2C9* variant alleles (e.g., *CYP2C9*2* and **3*) have been reported to have lower Z-endoxifen concentrations,[44] perhaps due to less conversion of tamoxifen to 4OHtam.[10] This is in line with our results, showing a moderately decreased (27%) estimated Z-endoxifen formation with decreased CYP2C9 activity from NM to PM.

Overall, clinical and non-CYP2D6 genetic variables explained < 20% of the total explained variability in Z-endoxifen formation. This estimate is consistent with other studies that reported relatively minor contributions from non-CYP2D6 variables on Z-endoxifen concentrations.[6,26] Regardless of the relatively minor contribution of these variables, our population pharmacokinetic

modeling approach incorporates new aspects compared with previously reported regression and pharmacokinetics models. Our final parent-metabolite model accounts for several levels of variability and can be used to predict Z-endoxifen concentrations. Importantly, our extended model now allows for the inclusion of studies regardless of whether only Z-endoxifen or only total endoxifen has been measured. Furthermore, our extended model is significantly more precise than our previously published model,[26] as indicated by the distribution of the datapoints in the observation vs. prediction plot around the line of identity, which served as a starting point for this analysis (**Figure 5a**). Another advantage of our model is that it can incorporate data from nonadherent patients, which enables a fit-for-purpose analysis of "real-world" data. Despite these advantages, given that the current model explains only a fraction of Z-endoxifen variability (18%), immediate use in clinical trials for the prediction of endoxifen levels is unlikely without further model refinement, for example, implementation of linear-scaled *CYP2D6* allele activities.

The CEPAM model will be used in future studies to indirectly validate the association of Z-endoxifen pharmacokinetics with efficacy of single-agent tamoxifen treatment using data from large clinical trials with prospectively collected recurrence and survival data (e.g., ATAC and BIG 1–98).[6,19,20] If the association can be validated, the endoxifen concentration prediction algorithm could be implemented clinically as a dose-prediction model for patients initiating tamoxifen treatment, similar to integrated dosing algorithms developed for warfarin.[45] Model-informed precision dosing[46] could ensure that all patients achieve target Z-endoxifen concentrations, thereby avoiding under-treatment and treatment failure. Of note, prospective studies have demonstrated the feasibility and safety of tamoxifen dose escalation in patients who have reduced activity *CYP2D6* genotypes or low Z-endoxifen systemic concentrations,[47,48] although no studies have demonstrated an efficacy benefit of this approach.[49]

This pooled analysis applied advanced pharmacokinetic modeling techniques to the largest international database of tamoxifen-treated patients with measured endoxifen concentration and comprehensive clinical and pharmacogenetic data. This enabled robust estimation of the contribution of clinical and genetic variables on tamoxifen bioactivation to Z-endoxifen. Despite these strengths, this study has some limitations that should be considered. Missing and inconsistent data are inherent limitations of pooled analyses, which can result both in bias and inflated *P* values of estimates. CEPAM had adequate availability of most clinical variables, but some variables including non-CYP2D6 genetics and co-medications had greater missingness (**Table 1**, **Tables S2, S3**). We imputed missing continuous data with the median value and missing categorical data with the most common category, the robustness of which were supported by our sensitivity analyses. Another weakness was that only one measurement of endoxifen was available per patient, which prevents assessment of variability between measurements for the same individual. Additionally, the CEPAM dataset included studies using a variety of bioanalytical assays that measured Z-endoxifen or total endoxifen. We accounted for this by reviewing assay methods and including the measured isoform in our model. Finally, studies differed in *CYP2D6* genotyping, ranging from a single variant to multiplexed assays that analyzed 33 *CYP2D6* star (*) alleles, with or without copy number interrogation. Default assignment of untested alleles to *CYP2D6* wild-type could inflate unexplained variability, which is one possible reason the variability explained by *CYP2D6* in this study is lower than that reported in previous studies.[30] This model used the consensus CYP2D6 AS system[50] to accurately quantify the activity of the alleles available; however, future work will use this uniquely large dataset to estimate CYP2D6 activity per individual star (*) allele on a continuous scale,[51] which likely will improve the contribution of independent *CYP2D6* diplotypes to the interindividual variability of endoxifen plasma levels.

In conclusion, this pooled analysis of the largest ever database of tamoxifen-treated patients generated a novel precision algorithm that integrates pharmacogenetic and clinical factors to predict Z-endoxifen concentration during tamoxifen treatment. Validation of the association of predicted Z-endoxifen concentration with tamoxifen treatment efficacy in available clinical trial cohorts will enable personalized tamoxifen treatment to achieve therapeutic Z-endoxifen concentrations and improve treatment outcomes in patients with hormone receptor-positive breast cancer.[6]

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST

C.K. reports grants from an industry consortium (AbbVie Deutschland GmbH & Co. K.G., AstraZeneca, Boehringer Ingelheim Pharma GmbH & Co. KG., F. Hoffmann-La Roche Ltd., Merck KGaA, Novo Nordisk A/S and Sanofi) for the graduate research training program PharMetrX. In addition, C.K. reports research grants from the Innovative Medicines Initiative-Joint Undertaking ("DDMoRe"), from H2020-EU.3.1.3 ("FAIR"), Diurnal Ltd. and the Federal Ministry of Education and Research within the Joint Programming Initiative on Antimicrobial Resistance Initiative ("JPIAMR"), all outside the submitted work. D.F.H. reports support unrelated to this study but provided to his institution in the last 24 months during conduct and analysis of this study from Astra Zeneca, Menarini Silicon Biosystems, Merrimack Pharmaceuticals, and Pfizer. D.F.H. reports personal income related to consulting or advisory board activities from Arvenis, BioVeca, BioTheranostics an Hologic Company, Cellworks, Centrix, Cepheid, EPIC Sciences, EXACT Sciences, Freenome, Guardant, L-Nutra, Macrogenics, Oncocyte, Predictus BioSciences, Stratipath, Tempus, Turnstone Biologics, and Xilis. All other authors declared no competing interests for this work.

AUTHOR CONTRIBUTION

A.M.M., T.H., F.K., C.K., and D.L.H. wrote the manuscript. C.K. and D.L.H. designed the research. A.M.M., T.H., and F.K. performed

the research. A.M.M. and F.K. analyzed the data. All other authors contributed datasets, and assisted with revising and approval of the manuscript.

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APPENDIX

CEPAM Consortium Contributors: Anna M. Mc Laughlin, anna.mclaughlin@fu-berlin.de. Thomas Helland, thms.helland@gmail.com. Fenja Klima, fenja.klima@fu-berlin.de. Stijn L.W. Koolen, s.koolen@erasmusmc.nl. Ron H.N. van Schaik, r.vanschaik@erasmusmc.nl. Ron H.J. Mathijssen, a.mathijssen@erasmusmc.nl. Patrick Neven, patrick.neven@uzleuven.be. Jesse J. Swen, j.j.swen@lumc.nl. Henk-Jan Guchelaar, h.j.guchelaar@lumc.nl. Florence Dalenc, dalenc.florence@iuct-oncopole.fr. Melanie White-Koning, melanie.white-koning@univ-tlse3.fr. Robin Michelet, robin.michelet@fu-berlin.de. Gerd Mikus, gerd.mikus@fu-berlin.de. Werner Schroth, werner.schroth@ikp-stuttgart.de. Thomas Mürdter, thomas.muerdter@ikp-stuttgart.de. Hiltrud Brauch, hiltrud.brauch@ikp-stuttgart.de. Matthias Schwab, matthias.schwab@ikp-stuttgart.de. Håvard Søiland, hsoiland@gmail.com. Gunnar Mellgren, gunnar.mellgren@uib.no. Fabienne Thomas, thomas.fabienne@iuct-oncopole.fr. Charlotte Kloft, charlotte.kloft@fu-berlin.de. Daniel L. Hertz, dlhertz@med.umich.edu. Bram C. Agema, b.agema@erasmusmc.nl, 0000-0003-1825-2911, Department of Medical Oncology, Erasmus MC Cancer Institute, Erasmus University Medical Center, Rotterdam, The Netherlands. Department of Hospital Pharmacy, Erasmus University Medical Center, Rotterdam, the Netherlands. Anabel Sanchez-Spitman, a.spitman@protonmail.com 0000-0003-2073-5648, Department of Clinical Pharmacy & Toxicology, Leiden University Medical Center, Leiden, The Netherlands. Thais Almeida, thaisaalmeida@gmail.com, Clinical Oncology Department, Erasto Gaertner Hospital, Curitiba, Brazil. Pontifical Catholic University of Parana, Curitiba, Brazil. Jeanine Nardin, jnardin@erastogaertner.com.br, Clinical Research Department, Erasto Gaertner Hospital, Curitiba, Brazil. School of Health Science, UniBrasil, Curitiba, Brazil. José Claudio Casali-da-Rocha, casalidarocha@gmail.com, Pontifical Catholic University of Parana, Curitiba, Brazil. Department of Oncogenetics, Erasto Gaertner Hospital, Curitiba, Brazil. Rosa Esther Moo-Puc, PhD, moopuc@gmail.com 0000-0001-9204-6062, Unidad de Investigación Médica Yucatán, Unidad Médica de Alta Especialidad, Centro Médico Ignacio García Téllez, Instituto Mexicano del Seguro Social (IMSS), México. Jorge Aarón Rangel-Mendez, PhD, ja.rangel@correo.uady.mx 0000-0003-0931-7998, Unidad de Investigación Médica Yucatán, Unidad Médica de Alta Especialidad, Centro Médico Ignacio García Téllez, Instituto Mexicano del Seguro Social (IMSS), México. Gwendolyn McMillin, gwen.mcmillin@aruplab.com, University of Utah, Department of Pathology, and ARUP Laboratories, Salt Lake City, UT. Ewa E. Hennig, ewa.hennig@nio.gov.pl 0000-0002-6388-2237, Department of Gastroenterology, Hepatology and Clinical Oncology, Centre of Postgraduate Medical Education, Warsaw, Poland. Department of Genetics, Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Poland. Elżbieta Brewczyńska, elzbieta.brewczynska@nio.gov.pl 0009-0008-7499-1691, Department of Breast Cancer and Reconstructive Surgery, Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Poland. Marina Venzon Antunes, marinaantunes@feevale.br 0000-0001-9157-9510, Graduate Program on Toxicology and Analytical Toxicology, Feevale University, Novo Hamburgo, Brazil. Vincent Haufroid, vincent.haufroid@uclouvain.be 0000-0001-5040-9806, Louvain Centre for Toxicology and Applied Pharmacology (LTAP), Université Catholique de Louvain and Department of clinical chemistry, Cliniques Universitaires Saint-Luc, Brussels, Belgium. Linda Thorén, linda.thoren@ki.se 0000-0003-1541-6191, Department of Clinical Science and Education at Södersjukhuset, Karolinska Institutet, Stockholm, Sweden. Department of Oncology, Södersjukhuset, Stockholm, Sweden. Lisa Madlensky, lmadlensky@health.ucsd.edu 0000-0001-7469-4736. Dept of Medicine and Moores Cancer Center, University of California San Diego. John Pierce, jppierce@health.ucsd.edu 0000-0002-0075-7471. Herbert Wertheim School of Public Health & Human Longevity Science and Moores Cancer Center, University of California San Diego. Yusuke Nakaumra, yusuke.nakamura@jfcr.or.jp. RIKEN Center for Genomic Medicine Yokohama, Kanagawa, Japan. Michiaki Kubo, michiaki.kubo@riken.jp. RIKEN Center for Genomic Medicine Yokohama, Kanagawa, Japan. Hitoshi Zembutsu, MD, PhD hzenbuts@ncc.go.jp. Department of Clinical Genomics, National Cancer Center Research Institute, Tokyo, 1040045, Japan. João Paulo Bianchi Ximenez, joaopaulo.ximenez@usp.br. Department of Clinical Analyses, Toxicology and Food Science, School of Pharmaceutical Sciences of Ribeirao Preto, University of Sao Paulo, Ribeirao Preto, SP, Brazil. Vera Lucia Lanchote, lanchote@fcfrp.usp.br. Department of Clinical Analyses, Toxicology and Food Science, School of Pharmaceutical Sciences of Ribeirao Preto, University of Sao Paulo, Ribeirao Preto, SP, Brazil. James M. Rae, PhD jimmyrae@med.umich.edu 0000-0002-3412-8219. Department of Internal Medicine, Division of Hematology/Oncology, University of Michigan Medical School. Daniel F Hayes, MD hayesdf@med.umich.edu 0000-0003-3620-7575. Department of Internal Medicine, Division of Hematology/Oncology, University of Michigan Medical School. Vered Stearns, MD vstearn1@jhmi.edu. Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins School of Medicine, Baltimore, MD. Todd C Skaar, tskaar@iu.edu. Department of Medicine, Division of Clinical Pharmacology, Indiana University School of Medicine, Indianapolis, IN 46202. Zeruesenay Desta, zdesta@iu.edu 0009-0001-1513-1295. Department of Medicine, Division of Clinical Pharmacology, Indiana University School of Medicine, Indianapolis, IN 46202. Stuart A Scott, sascott@stanford.edu 0000-0001-5720-1864. Department of Pathology, Stanford University, Stanford, CA; Clinical Genomics Laboratory, Stanford Medicine, Palo Alto, CA. Robert J Desnick, robert.desnick@mssm.edu. Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY. In Hae Park, parkinhae@gmail.com 0000-0001-7894-8772. Department of Hemato-oncology,

Korea University College of Medicine, Guro Hospital, Seoul, Korea. National Cancer Center, Center for Breast Cancer, Korea. Hye In Woo, hyein.wooo@gmail.com. Department of Laboratory Medicine & Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea. Soo-Youn Lee, suddenbz@skku.edu 0000-0001-7595-4042. Department of Laboratory Medicine & Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea. Ana Fernandez-Santander, ana.fernandez@universidadeuropea.es 0000-0001-7545-1170. Human Genetic Variability Group, Hospital La Paz Institute for Health Research–IdiPAZ (La Paz University Hospital–Universidad Autónoma de Madrid–Getafe University Hospital–Universidad Europea de Madrid), 28046 Madrid, Spain. Biomedical and Health Sciences Faculty, Department of Medicine, Universidad Europea de Madrid, 28670 Madrid, Spain. Alicia Romero-Lorca, alicia.romero@universidadeuropea.es. Human Genetic Variability Group, Hospital La Paz Institute for Health Research–IdiPAZ (La Paz University Hospital–Universidad Autónoma de Madrid–Getafe University Hospital–Universidad Europea de Madrid), 28046 Madrid, Spain. Biomedical and Health Sciences Faculty, Department of Medicine, Universidad Europea de Madrid, 28670 Madrid, Spain. Apolonia Novillo Villajos, aponovil@ucm.es 0000-0003-0902-0991. Human Genetic Variability Group, Hospital La Paz Institute for Health Research–IdiPAZ (La Paz University Hospital–Universidad Autónoma de Madrid–Getafe University Hospital–Universidad Europea de Madrid), 28046 Madrid, Spain. Medicine Faculty, Cellular Biology Section Department, Complutense University, 28040 Madrid, Spain. María Gaibar Alonso, maria.gaibar@ucjc.edu 0000-0003-3881-8345. Human Genetic Variability Group, Hospital La Paz Institute for Health Research–IdiPAZ (La Paz University Hospital–Universidad Autónoma de Madrid–Getafe University Hospital–Universidad Europea de Madrid), 28046 Madrid, Spain. Facultad HM de Ciencias de la Salud, Universidad Camilo José Cela, Villanueva de la Cañada 28692, Madrid, Spain. Harriet Johansson, harriet.johansson@ieo.it 0000-0002-4131-2030. IEO, European Institute of Oncology IRCCS, Milan, Italy. Bernardo Bonanni, bernardo.bonanni@ieo.it 0000-0003-3589-2128. IEO, European Institute of Oncology IRCCS, Milan, Italy. Andrea DeCensi, andrea.decensi@galliera.it 0000-0003-2635-4491. Ente Ospedaliero Ospedali Galliera, Genoa, Italy. Wolfson Institute of Population Health, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom. Howard Gurney, howard.gurney@mq.edu.au 0000-0003-0217-5261. Faculty of Medicine, Health and Human Sciences, Macquarie University, Sydney, Australia. Medical Oncology, Westmead Hospital, Sydney, Australia. Rosemary Balleine, rosemary.balleine@sydney.edu.au 0000-0003-2864-4345. The Westmead Institute for Medical Research, Faculty of Medicine and Health, University of Sydney, Sydney, Australia. William J. Irvin Jr., william_irvin@bshsi.org 0009-0000-4033-9541. Bon Secours Cancer Institute, Richmond, Virginia, USA. Howard L. McLeod, hmcleod1965@gmail.com 0000-0002-9004-9232. Utah Tech University, St George, Utah, USA. Matthew P. Goetz goetz.matthew@mayo.edu. Department of Oncology, Mayo Clinic, Rochester, MN, USA. Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, MN, USA. Joel M. Reid reid@mayo.edu. Department of Oncology, Mayo Clinic, Rochester, MN, USA. Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, MN, USA. Vera J. Suman suman@mayo.edu. Department of Health Sciences Research, Mayo Clinic, Rochester, MN, USA. Nutthada Areepium nutthada.a@pharm.chula.ac.th 0000-0002-9917-833X. Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand. Wanaporn Charoenchokthavee wanaporn@nmu.ac.th. 0000-0003-3417-460X, Department of Pharmacy, The Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Bangkok, Thailand. Diana Eccles, d.m.eccles@soton.ac.uk 0000-0002-9935-3169. Faculty of Medicine, Cancer Sciences Academic Unit and University of Southampton Clinical Trials Unit, University of Southampton, Southampton, United Kingdom. University Hospital Southampton NHS Foundation Trust, Southampton, United Kingdom. William Tapper, w.j.tapper@soton.ac.uk. Faculty of Medicine, Cancer Sciences Academic Unit and University of Southampton Clinical Trials Unit, University of Southampton, Southampton, United Kingdom. University Hospital Southampton NHS Foundation Trust, Southampton, United Kingdom. Balram Chowbay, ctebal@nccs.com.sg. Clinical Pharmacology, SingHealth, Singapore, Singapore. Clinical Pharmacology Laboratory, Division of Cellular & Molecular Research, National Cancer Centre, Singapore, Singapore. Centre for Clinician Scientist Development, Duke-NUS Medical School, Singapore and Singapore Immunology Network, Agency for Science, Technology & Research (A*STAR) Singapore. Chiea Chuen Khor, khorcc@gis.a-star.edu.sg. Genome Institute of Singapore (GIS), Agency for Science, Technology and Research (A*STAR), 60 Biopolis Street, Genome #02-01, Singapore 138672, Republic of Singapore. Elaine Lim Hsuen, elaine.lim.hsuen@singhealth.com.sg. Department of Medical Oncology Singapore. Arafat Tfayli, at35@aub.edu.lb. Hematology-Oncology Division, Department of Internal Medicine, Faculty of Medicine, American University of Beirut, Beirut, Lebanon. Nathalie K. Zgheib, nk16@aub.edu.lb 0000-0002-0571-768X. Faculty of Medicine, Department of Pharmacology and Toxicology, American University of Beirut, Beirut, Lebanon. Cécile Arellano, cecile.arellano@univ-tlse3.fr. Cancer Research Center of Toulouse (CRCT), Inserm U1037, Université Paul Sabatier, Toulouse, France.