ARTICLE

Application of PBPK Modeling and Virtual Clinical Study Approaches to Predict the Outcomes of CYP2D6 Genotype-Guided Dosing of Tamoxifen

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The Tamoxifen Response by CYP2D6 Genotype-based Treatment-1 (TARGET-1) study ($n = 180$) was conducted from 2012–2017 in Japan to determine the efficacy of tamoxifen dosing guided by cytochrome P450 2D6 (CYP2D6) genotypes. To predict its outcomes prior to completion, we constructed the comprehensive physiologically based pharmacokinetic (PBPK) models of tamoxifen and its metabolites and performed virtual TARGET-1 studies. Our analyses indicated that the expected probability to achieve the end point (demonstrating the superior efficacy of the escalated tamoxifen dose over the standard dose in patients carrying CYP2D6 variants) was 0.469 on average. As the population size of this virtual clinical study (VCS) increased, the expected probability was substantially increased (0.674 for $n = 260$). Our analyses also informed that the probability to achieve the end point in the TARGET-1 study was negatively impacted by a large variability in endoxifen levels. Our current efforts demonstrate the promising utility of the PBPK modeling and VCS approaches in prospectively designing effective clinical trials.

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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Therapeutic efficacy of tamoxifen requires its conversion to endoxifen via CYP2D6. To develop CYP2D6 genotype-guided tamoxifen dosing for the treatment of hormone receptor-positive breast cancer, the TARGET-1 study was conducted in Japan, with no outcomes revealed yet.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ That we can prospectively predict the outcome of the TARGET-1 study using PBPK-based VCS approaches.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ The outcome of the TARGET-1 study may be significantly impacted by patient numbers enrolled in the trial and variability of endoxifen levels based on prospective simulations using PBPK-based VCS approach.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

☑ Prospective prediction using PBPK-based VCS approach provides mechanistic and critical insights for the effective and efficient design of clinical trials.

Tamoxifen, a selective estrogen-receptor modulator, is widely used for treatment of estrogen receptor (ER) or progesterone receptor-positive breast cancer. Therapeutic efficacy of tamoxifen requires its conversion to more potent anti-estrogenic metabolites (with at least 100-fold greater affinity to ER than tamoxifen), which include 4-hydroxytamoxifen (4-OHT) and 4-hydroxy-N-desmethyltamoxifen (endoxifen).[1,2] Endoxifen is considered to be the predominant metabolite that exists in ~10-fold excess over 4-OHT in human plasma.[2,3] The cytochrome P450 2D6 (CYP2D6) is a key player in the formation of endoxifen.[4] The plasma levels of endoxifen were reported to be lower in patients with variant alleles of CYP2D6 than those with wild-type alleles. Yet, the results from retrospective studies have been inconsistent, not reaching a consensus conclusion regarding the association between CYP2D6 enzyme activity and tamoxifen efficacy for treating breast cancer. When the International Tamoxifen Pharmacogenomics Consortium performed meta-analysis on the data from heterogeneous study populations (12 globally distributed sites), the CYP2D6 genotypes were associated with poor outcomes only by using strict inclusion criteria.[5] These results prompted further prospective validation of the CYP2D6 genotype in guiding tamoxifen therapy.

The frequencies of CYP2D6 polymorphic variants substantially vary among different ethnic groups. For instance, East Asians, including Japanese, display a much higher

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*Footnote: The results from the actual TARGET-1 study are not revealed at the time of performing the analysis and preparing the manuscript. The authors who performed PBPK modeling and VCS analyses (T.N., K.T., W.L., and Y.S.) obtained the publicly available protocol of the TARGET-1 study, but had no access to the results of interim and final analyses at any given time.

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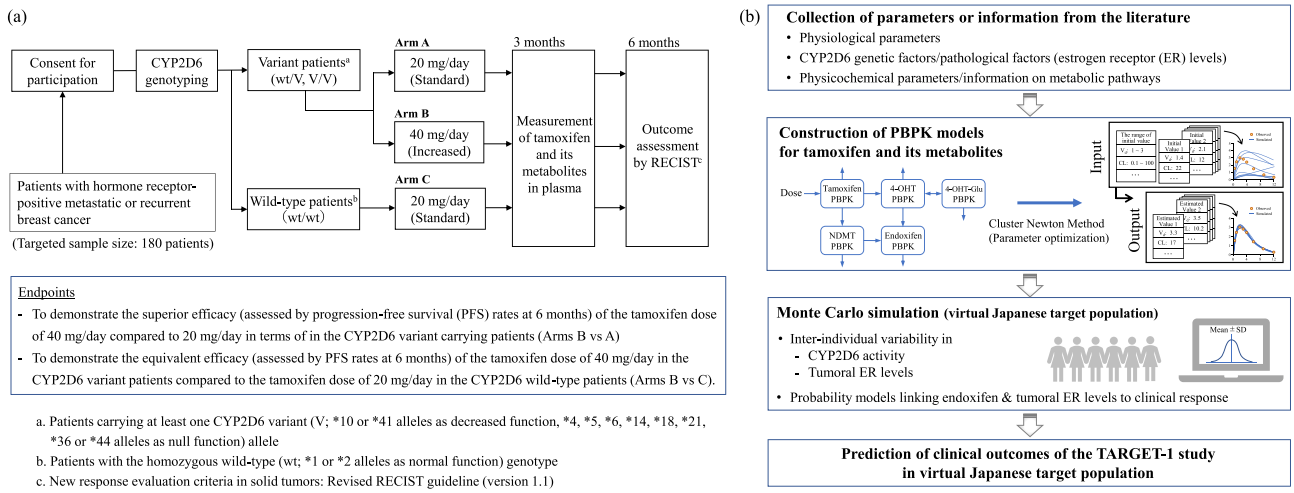

Figure 1 Schema of the Tamoxifen Response by CYP2D6 Genotype-based Treatment-1 (TARGET-1) clinical study and workflow of the virtual TARGET-1 clinical study approach. (**a**) Study schema of the TARGET-1 clinical study which investigated cytochrome P450 (CYP)2D6 genotype-guided tamoxifen dose adjustment in patients with hormone receptor-positive breast cancer. The two arms for patients harboring CYP2D6 variants were set to have an equal allocation of patients with tumors of high tumoral estrogen receptor expression levels (≥50% by immunohistochemical staining). (**b**) Overall workflow implemented for the prediction of the outcomes from the TARGET-1 clinical study using the physiologically based pharmacokinetic (PBPK) modeling and virtual clinical study approaches. 4-OHT, 4-hydroxytamoxifen; NDMT, N-desmethyltamoxifen; RECIST, Response Evaluation Criteria in Solid Tumors.

allelic frequency for CYP2D6*10 associated with the decreased enzymatic activity, thus likely having lower endoxifen levels than other ethnic groups.[6] It was reported that an increased tamoxifen dose in patients with CYP2D6*1/*10 and CYP2D6*10/*10 genotypes yields plasma endoxifen levels comparable to those observed in patients with CYP2D6*1/*1 genotype and the standard tamoxifen dose (20 mg/day) without increasing toxicity.[7] To evaluate the CYP2D6 genotype-guided tamoxifen dose adjustment in hormone receptor-positive metastatic or recurrent breast cancer patients, the randomized phase II clinical trial Tamoxifen Response by CYP2D6 Genotype-based Treatment-1 (TARGET-1) study was conducted from 2012–2017 in Japan (UMIN ID: UMIN000009155).[8] In this TARGET-1 study, patients carrying at least one CYP2D6 variant allele were randomized to receive different treatments based on pretreatment CYP2D6 genotyping results (**Figure 1a**). The end point was to demonstrate the superior efficacy (assessed by progression-free survival (PFS) rates at 6 months after patient randomization) of the increased tamoxifen dose (40 mg/day) over the standard dose (20 mg/day) in patients carrying at least one variant allele of CYP2D6. The TARGET-1 study also examined whether the efficacy is equivalent between the tamoxifen dose of 40 mg/day in those carrying at least one CYP2D6 variant allele and the tamoxifen dose of 20 mg/day in those with homozygous wild-type CYP2D6 genotype. The outcomes of the TARGET-1 study are not yet revealed.

Physiologically based pharmacokinetic (PBPK) modeling is a powerful tool to predict/simulate drug disposition profiles in a variety of potential clinical scenarios. The PBPK modeling has also been increasingly applied to conduct a virtual clinical study (VCS) in a virtual target population constructed by incorporating variabilities in various physiological and pharmacokinetic (PK) aspects.[9] Previously, PBPK modeling and VCS simulation approaches were applied to predict the levels of tamoxifen and endoxifen in patients with impaired CYP2D6 metabolism.[10,11] Yet, these previous modeling efforts did not adequately capture interindividual variability in endoxifen levels observed in clinical settings and more importantly did not link varying endoxifen levels to clinical response of tamoxifen therapy.

In the current study, we constructed comprehensive PBPK models of tamoxifen and its metabolites using a Cluster Newton method (CNM) for parameter optimization.[12] Via these efforts, we successfully captured interindividual variability in endoxifen levels in virtual Japanese patients with breast cancer with differing CYP2D6 genotypes and calculated the expected outcomes of the TARGET-1 study prior to its completion (our approach is outlined in **Figure 1b**). Our current efforts demonstrate the promising utility of the PBPK modeling and VCS approaches in designing effective clinical trials.

METHODS

Development of the PBPK model of tamoxifen and its metabolites

Basic PBPK modules were constructed as previously described (**Figure 2a**).[13–15] Individual PBPK modules constructed for tamoxifen and its four metabolites (i.e., N-desmethyltamoxifen (NDMT), 4-OHT, 4-OHT-glucuronide, and endoxifen) were inter-connected, as shown in **Figure 2b**. Based on the available information regarding the formation and disposition of tamoxifen and its metabolites, differential equations were derived for individual compounds and organs (detailed description provided in the **Supplementary Methods**).

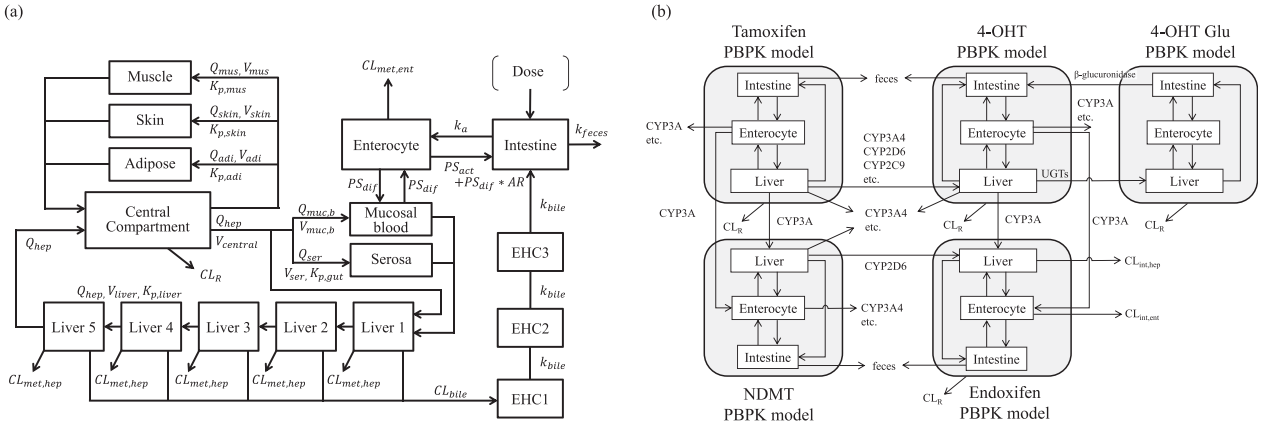

Figure 2 Structures of the physiologically based pharmacokinetic (PBPK) models of tamoxifen and its metabolites. (**a**) Schematic representation of a basic PBPK model that was developed assuming a perfusion rate-limited distribution. The liver compartment was further divided into five subcompartments to construct a model similar to the dispersion model and enterohepatic circulation was incorporated. (**b**) Individual PBPK modules developed for tamoxifen and its metabolites were inter-connected as shown here. 4-OHT, 4-hydroxytamoxifen; CYP, cytochrome P450; NDMT, N-desmethyltamoxifen; UGT, uridine 5′-diphosphate glucuronosyltransferase.

Parameter optimization by CNM and selection of the final sets of parameters for PBPK models

To obtain unknown parameters for the constructed PBPK models (the list of parameters provided in **Table S1**), we utilized the observed PK profiles of tamoxifen, NDMT, 4-OHT, and endoxifen in healthy postmenopausal women ($n = 30$) who lived in the United States.[16] The unknown parameters (compound-specific or metabolic pathway-dependent) were optimized by CNM-based processes, as similar to our previous report.[15] Briefly, 1,000,000 different initial sets of parameters were generated and subjected to the CNM-based process. The top 50 sets of parameters that adequately captured the observed PK profiles were identified. In selecting the final sets of parameters, sensitivity analysis was performed to examine whether the set of parameters can reproduce the reported differences in endoxifen levels between the groups of CYP2D6*1/*1 and CYP2D6*10/*10 genotypes (i.e., the ratio of 0.397–0.472).[7,17,18] Based on these criteria, the eight sets of parameters were selected (detailed description provided in the **Supplementary Methods**).

Calculation of parameter distribution reflecting interindividual variability in terms of CYP2D6 activity and tumoral ER expression

For differing CYP2D6 allele-based genotypes, the relative enzymatic activity and associated interindividual variability (% coefficient of variation (%CV)) have been reported based on the experimental results.[19] In order to inter-relate such information to clinical CYP2D6 phenotypes, individual alleles were grouped into four subclasses based on the predicted CYP2D6 function (increase, normal, decrease, and null). For seven diplotypes (increase/normal, normal/normal, normal/decrease, normal/null, decrease/decrease, decrease/null, and null/null), their relative activity scores and interindividual variabilities were deduced from the reported data on CYP2D6 allele-based genotype groups.[19] The detailed description is available in the **Supplementary Methods** and the results are summarized in **Table 1**.

Distribution of varying tumoral ER levels was also considered, based on its importance as a predictive biomarker of tamoxifen therapy; higher tumoral ER levels have been associated with better clinical response to tamoxifen.[20–23] Information on varying tumoral ER expression levels assessed by immunohistochemical staining was available for patients in the United States[24] and in Japan.[22] Based on these reports, the frequency distribution was calculated for differing tumoral ER expression levels (**Table 1**). The TARGET-1 study enrolled patients with ER-positive breast cancer, based on the immunohistochemical staining score $\geq$10% (thus, no patients with tumoral ER expression level $<$10%). Given that tumoral ER expression levels may well impact clinical response in the TARGET-1 study, the frequency distribution for tumoral ER expression in the target population (i.e., distribution of virtual patients with moderate ($\geq$10% and $<$50%) or strong ($\geq$50%) tumoral ER expression levels) was set to be comparable in each treatment arm.

Calibration of a probability model that links endoxifen and tumoral ER levels to clinical response

In predicting the outcomes of the TARGET-1 study, it is essential to link known predictive variables (i.e., endoxifen levels and tumoral ER levels) to clinical response of tamoxifen therapy. The TARGET-1 study is designed to compare the PFS rate at 6 months as an efficacy end point (**Figure 1a**). The international phase III clinical trials on tamoxifen therapy were conducted mainly in the United States and Europe, reporting the PFS rate at 6 months to be $\sim$0.5.[25] Another multicenter phase III clinical trial on tamoxifen therapy also reported a similar PFS rate.[26] The results from these reports were used to develop and calibrate our model to predict clinical response from tamoxifen therapy with the assumption that the reported clinical trials largely represent a white population.

Table 1 Distribution of parameters reflecting interindividual variability in CYP2D6 activity and tumoral ER expression.

Distribution of CYP2D6 activity-based diplotypes (relative activity and frequency)				
	CYP2D6 activity[a]		**Frequency, %[b]**	
Classification	**Relative activity score**	**%CV**	**White**	**Japanese**
Increase/normal	1.13	64.3	2.4	1.4
Normal/normal	0.89	47.9	43.1	33.4
Normal/decrease	0.71	53.6	11.2	37.3
Normal/null	0.42	99.0	31.8	9.5
Decrease/decrease	0.24	66.0	1.1	12.5
Decrease/null	0.20	109.0	4.3	5.5
Null/null	0.004	103.0	6.2	0.5

Tumoral ER expression frequency[c]				
	Frequency in US patients		**Frequency in Japanese patients**	
Classification	**Overall population**	**TARGET-1 study population**	**Overall population**	**TARGET-1 study population**
ER weak, $<10\%$	0.443	-	0.315	-
ER moderate, $\geq 10\%$ and $<50\%$	0.124	0.222	0.135	0.198
ER strong, $\leq 50\%$	0.434	0.778	0.550	0.802

%CV, (% coefficient of variation; CYP, cytochrome P450; ER, estrogen receptor; TARGET-1, Tamoxifen Response by CYP2D6 Genotype-based Treatment-1.
[a]Relative activity scores (using the activity of CYP2D6*1/*1 as a reference) and interindividual variabilities (%CV) were calculated using the data from literature.[19]
[b]The CYP2D6 genotypic frequencies in white and Japanese populations. Detailed description is provided in the **Supplementary Methods**.
[c]Distribution of varying tumoral ER expression levels was obtained from the literature of US patients[24] or Japanese patients.[22] Because the TARGET-1 study was designed to enroll patients with ER-positive breast cancer, the target population did not include patients with weak ER expression ($<10\%$ ER positivity by immunohistochemical staining).

We assumed that the overall probability to achieve clinical response corresponds to the products of the two probability scores (one for tumoral ER expression levels and the other for endoxifen levels). For patients with moderate ($\geq 10\%$ and $<50\%$) and high ($\geq 50\%$) tumoral ER levels, the probability scores of 0.5 and 1.0 were assigned, respectively. For endoxifen levels, the probability scores were assigned in a similar stepwise manner, and the lower and higher cutoff values were assigned 14.3 and 35.1 nM steady-state endoxifen levels in blood, respectively, based on the WHEL study.[21] For patients with low (<14.3 nM), intermediate (≥ 14.3 nM and <35.1 nM), and high (≥ 35.1 nM) endoxifen levels, the probability scores of 0.1, 0.53, and 1.0 were assigned, respectively (detailed description of our probability model provided in **Supplementary Methods**).

Using both the probability model for clinical response and the simulated endoxifen concentrations in a virtual white population (detailed description provided in **Supplementary Methods**), the calibration was performed so that the overall probability to obtain clinical response (PFS at 6 months) would approximate to the reported value of 0.5.[25,26] The calibrated probability model for clinical response was used for virtual TARGET-1 study in the virtual target population of Japanese patients.

Virtual TARGET-1 study

Detailed information on the TARGET-1 study (UMIN ID: UMIN000009155) is available on the website at the University Hospital Medical Information Network (UMIN).[8] Briefly, patients with hormone receptor-positive metastatic or recurrent breast cancer were enrolled and subjected to CYP2D6 genotyping. The CYP2D6 alleles were classified into either wild-type ("wt"; *1 or *2 as normal function) or variant ("V"; *10 or *41 as decreased function, *4, *5, *6, *14, *18, *21, *36, or *44 as null function). As outlined in **Figure 1a**, the TARGET-1 study consisted of the following three arms: arm A) patients who carry at least one CYP2D6 variant allele and receive the standard tamoxifen dose (wt/V, V/V, 20 mg/day, 24 weeks); arm B) patients who carry at least one CYP2D6 variant allele and receive the increased tamoxifen dose (wt/V, V/V, 40 mg/day, 24 weeks); and arm C) patients who are homozygous for CYP2D6 wild-type allele and receive the standard tamoxifen dose (wt/wt, 20 mg/day, 24 weeks). The end point was to demonstrate the superior efficacy (assessed by PFS rate at 6 months after patient randomization) of the tamoxifen dose of 40 mg/day over 20 mg/day in patients carrying at least one variant allele of CYP2D6 (arm A vs. B). The TARGET-1 study also determined equivalent efficacy between the tamoxifen dose of 40 mg/day in those carrying at least one variant allele and 20 mg/day in those with homozygous wild-type genotype (arm B vs. C).

In designing the actual TARGET-1 study (which had been completed prior to and independent of our current study), power analysis was performed to determine the targeted sample size. Based on the previous clinical data, variant patients (wt/V and V/V groups) were assumed to have the following probabilities of achieving PFS at 6 months: 40% and 60% for tamoxifen doses of 20 and 40 mg/day, respectively (arm A vs. B; **Figure 1a**). To detect a difference of 20% (60% in arm B vs. 40% in arm A) with 70% statistical power and one-sided significance level $\alpha = 0.05$ (type I error of 0.1), the sample size was estimated to be 136 (68 for

arm A and 68 for arm B; considering exclusion cases such as withdrawal). Based on the reported CYP2D6 genotype frequencies in the Japanese population, it was estimated that pretreatment genotyping will identify 44 wild-type patients (wt/wt genotype) until the accrual goal of 136 patients with wt/V and V/V genotypes is met (yielding the total targeted sample size of $n = 180$ patients).

In running a virtual TARGET-1 study, 180 of virtual Japanese patients were generated with the calculated frequency distribution for CYP2D6 activity and tumoral ER expression status and allocated to three different arms. The input parameters in virtual Japanese patients differed from the white population with regard to body weight, frequency of CYP2D6 genotypes, and tumoral ER expression. The patients who carry at least one CYP2D6 variant allele were assigned to arms A or B and the distribution of tumoral ER expression status (moderate ($\geq$10% and $<$50%) or high ($\geq$50%)) was set to be nearly equal between arms A and B, similar to the actual TARGET-1 study. Individual endoxifen levels were simulated using the constructed PBPK models. The calibrated probability model for clinical response was used to predict the overall probability to achieve clinical response in virtual Japanese patients. For the comparison between the two arms of the CYP2D6 variant groups (arm A vs. B; 20 mg/day vs. 40 mg/day), the χ^2 goodness-of-fit test was performed for the overall probability scores of clinical responses in the respective arms. The P values < 0.05 were deemed statistically significant. Virtual TARGET-1 studies were performed 200 times in different virtual Japanese patient populations ($n = 180$/run). For the comparison between arms B and C, the differences in their PFS rates were calculated. If the difference was <0.1, clinical efficacy between arms B and C were deemed equivalent. The VCS program for TARGET-1 study using R is available in **Supplementary Methods**.

Software. The simultaneous ordinary differential equations were solved using the MATLAB (MathWorks, Natick, MA) with a Stiff ODE solver ODE15s on MATLAB version 8.0.0.783 (R2012b). Generation of virtual patient population and statistical analysis were performed using R version 3.3.2.

RESULTS

Analyses of PKs of tamoxifen and its metabolites via PBPK modeling and CNM-based parameter optimization

For the PBPK models constructed for tamoxifen and its metabolites (**Figure 2a,b)**, the parameters were obtained by the CNM-based process (**Table S2**). Simulated blood concentration-time profiles for tamoxifen and its three metabolites (NDMT, 4-OHT, and endoxifen) are shown along with the reported profiles (**Figure S1**).[16] The eight sets of parameters reasonably captured the reported blood concentration-time profiles of tamoxifen and its three metabolites. Accordingly, the observed and simulated results yielded comparable area under the curve (AUC) values for tamoxifen and its three metabolites. The peak plasma concentration (C_{max}) value of tamoxifen was, however, slightly underestimated; simulated results were 0.28-fold to 0.51-fold (0.38-fold on average) of the observed values.

Construction of virtual patient population with interindividual variability in CYP2D6 activity and tumoral ER expression levels

Using the data available on the activity/variability associated with CYP2D6 genotypes[19] and tumoral ER levels,[22,24] we calculated the expected frequency distributions of varying CYP2D6 activity levels and tumoral ER levels (**Table 1**). A virtual white population ($n = 1{,}000$) was then generated based on the results summarized in **Table 1** and **Tables S2 and S3**. The simulated results (blood concentrations at the steady state) of the virtual white population were in good agreement with the reported distribution of 4-OHT and endoxifen levels by CYP2D6 genotypes (**Figure 3** and **Figure S2**).[27]

Calibration of the overall probability to obtain clinical response in the virtual TARGET-1 study

In order to predict clinical outcomes of the TARGET-1 study, we developed a probability model that links endoxifen levels and tumoral ER levels to clinical responses. For our current study, it was assumed that the pharmacological activity of equivalent endoxifen levels does not vary among different ethnic groups. The model was calibrated to match with the reported PFS rate at 6 months of 0.5 in the virtual white population, based on the reported results from phase III clinical trials.[25,26] Of eight sets of parameters obtained by CNM-based processes, seven sets of parameters successfully yielded the average overall probability of 0.50 ± 0.03, but one set (ID 3, which showed the lowest AUC_{inf} value among the sets selected) yielded the overall probability of 0.395, substantially different from the rest (deemed a likely outlier per Grubbs' test for outliers). Thus, in subsequent analyses, seven sets of parameters (excluding the set of ID 3) were utilized.

Clinical outcomes predicted from the virtual TARGET-1 study

Two hundred runs of virtual TARGET-1 studies ($n = 180$ virtual patients/run) were performed using the seven sets of parameters and the results are shown in **Figure 4** and **Table 2**. Using the constructed PBPK models, the steady-state blood tamoxifen and endoxifen levels were simulated. When compared with the observed data,[7] the simulated results seemed to provide reasonable agreement in terms of blood concentrations and variability of tamoxifen and its metabolites in the Japanese population, despite some differences in the genotype classification among patients (**Figure S3**). The simulated endoxifen concentrations were then utilized to calculate the overall probability to achieve clinical response. As summarized in **Table 2**, the expected probability of demonstrating the superior efficacy of arm B (40 mg/day tamoxifen dose) over arm A (20 mg/day tamoxifen dose) ranged from 0.400–0.515 (with the average probability 0.469 ± 0.040). When virtual TARGET-1 studies demonstrated the superior efficacy of arm B over arm A,

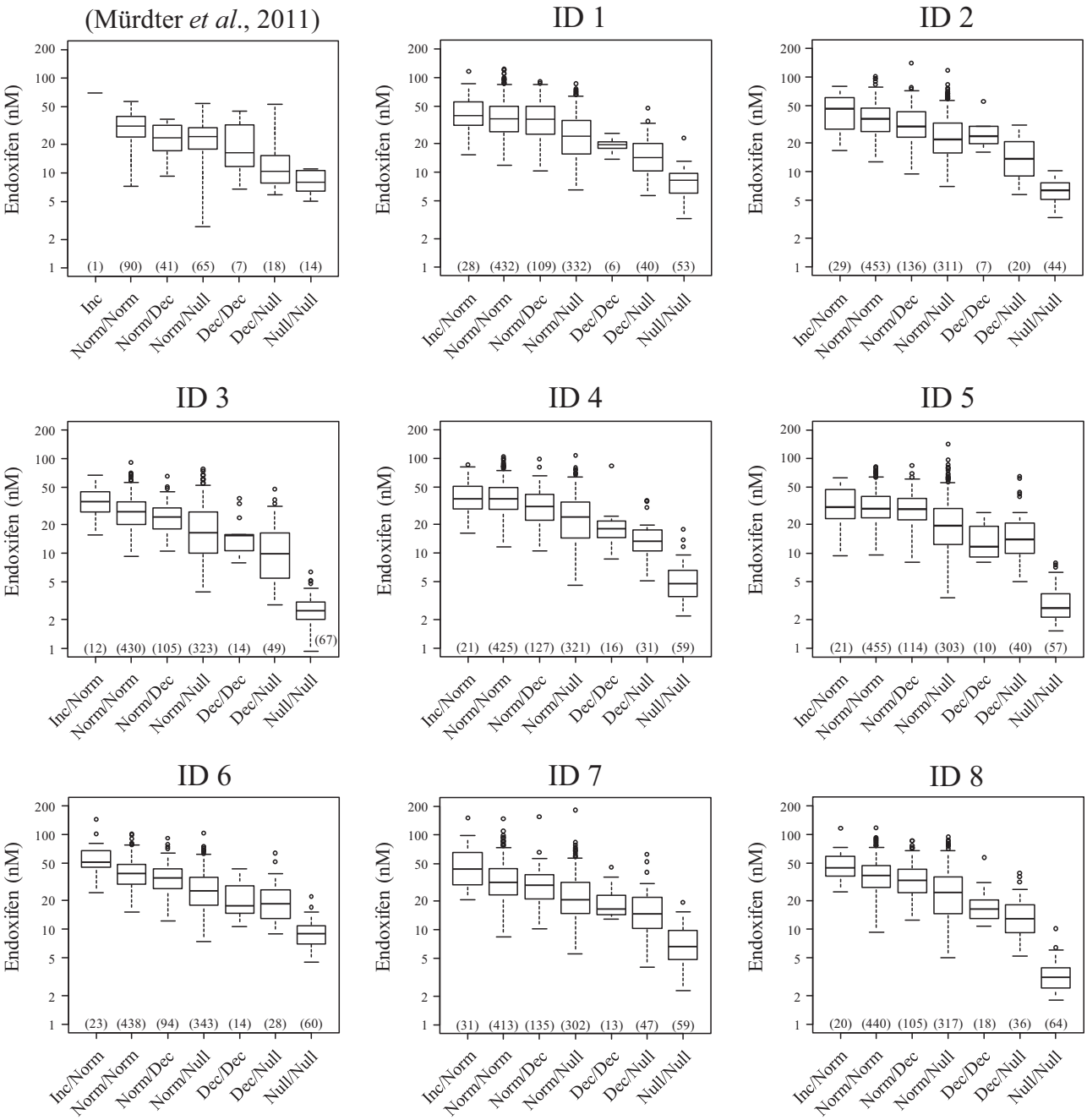

Figure 3 Steady-state blood levels of endoxifen in virtual patients with differing cytochrome P450 (CYP)2D6 activities. The top-left panel shows the data from the literature.[27] The rest of panels are the simulated results using the parameters of the indicated sets. The numbers in parentheses denote the number of patients in the respective group. Dec, decrease; Inc, increase.

the expected probability to demonstrate the equivalent efficacy between arms B and C ranged from 0.398–0.713.

Given that the expected probability of demonstrating the superior efficacy of arm B over arm A did not exceed 0.469, we examined how the expected probability would be impacted by the increase in the number of patients per run. As the number of patients increased from 140, 180, and 220 to 260, the expected probability to achieve the end point substantially increased (0.363 ± 0.055, 0.469 ± 0.040, 0.621 ± 0.049, and 0.674 ± 0.058, respectively; **Figure 5**).

DISCUSSION

In the current study, we developed the comprehensive PBPK models of tamoxifen and its metabolites using the

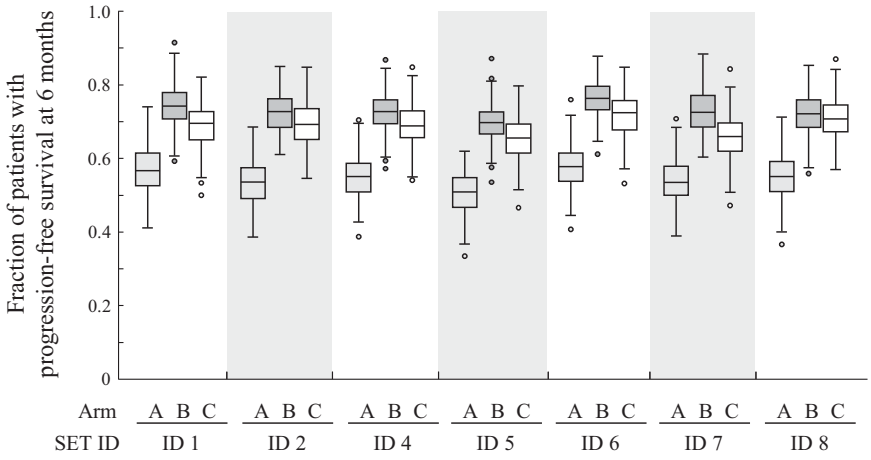

Figure 4 Prediction results of clinical response (efficacy assessed by progression-free survival rates at 6 months) in the treatment arms A, B, and C from seven runs of virtual tamoxifen response by CYP2D6 Genotype-based Treatment-1 (TARGET-1) studies.

Table 2 Summary of predicted outcomes from virtual clinical TARGET-1 studies (180 virtual patients/run, and 200 virtual studies/set)

$N = 180$[a]	Arm A vs. B[b]		Arm B vs. C[c]	
Set ID	No. of virtual studies demonstrating superiority of arm B	Expected probability	No. of virtual studies demonstrating equivalency	Expected probability
ID 1	87	0.435 (87/200)	49	0.563 (49/87)
ID 2	99	0.495 (99/200)	63	0.636 (63/99)
ID 4	91	0.455 (91/200)	63	0.692 (63/91)
ID 5	97	0.485 (97/200)	68	0.701 (68/97)
ID 6	99	0.495 (99/200)	63	0.636 (63/99)
ID 7	103	0.515 (103/200)	41	0.398 (41/103)
ID 8	80	0.400 (80/200)	57	0.713 (57/80)

TARGET-1, Tamoxifen Response by CYP2D6 Genotype-based Treatment-1.
[a]The results ($n = 140$, 220, and 260) are provided in **Table S4**.
[b]To demonstrate the superior efficacy (assessed by progression-free survival (PFS) at 6 months) of the tamoxifen dose of 40 mg/day over the tamoxifen dose of 20 mg/day in the cytochrome P450 (CYP)2D6 variant group (arm A vs. B; $P < 0.05$).
[c]To demonstrate the equivalent efficacy of the tamoxifen dose of 40 mg/day in the CYP2D6 variant group compared to the tamoxifen dose of 20 mg/day in the CYP2D6 wild-type group (arm B vs. C; PFS difference <0.1).

parameters obtained by the CNM, which allows for the estimation of multiple sets of parameters with wide initial ranges. Our PBPK models successfully captured the reported interindividual variability in endoxifen levels, impacted in part by CYP2D6 genotypes. The constructed PBPK models were then applied for simulation in the virtual Japanese patient population that reflects varying degrees in CYP2D6 activity (in terms of the relative activity and interindividual variability per genotype) and tumoral ER expression levels. The results from the virtual TARGET-1 studies (200 runs) yielded the average probability of 0.469 (ranging from 0.400–0.515) to achieve the end point of the TARGET-1 study (demonstrating the superior efficacy of 40 mg/day tamoxifen dose over 20 mg/day in CYP2D6 variant groups, arm B over arm A; **Table 2**). Given that the real TARGET-1 study is to be run only once, our prediction results suggest that the real TARGET-1 study has a marginally unfavorable chance of achieving the end point. When virtual TARGET-1 studies were performed with a greater sample size, the average probability to achieve the end point substantially increased (0.621 and 0.674 for $n = 220$ and 260, respectively). As the cutoff P values increased, the average probability to achieve the end point also increased (in the case of $n = 180$, the cutoff P value of 0.05 and 0.1 yielded the average probability of 0.469 and 0.596, respectively; **Figure 5**). These prediction results will soon be compared with the actual outcomes of the TARGET-1 study as they become available. It is expected that the PBPK modeling and VCS approaches will increasingly offer aid in the identification of potential factors impacting the clinical outcome of drug therapy and in the design of cost-effective clinical trials.

Our prediction results from the virtual TARGET-1 studies indicated a marginally unfavorable chance of achieving the end point in the TARGET-1 study. Although it awaits further comparison with the outcomes from the real TARGET-1 study, our current results support that personalized tamoxifen dosing strategy needs to consider not only CYP2D6 genotypes but also the extent of variability in endoxifen levels that persists even within the individuals of the same CYP2D6 genotype.[27,28] In fact, when the previous efforts of

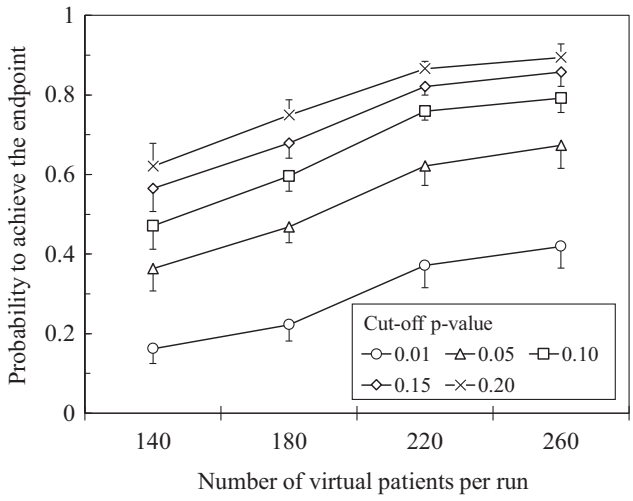

Figure 5 Relationship between the expected probability to demonstrate the superior efficacy of arm B over arm A in the virtual clinical Tamoxifen Response by CYP2D6 Genotype-based Treatment-1 (TARGET-1) study and the number of virtual patients. Expected probabilities were calculated with differing cut-off *P* values (ranging from 0.01–0.20) and virtual population sizes (ranging from 140–260). Data are shown as mean ± SD.

PBPK modeling and VCS approaches assumed an equal extent of variability across CYP2D6 genotypes, the simulation results did not adequately capture interindividual variability in endoxifen levels observed in clinical settings.[10,11] We constructed the virtual population that reflects the experimentally observed variability in the CYP2D6 activity (i.e., varying extent of variability among different CYP2D6 activity-based genotypes).[19] By doing so, we successfully captured the reported distribution of 4-OHT and endoxifen levels by CYP2D6 genotypes (**Figure 3** and **Figure S2)**.[27] Yet, our analyses suggest the substantial variability in endoxifen levels (even within the same treatment arm) as a major impediment in achieving the endpoint of the TARGET-1 study (**Figure S4**). Other factors (e.g., the activity of metabolizing enzymes other than CYP2D6, obesity, or age) may be considered as a covariate to account for the variability in endoxifen levels, thereby improving the prediction accuracy of virtual TARGET-1 studies.

Our prospective VCS approach uses the population of virtual patients reflecting interindividual variabilities based on the information available (typically from the literature; various factors, including physiological parameters). Subsequently, the PK profiles of drugs are simulated for individual virtual patients in a given population. The VCS approach does not utilize actual clinical PK data and may offer advantages in providing insights and prospective prediction when no clinical PK data is available in particular clinical settings. Currently, no report is available to provide sufficient clinical PK data for tamoxifen in Japanese patients. We believe that our current study provides a proof-of-concept for the utility of the VCS approach in the outcome prediction and design of clinical trials. In addition, our VCS approach differs from conventional power analysis in that the impact of sample sizes were calculated by taking various factors (i.e., variabilities of endoxifen concentrations, CYP2D6 genotype frequencies, and ER expression levels) into consideration. Our VCS approach may also be considered a more dynamic and quantitative approach than conventional power analysis.

In our current study, we utilized the CNM-based processes to obtain multiple parameters for our PBPK models. Despite extensive *in vitro* investigations of tamoxifen and its metabolites, the information is rather limited to describe their PK profiles in humans.[3,29,30] Previously, the CNM-based parameter optimization was successfully implemented for cases in which multiple sets of parameters were simultaneously optimized with wide initial ranges.[12,15,31] In those cases, the CNM-based process first identified a large number of parameter sets that can adequately describe the observed data. In the current study, we utilized the observed profiles of tamoxifen and its metabolites and selected the final sets of parameters from sensitivity analyses for our analyses. This type of parameter optimization and workflow can be implemented for other complex cases of PBPK modeling.

A potential limitation of our current study may be related to the use of a simplified probability model that we adapted to link endoxifen levels and tumoral ER expression levels to clinical response. Considering the complex/compensatory nature of cellular signaling pathways influencing tumoral response to tamoxifen therapy, the current model represents a reductionist approach with minimal components. Our current model may, however, serve as a starting point toward further refinement (e.g., changes in upper/lower boundary values, and use of nonlinear functions) and validation using the datasets with larger sample sizes and longer follow-up data on endoxifen and tumoral ER levels and clinical response. In addition, further validation is warranted for our current assumption that the pharmacological activity of endoxifen levels is comparable among different ethnic groups.

In conclusion, we successfully constructed comprehensive PBPK models of tamoxifen and its metabolites and performed virtual TARGET-1 studies in virtual Japanese patients with breast cancer with differing CYP2D6 genotypes and obtained the expected outcomes of the TARGET-1 study prior to its completion. Our current efforts demonstrate the promising utility of the PBPK modeling and VCS approaches in gaining additional insights into factors influencing the outcomes and designing effective clinical trials.

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