Citation: CPT: Pharmacometrics & Systems Pharmacology (2013) 2, e66; doi:10.1038/psp.2013.43

www.nature.com/psp

ORIGINAL ARTICLE

Pharmacometric Analyses to Support Early Development Decisions for LY2878735: A Novel Serotonin Norepinephrine Reuptake Inhibitor

E Raddad[1], MR Melhem[2], JS Sloan-Lancaster[1], JW Miller[1], SA Van Wart[2] and CM Rubino[2]

LY2878735 is a novel dual serotonin (5-hydroxytryptamine (5-HT)) and norepinephrine (NE) reuptake inhibitor (SNRI) in development for chronic pain indications. *In vitro* profile suggests a more balanced profile as compared with other SNRI's, which is expected to confer superior clinical efficacy. LY2878735 is metabolized partly by the genetically polymorphic cytochrome P450 (CYP) 2D6 pathway, raising pharmacokinetic (PK) variability concerns. Phase 1 PK and biomarker data were analyzed by pharmacometric methods to characterize the balance between dual-target engagement and adverse effects on heart rate (HR) and blood pressure (BP). A narrow range of plasma LY2878735 levels was associated with an acceptable balance. As compared with poor metabolizers (PM), CYP2D6 extensive metabolizers (EM) have 21- and threefold higher clearance and distribution volume, respectively. Even with a CYP2D6-based dosing paradigm, a superior therapeutic index comparable to duloxetine, a widely used SNRI, was not achievable and LY2878735 development was thus terminated. Model-based approach effectively synthesizes PK-pharmacodynamic (PD) relationships, enabling efficient early development decisions.

CPT: Pharmacometrics & Systems Pharmacology (2013) **2**, e66; doi:10.1038/psp.2013.43; published online 21 August 2013

Pharmacometric analyses have demonstrated great value in all the stages of drug development,[1–6] especially in Phase 2 and 3 patient studies. Although the use of pharmacokinetic-pharmacodynamic (PK-PD) modeling in Phase 1 drug development has a long history, the impact has generally been limited.[7] Aarons *et al.* (2001) identified situations where PK-PD modeling and simulation offers great value in drug development, integrating knowledge across multiple studies to explore potential drug properties in different dosing regimens or subpopulations. This report illustrates such value for LY2878735, a novel serotonin norepinephrine (NE) reuptake inhibitor (SNRI) in development for chronic pain indications.

SNRIs bind to serotonin (5-hydroxytryptamine (5-HT)) and NE transporters (NET) to selectively inhibit the reuptake of both the neurotransmitters from the synaptic clefts, thereby increasing the availability of serotonin and NE within the central nervous system.[8] This drug class includes: venlafaxine and its active metabolites desvenlafaxine, duloxetine, and milnacipran. Because monoamine inhibitors of both the 5-HT and NE are effective in treating depression,[9–12] SNRIs were initially developed for such conditions. SNRIs have now been tested for efficacy in a number of clinical trials for major depressive disorder, general anxiety disorder, social anxiety disorder, and panic disorder in adults.[8] Observed improvements in response rates of SNRIs over selective serotonin reuptake inhibitors in these indications have been widely believed to be due to the addition of NET inhibition.[8]

Subsequently, interest grew in the potential use of SNRIs for specific chronic pain conditions. Milnacipran was shown to be effective in the management of fibromyalgia,[13] whereas duloxetine was shown to be effective in fibromyalgia, diabetic peripheral neuropathic pain, and chronic musculoskeletal pain.[14] However, available SNRIs carry some undesired properties, including exposure variability, NET-mediated adverse effects on blood pressure (BP) and heart rate (HR), and the need for close monitoring in specific populations.[13,14] Therefore, there is still room for improvement of this class of drugs.

LY2878735 is a novel potent and selective SNRI which was designed with key differentiating features as compared with existing SNRIs. LY2878735 possesses high affinity to both the serotonin transporter (SERT; K_i (inhibition constant) = 0.20 nmol/l) and the NET (K_i = 1.1 nmol/l).[15] Moreover, the relative affinity of LY2878735 to SERT vs. NET *in vitro* is 1:5, which is more balanced than that of venlafaxine (1:30) or duloxetine (1:9), and is more selective toward SERT vs. milnacipran (1:1.6).[8] Either SERT- or NET-related adverse effects can be dose-limiting, contributing to the generally low therapeutic index of SNRIs. Therefore, the more balanced SERT and NET affinity of LY2878735 provide the basis for potentially improved therapeutic index if consistent and optimal levels of drug exposure can be achieved *in vivo*. Furthermore, LY2878735 has lower potential for as-perpetrator cytochrome P450 (CYP)-based drug–drug interactions. The IC_{50} (half-maximal inhibitory concentration) for inhibition of CYP2D6, CYP2C9, CYP1A2, and CYP3A4 was much larger than the SERT- and NET-binding affinities, indicating low inhibition potential for these isoforms in humans, in comparison to the moderate CYP inhibition liability of venlafaxine and duloxetine.[8] CYP2D6 appeared to be associated with the highest rate of substrate depletion *in vitro*, however, its contribution to *in vitro*-estimated human clearance was <30%. Nonetheless,

[1]Chorus, Lilly Research Laboratories, Eli Lilly & Company, Indianapolis, Indiana, USA; [2]Institute of Clinical Pharmacodynamics, Inc., Latham, New York, USA. Correspondence: E Raddad (eyas@lilly.com)
Received 1 April 2013; accepted 23 June 2013; advance online publication 21 August 2013. doi:10.1038/psp.2013.43

this highlighted the risk for high pharmacokinetic variability and CYP2D6-based as-victim drug interaction potential, and warranted early assessment during the clinical development of LY2878735.

In this report, we illustrate the value of pharmacometric analysis of Phase 1 clinical data of LY2878735. The results of such analyses demonstrate an example of a decision-focused integration of PK and PD information from two small Phase 1 studies to aid understanding of clinical utility. Development decision criteria were: The SERT:NET relative potency position to be more balanced than duloxetine, and the magnitude of unexplained PK variability can enable a dosing regimen that achieves a favorable window between the SERT/NET target engagement and the unwanted effects on vital signs. Duloxetine was used as the benchmark because it is one of the most widely used SNRIs in chronic pain,[16] and the one with best understood PK-PD relationships. Models were the vehicle in which PK/PD information were synthesized for decision making.

RESULTS

Patients and data

A total of 1,096 quantifiable concentration data points from 57 subjects were used in the population PK analysis. A reasonably wide range of LY2878735 doses (1.0–25 mg) were administered across the two studies. A total of 233, 237, and 676 measurements from 57 subjects were available for 5-HT uptake inhibition, NE uptake inhibition, and plasma dihydroxyphenylglycol (DHPG) time course analyses, respectively.

In total, 26 SERT occupancy measurements were available from 14 healthy subjects, whereas 934 HR, systolic BP (sBP), and diastolic BP (dBP) measurements from 57 healthy subjects were available for the PK-PD analyses of changes in vital signs. Overall, data for 7 poor metabolizers (PM), 17 intermediate metabolizers, 32 extensive metabolizers (EM), and 1 ultra-rapid metabolizer were available from both studies. CYP2D6 distribution by dose group and study is reported in the **Supplementary Table S1** online.

Safety and tolerability

The treatment-emergent adverse event profile of LY2878735 was consistent with the serotonin and NE reuptake inhibition. Upon multiple dose administration (SNAB study), the most frequent treatment-emergent adverse events were nausea/vomiting, dizziness, headache, erectile dysfunction, and fatigue. These events occurred mainly after the repeated administration of doses of 2.5 mg or more. Nausea and vomiting generally began within 0.5–3 hours and subsided within 12 h. Hot flush, palpitations, mydriasis, diarrhea, dysuria, and hyperhidrosis also occurred following single doses of 10 and 25 mg LY2878735. All reported treatment-emergent adverse events were mild or moderate in nature. Titrated dosing and dosing in the fed state were generally better tolerated.

Population model development

PK model. LY2878735 concentrations peaked at a median of 3–6 h, exhibited biphasic decline, and had a two- to fourfold mean accumulation. As shown in **Figure 1a**, dose-normalized observed LY2878735 exposure measures were highly dependent on the CYP2D6 genotype. A two-compartment open model with first-order lagged absorption and linear elimination best described the PK data. Separate apparent clearance (CL/F) and apparent central volume of distribution (V_c/F) terms were estimated for the different CYP2D6 metabolizer genotypes. No significant food effect was detected on either the rate or the extent of drug absorption. Most model parameters and associated interindividual variability (IIV) (**Table 1**) were estimated with acceptable precision, except for IIV on V_c/F. The model adequately described the central tendencies in the observed data (**Figure 1b**). Plots of the observed vs. individual-predicted data and population-level residuals over time (**Figure 2**) were generally unbiased.

PK-PD models for SERT occupancy, NET inhibition, and DHPG. From **Figure 3a**,**b**, it is apparent that a wide dynamic range of response in all the PD markers was observed. The response generally plateaued at high LY2878735 concentrations. Direct simple or sigmoidal (E_{max}) model structures adequately described the relationships between LY2878735 concentrations and *ex vivo* 5-HT and NE uptake inhibition, as well as SERT occupancy. For all the three biomarkers, model parameters were estimated with acceptable precision (**Table 1**). The E_{max} in the SERT occupancy model was fixed to 100% to facilitate direct comparison to the published model for duloxetine.[17] Plots of the observed vs. individual-predicted data and population-level residuals over time (**Figure 2**) were generally unbiased. The pattern in the residuals for the SERT occupancy suggests positive prediction bias at higher end of the relationship. This is mainly attributed to forcing a fixed E_{max} at 100% to facilitate comparison to duloxetine published model. Similar bias can also be observed in Takano *et al.*[17] The visual predictive check (**Figure 3b**) provided evidence that these models adequately capture the central tendencies and distribution characteristics of the observed data.

An indirect response model adequately described the DHPG plasma concentrations for the pooled data. All the parameters and the associated IIV values (**Table 1**) were estimated with acceptable precision, except for IIV on the inhibitory concentration at which E_{max} is attained (IC_{50}). Plots of the observed vs. individual-predicted data and population-level residuals over time (**Figure 2**) were unbiased.

PK-PD models of vital signs. PK-PD models describing the relationship between the plasma LY2878735 concentrations and the supine HR, sBP, and dBP data were direct effect models with oscillating baselines. The population estimates of the mean pretreatment vital signs were consistent with the observed values. Moreover, the estimates of the amplitude (**Table 1**) for all vital signs were consistent with the trends in the observed data. Plots of the observed vs. individual predicted data and population-level residuals over time (**Figure 2**) were unbiased.

The circadian rhythm amplitude for HR was ~6%, whereas LY2878735 dosing contributed an additional effect up to maximum increase of ~37.7%. In addition, a maximum increase of ~2.43% in sBP was estimated without treatment, whereas the additional maximum increase due to LY2878735 treatment was estimated to be 7.19%.

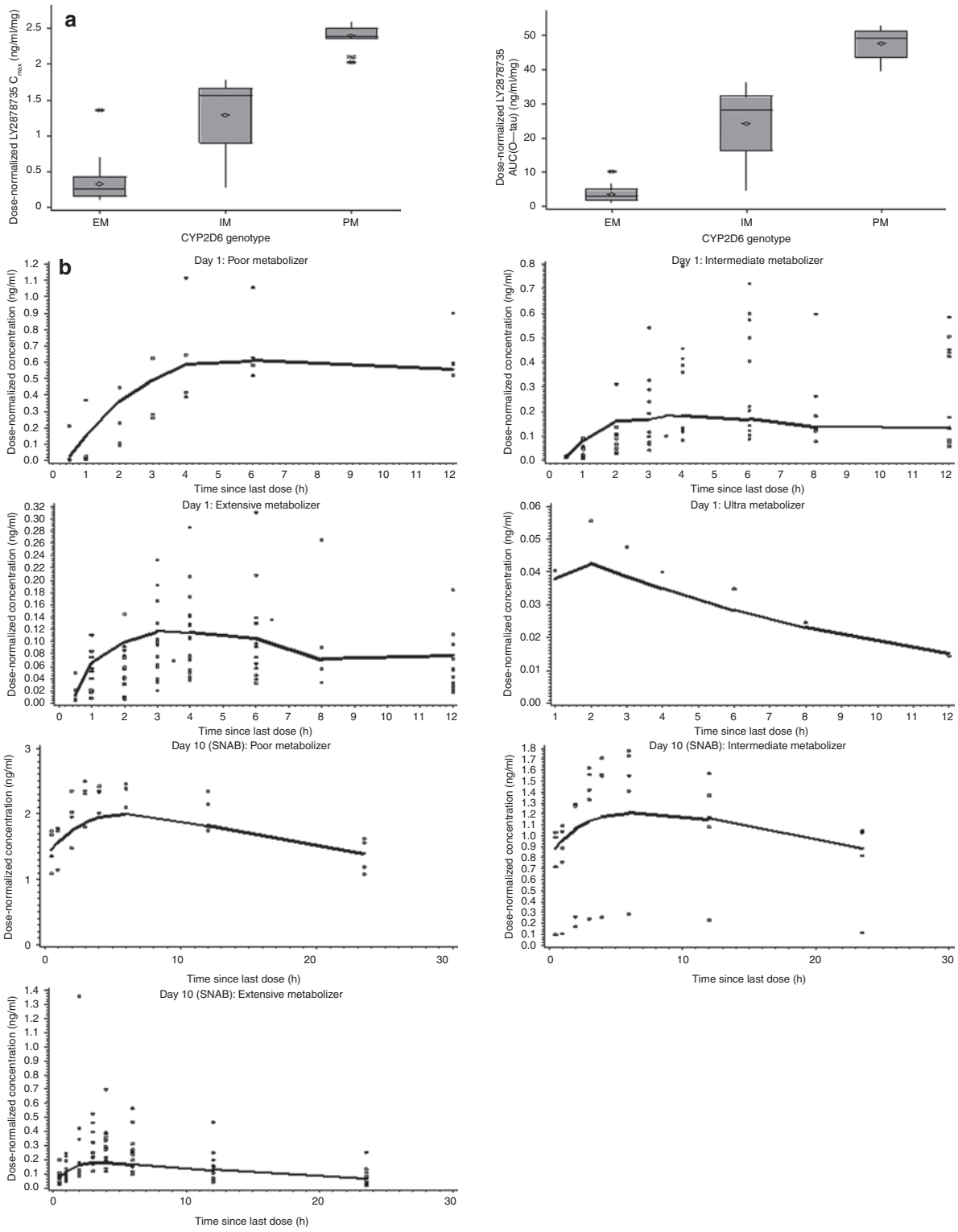


Figure 1 Effect of CYP2D6 genotype on LY2878735 exposure. (**a**) The effect of CYP2D6 genotype on dose-normalized C_{max} and $AUC_{(0–tau)}$ at steady state (day 10, SNAB study). (**b**) Dose-normalized plasma LY2878735 concentrations vs. time since last dose. Scattered symbols represent observations and lines represent the population median predictions. $AUC_{(0–tau)}$, area under the plasma concentration–time curve during the dosing interval of 24 h; C_{max}, maximum plasma concentration; EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer.

Model-based simulations. Graphical presentations for the simulation results were based on day 15 data, representing steady-state conditions (refer to **Supplementary Data** online). **Figure 4** shows boxplots of the maximum SERT occupancy, area above the percent change from baseline curve for DHPG, maximum change in HR, sBP, and dBP

Table 1 Parameter estimates and associated SEs from the final population PK or PK-PD model fit to pooled data from SNAA and SNAB studies

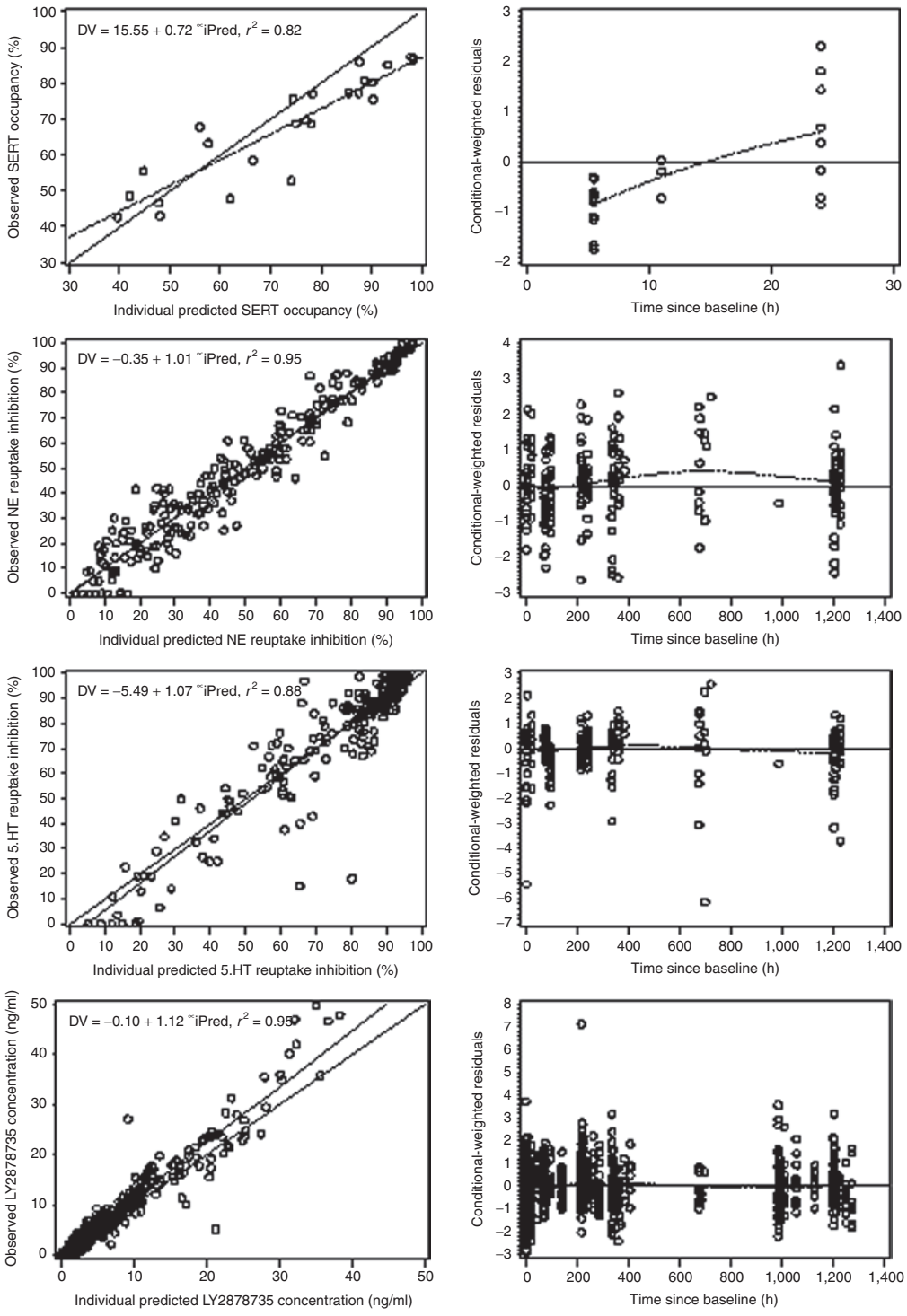
	Population mean		Interindividual variability (%CV)	
Parameters	**Final estimate**	**%SEM**	**Final estimate**	**%SEM**
Population PK				
K_a (h^{-1})	0.578	14.26	89.21	31.28
$V_{c\ (PM)}$/F (l)	902	28.29	50.2	58.33
$V_{c\ (IM)}$/F (l)	3,020	19.74	57.79	29.45
$V_{c\ (EM)}$/F (l)	6,330	11.8	68.70	25.96
$V_{c\ (UM)}$/F (l)	771	5.58	NE	NA
Q/F (l/h)	55.4	33.03	NE	NA
V_p/F (l)	2,090	15.55	NE	NA
Lag time (h)	0.415	7.47	NE	NA
$CL_{(PM)}$/F (l/h)	18.3	4.54	NE	NA
$CL_{(IM)}$/F (l/h)	122	22.62	106.77	20.61
$CL_{(EM)}$/F (l/h)	392	12.32	67.53	19.21
$CL_{(UM)}$/F (l/h)	2,090	15.55	NE	NA
Proportional residual error (%CV)	33.17	13.09	NA	NA
Ex vivo 5-HT uptake inhibition				
$LOGIT_{Emax}$	7.2	4.60	1.23 (SD)	67.50
EC_{50} (ng/ml)	0.11	18.60	82.20	23.10
Hill coefficient	1.74	14.30	NE	NA
E_0 (%)	0 (Fixed)	NA	NE	NA
Additive residual error (%)	10.58 (SD)	36.90	NE	NA
Ex vivo NE uptake inhibition				
$LOGIT_{Emax}$	30.5	8.80	NA	NA
EC_{50} (ng/ml)	0.727	8.40	49.40	29.90
Hill coefficient	0.94	5.60	25.50	73.70
E_0 (%)	0 (Fixed)	NA	NA	NA
Additive residual error (%)	7.90 (SD)	17.50	NA	NA
Plasma DHPG time course				
Baseline DHPG (pg/ml)	1,140	3.2	21.95	20.95
K_{out} (h^{-1})	0.39	8.69	29.10	65.05
I_{max}	0.387	4.94	18.66	41.09
IC_{50} (ng/ml)	0.215	14.79	47.33	79.91
Hill coefficient	1.38	19.13	NE	NA
Proportional residual error (%CV)	9.72	9.84	NE	NA
SERT occupancy				
E_{max} (%)	100 (Fixed)	NA	NA	NA
EC_{50} (ng/ml)	0.185	15.78	28.28	53.45
Hill coefficient	1 (Fixed)	NA	NE	NA
E_0 (%)	0 (Fixed)	NA	NA	NA
Proportional residual error (%CV)	14.87	31.72	NE	NA
Supine HR time course				
Mean baseline HR (beats/min)	59.7	1.93	12.70	22.40
Amplitude (fraction of baseline)	0.0604	8.64	NE	NA
Peak time shift (h)	16 (Fixed)	NA	16.70	22.60
Cycle duration (h)	24 (Fixed)	NA	NE	NA
E_{max} (fraction)	0.377	15.2	NE	NA
EC_{50} (ng/ml)	4.47	38.3	158.0	38.50
Hill coefficient	1 (Fixed)	NA	NE	NA
Proportional residual error (%CV)	10.2	9.52	NE	NA
Supine sBP time course				
Mean baseline sBP (mmHg)	119	1.08	6.70	31.80
Amplitude (fraction of baseline)	0.0243	17.7	NE	NA
Peak time shift (h)	16 (Fixed)	NA	29.40	42.0
Cycle duration (h)	24 (Fixed)	NA	NE	NA
E_{max} (fraction)	0.0719	14.9	62.0	45.8
EC_{50} (ng/ml)	0.993	23	81.70	46.2
Hill coefficient	3.98	16.8	NE	NA
Proportional residual error (%CV)	6.26	4.44	NE	NA
Supine dBP time course				
Mean baseline dBP (mmHg)	68.8	1.34	8.61	28.70
Amplitude (fraction of baseline)	0.03 (Fixed)	NA	47.30	56.20
Peak time shift (h)	14 (Fixed)	NA	36.20	50.80
Cycle duration (h)	24 (Fixed)	NA	NE	NA
E_{max} (fraction)	0.113	15.8	NE	NA
EC_{50} (ng/ml)	2.22	47.7	171.0	56.40
Hill coefficient	1.41	41.2	NE	NA
Proportional residual error (%CV)	6.99	4.35	NE	NA

CL/F, apparent systemic drug clearance; CV, coefficient of variation; dBP, diastolic blood pressure; DHPG, dihydroxyphenylglycol; E_0, baseline *ex vivo* 5-HT uptake inhibition, the baseline *ex vivo* NE uptake inhibition, or baseline SERT occupancy; EC_{50}, drug concentration at which half-maximal inhibition was achieved; EM, extensive cytochrome P450 2D6 metabolizer; E_{max}, maximum *ex vivo* 5-HT uptake inhibition, maximum *ex vivo* NE uptake inhibition, maximum inhibition rate of DHPG production, or the maximal change in the vital sign measurement with treatment; IC_{50}, half-maximal inhibitory concentration; IM, intermediate cytochrome P450 2D6 metabolizer; I_{max}, maximum inhibition rate of DHPG production; K_a, first-order absorption rate constant; K_{out}, first-order rate constant for DHPG elimination from plasma; Lag time, absorption lag time; NA, not applicable; NE, not estimated; NE uptake, norepinephrine uptake; PD, pharmacodynamics; PK, pharmacokinetics; PM, poor cytochrome P450 2D6 metabolizer; Q/F, apparent intercompartment clearance; sBP, systolic blood pressure; SERT, serotonin transporter; UM, ultra-fast cytochrome P450 2D6 metabolizer; V_c/F, apparent volume of distribution of the central compartment; V_p/F, apparent volume of distribution of the peripheral compartment; 5-HT, 5-hydroxytryptamine.

against dose for the whole population and for the different metabolizer genotypes. In CYP2D6 EMs, a LY2878735 dose of 5.5 mg would be expected to achieve equipotent SERT occupancy and plasma DHPG activity as 60 mg of duloxetine.

Given the trends in the observed data and the nature of the optimal PK-PD models, the maximum change in each of the vital signs increased with increasing dose, but reached a plateau at the highest exposures. **Figure 5** depicts predicted steady-state LY2878735 concentration–response relationships with plasma DHPG reduction (%), SERT occupancy, and vital signs. As shown in the figure, a maximum response in all the biomarkers and the vital signs was achieved at LY2878735 concentrations higher than 5 ng/ml and a dose of ~5 mg.

Consolidated graphs of the median effects of LY2878735 on several endpoints are provided in **Figure 6**. These plots show that an E_{max} of 21 mmHg was expected for



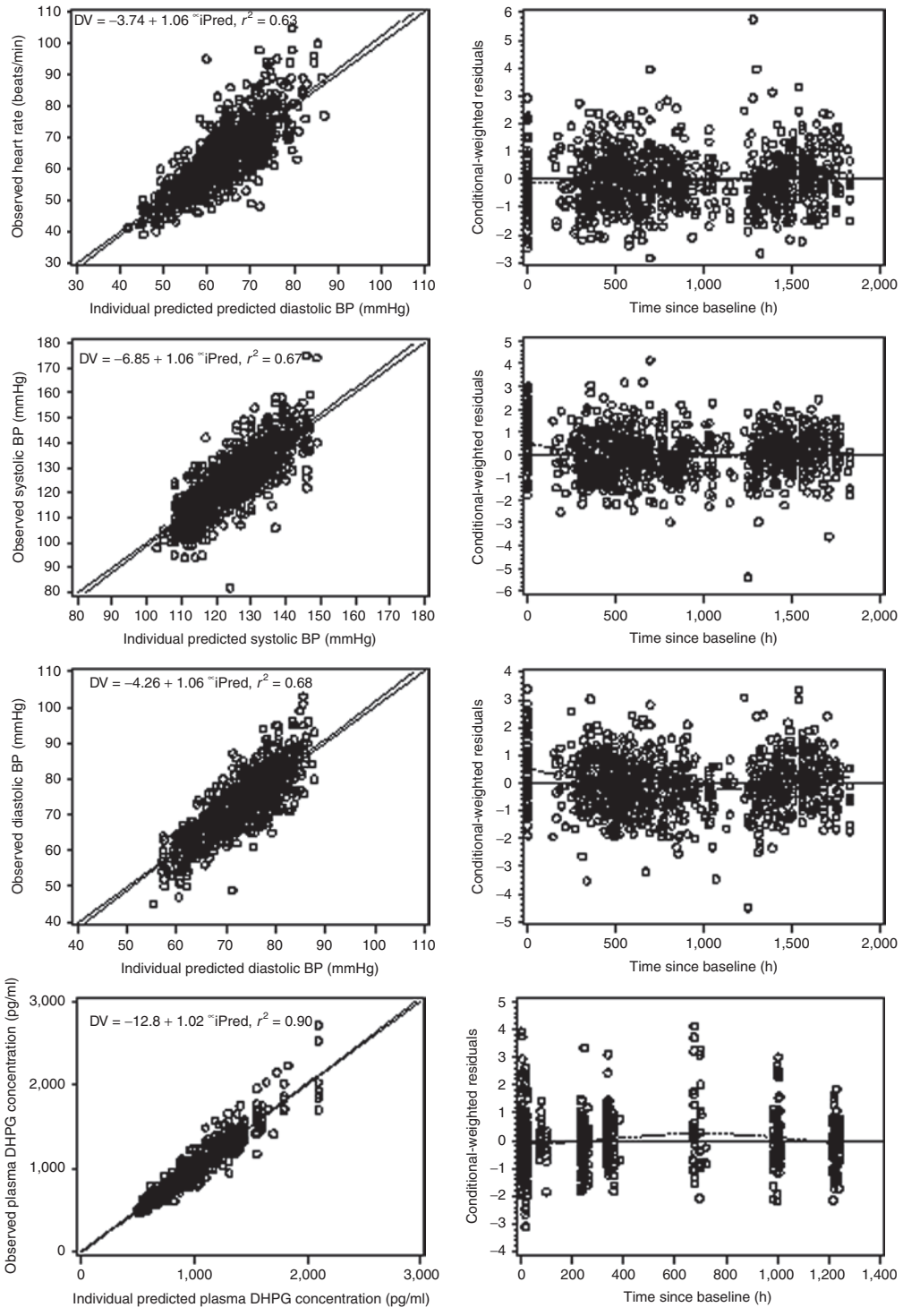


Figure 2 Goodness-of fit plots for the final population PK model and PK-PD models describing SERT occupancy, *ex vivo* NE inhibition, *ex vivo* 5-HT inhibition, DHPG time course, HR, sBP and dBP time course. dBP, diastolic blood pressure; DHPG, dihydroxyphenylglycol; HR, heart rate; NE, norepinephrine; PD, pharmacodynamics; PK, pharmacokinetics; sBP, systolic blood pressure; SERT, serotonin transporter; 5-HT, 5-hydroxytryptamine.

sBP, DHPG levels, and 98% SERT occupancy at doses of 30 mg or higher. E_{max} was expected at doses of 5 mg or higher for PMs and intermediate metabolizers, when compared with EMs and ultra-rapid metabolizers. Moreover, for any clinically meaningful maximum change in sBP and at a given dose, the mean percentage of subjects attaining such an increase was expected to be significantly higher in PM as compared with other metabolizer subtypes. E_{max} was not attained for HR in any of the CYP2D6 metabolizer groups.

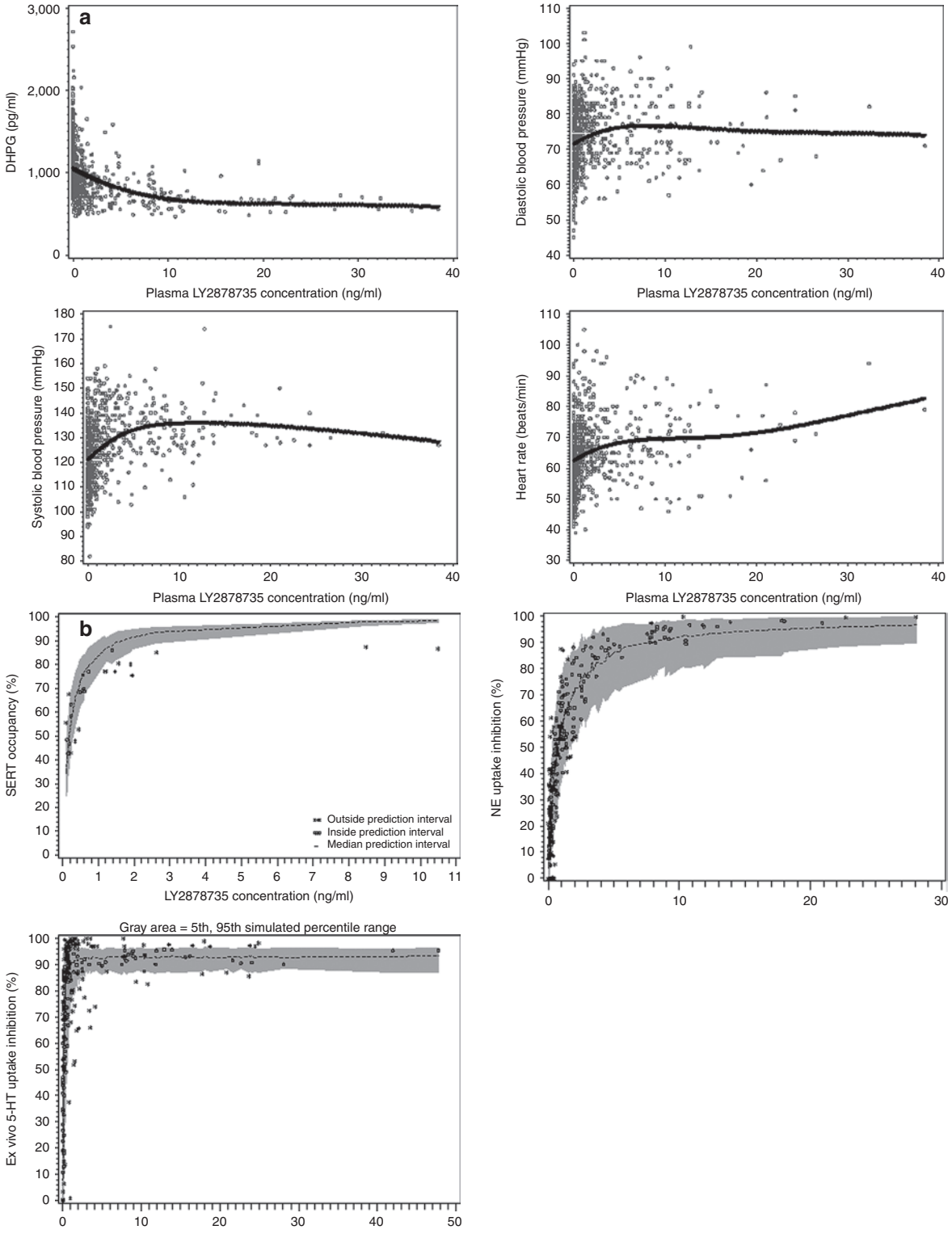


Figure 3 Relationship between LY2878735 plasma concentration and vital sign measurements or biomarker level. (**a**) Observed plasma DHPG concentration, dBP, sBP, and HR vs. LY2878735 plasma concentration. (**b**) Observed (symbols) and 90% prediction interval for the direct effect population PK-PD model describing the relationship between SERT occupancy, *ex vivo* 5-HT uptake inhibition, *ex vivo* NE uptake inhibition, and the LY2878735 plasma concentration. dBP, diastolic blood pressure; DHPG, dihydroxyphenylglycol; HR, heart rate; NE, norepinephrine; PD, pharmacodynamics; PK, pharmacokinetics; sBP, systolic blood pressure; SERT, serotonin transporter; 5-HT, 5-hydroxytryptamine.

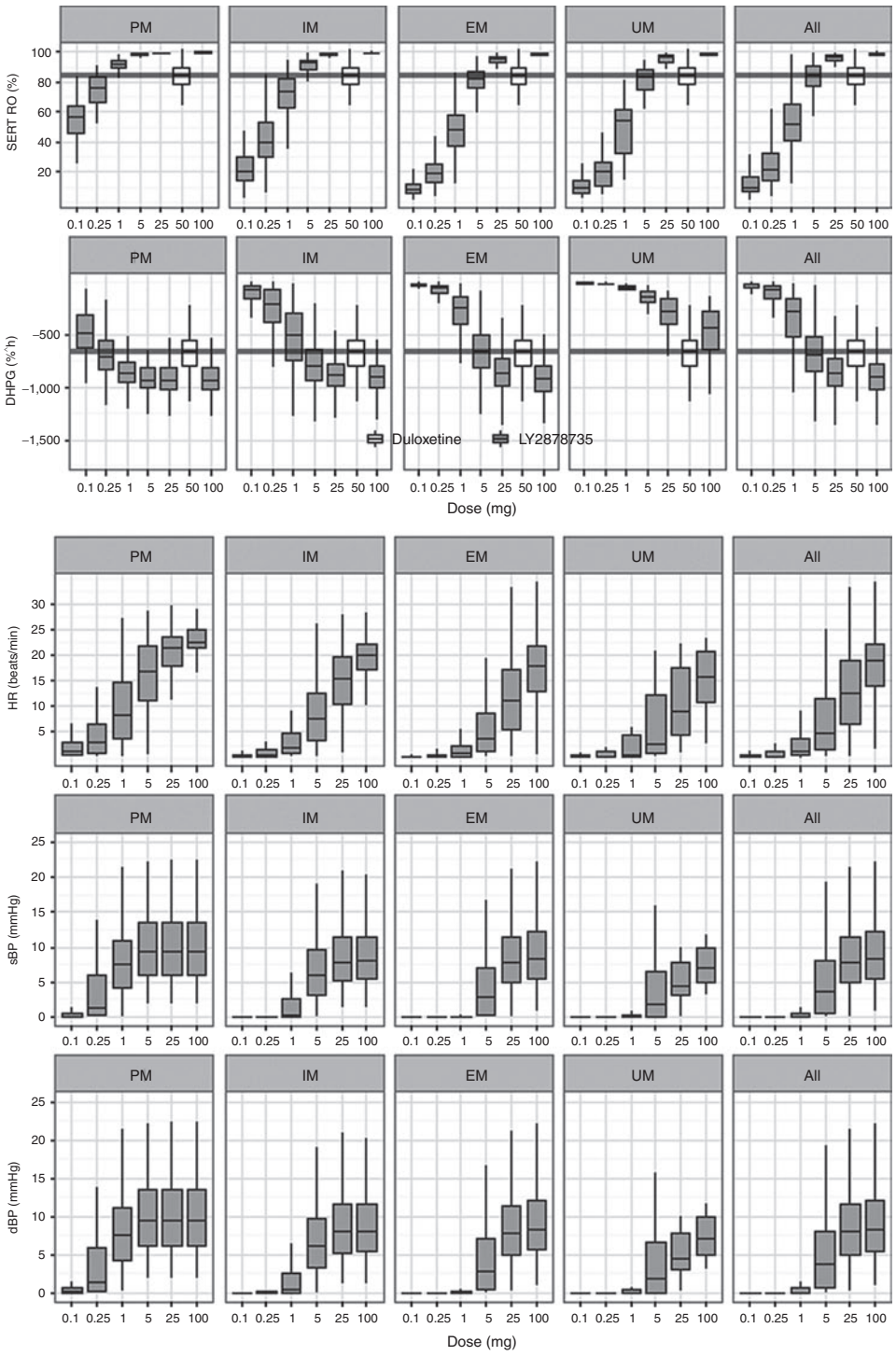


Figure 4 Boxplots of simulated maximum change in response (SERT occupancy (%), reduction in plasma DHPG from baseline (%), HR, sBP, and dBP) at different dose levels for the different CYP2D6 genotypes. dBP, diastolic blood pressure; DHPG, dihydroxyphenylglycol; EM, extensive metabolizer; HR, heart rate; IM, intermediate metabolizer; PM, poor metabolizer; RO, SERT receptor occupancy; sBP, systolic blood pressure; SERT, serotonin transporter; UM, ultra-rapid metabolizer.

DISCUSSION

Several factors contributed to the value of this analysis toward determining whether and how to pursue further development with LY2878735. First, well-accepted biomarkers of target engagement and PD effects were available. Second, the key determinants of success for LY2878735 as a novel SNRI agent were defined *a priori* and mapped to pharmacologic properties tested in the Phase 1 studies, i.e., relative potency, relationship to vital sign effects, PK variability, and

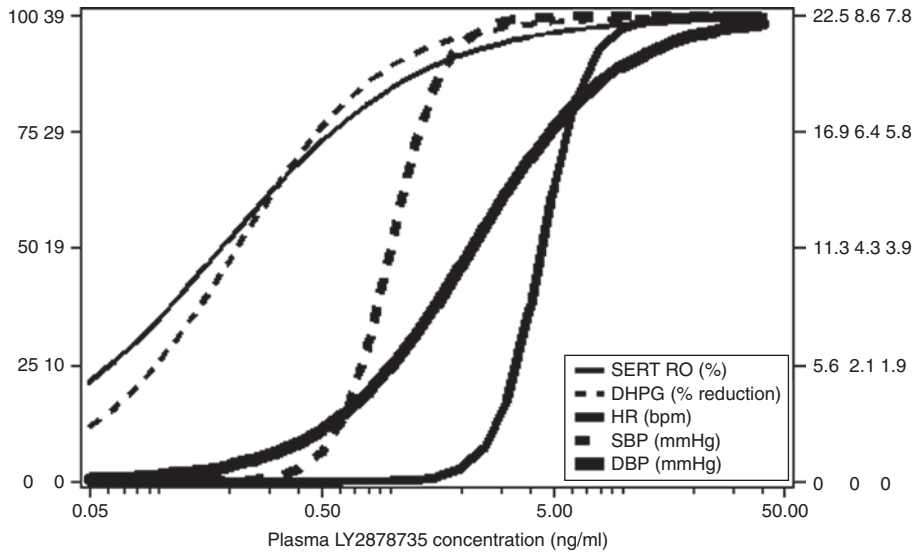


Figure 5 Model-predicted steady-state LY2878735 concentration–response relationships for plasma DHPG reduction (%), SERT occupancy (%), sBP, HR, and dBP. Axes values correspond from left to right with top to bottom legend order. bpm, beats per minute; dBP, diastolic blood pressure; DHPG, dihydroxyphenylglycol; HR, heart rate; RO, SERT receptor occupancy; sBP, systolic blood pressure; SERT, serotonin transporter.

the impact of CYP2D6 genotype. Third, rich prior information and models of a key SNRI comparator, duloxetine, existed.

The combined pharmacometric analysis of the two clinical studies provides key insight into the pharmacological properties of LY2878735. LY2878735 clearly acts as a dual 5-HT and NE reuptake inhibitor in humans, with a SERT:NET potency ratio that meets the intended position among other SNRI's as a more balanced molecule, but is associated with an unintended liability of high PK variability relating in part to CYP2D6 metabolism.

LY2878735 exhibited SERT occupancy in positron electron tomography imaging and reduced serum concentrations of DHPG, thus indirectly demonstrating the inhibition of NE reuptake. In addition, *ex vivo* measurement of 5-HT and NE uptake inhibition demonstrates that available LY2878735 plasma concentrations are at a range consistent with significant inhibition of 5-HT and NE reuptake.

LY2878735 appears to be significantly more potent than duloxetine at NET engagement. The estimated *in vivo* EC_{50} for the inhibitory effect on plasma DHPG for LY2878735 is 0.215 ng/ml (0.77 nmol/l), significantly lower than that for duloxetine (7.5 nmol/l).[18] SERT occupancy EC_{50} for LY2878735 (0.185 ng/ml, 0.66 nmol/l) is similar to that of duloxetine (0.8 nmol/l).[17] The NET:SERT EC_{50} ratio is ~1 for LY2878735 vs. 9 for duloxetine. The relative potencies (EC_{50}) of the *ex vivo* 5-HT and NE uptake inhibition measured in our clinical studies, 0.13 and 0.714 ng/ml, result in a NET:SERT potency ratio of 5 for LY2878735, as compared with the reported value of 2.6 for duloxetine.[19] Both the results are generally consistent with the *in vitro* K_i ratios, where LY2878735 was shown to have fourfold higher SERT-binding affinity as compared with duloxetine and sevenfold higher NET-binding affinity.[20] As such, LY2878735 clearly shows to be more NET-favoring relative to 5-HT as compared with duloxetine. The fact that potency comparison is across different endpoints and model structures is not a limitation given that the main interest is in comparing LY2878735 to duloxetine.

For LY2878735, the concentration–response relationships for SERT and DHPG suggest that only a narrow concentration window offers a high percentage of SERT and NET engagement without clinically concerning effects on vital signs (**Figure 5**).

LY2878735 PK appear to be highly variable, further aggravating the narrow concentration-based margin of safety. CYP2D6 appears to be the major pathway of clearance for LY2878735, and contributes to substantial PK variability. The CL/F of LY2878735 is ~21-fold faster, whereas the apparent V_c/F is approximately sevenfold larger, in CYP2D6 EMs as compared with PMs. Because there is no reason to anticipate a difference in the true V_c, it is likely that the difference in V_c/F is a result of differences in bioavailability due to a more efficient first-pass extraction with genotypes of higher CYP2D6-metabolizing capacity. Since V_c/F difference was approximately sevenfold, it is likely that EMs have about a sevenfold lower bioavailability than PMs. Similarly, it is likely that higher CL/F in EM is stemming not only from an increased clearance, but also from a reduced bioavailability. PMs lack a functional CYP2D6 enzyme, and hence the CL/F in PM represents LY2878735 clearance not attributed to CYP2D6. Based on these observations and assumptions, it is reasonable to expect that CYP2D6 clearance accounts for ~70% of total clearance in extensive CYP2D6 metabolizers. Although CYP2D6 is involved in the metabolism of duloxetine, its impact on PK variability is much less pronounced.[21]

The PK variability that is driven largely by CYP2D6 creates an opportunity and a challenge to the future development of LY2878735. On one hand, tailoring LY2878735 dosing to CYP2D6 metabolizer status significantly reduces the variability in drug exposure. The simulations presented in this report suggest that such tailoring strategy is necessary, and would be expected to result in improved balance between risk and benefit upon treatment with LY2878735. On the other hand, it appears that attaining similar median SERT and NET engagement levels as the recommended duloxetine dose is only possible at LY2878735 doses that are associated with median

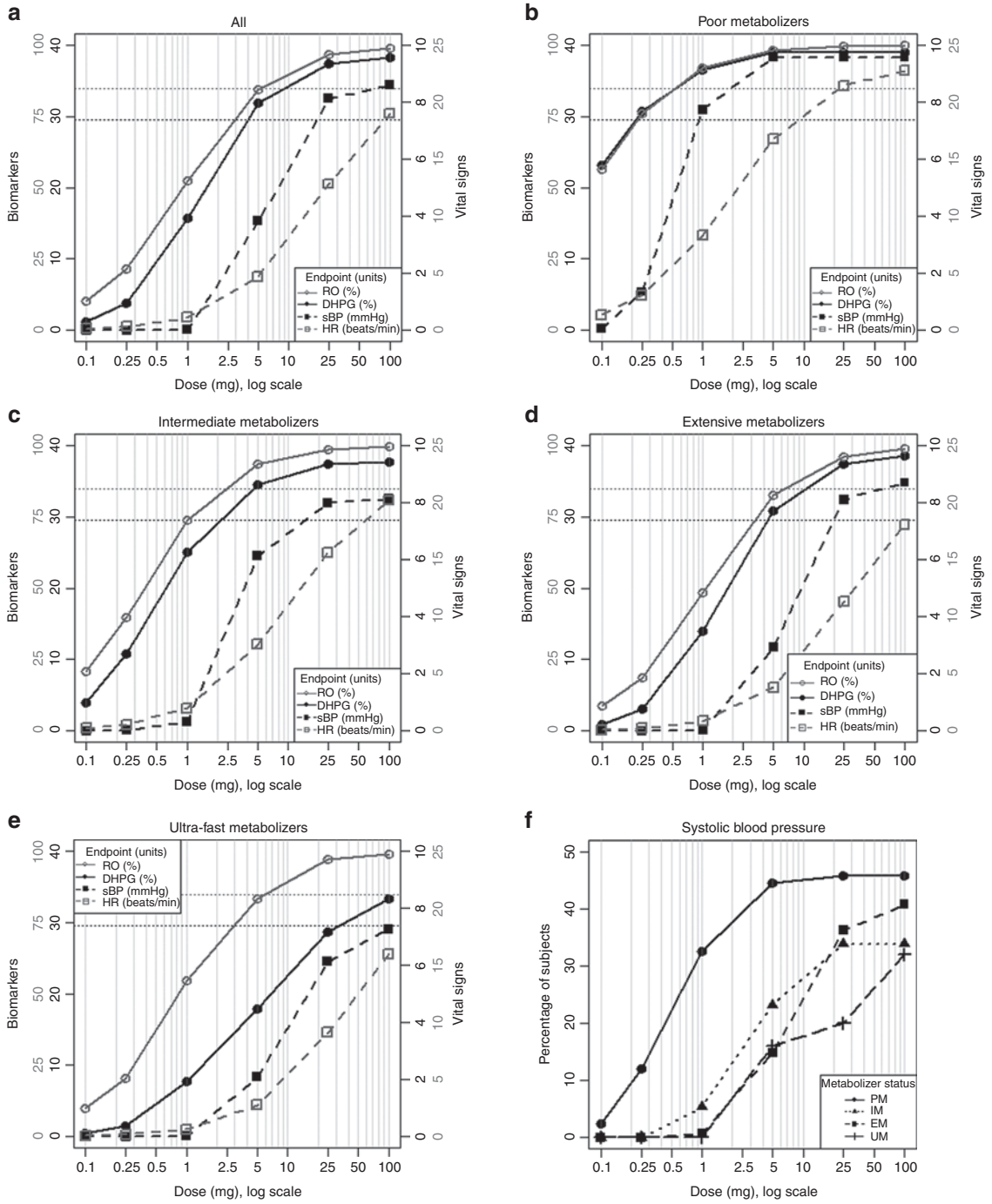


Figure 6 Simulations to illustrate the therapeutic index of LY2878735 as a function of CYP2D6 genotype. (**a**–**e**) Plots of median predicted response vs. LY2878735 dose, stratified by CYP2D6 metabolizer genotype, for different endpoints/biomarkers. (**f**) Line plots of percentage of subjects with a maximum increase in sBP of 10% or more vs. dose, stratified by CYP2D6 metabolizer status. DHPG, dihydroxyphenylglycol; EM, extensive metabolizer; HR, heart rate; IM, intermediate metabolizer; PM, poor metabolizer; RO, SERT receptor occupancy; sBP, systolic blood pressure; UM, ultra-rapid metabolizer.

HR and sBP effects in the vicinity of 5 beats per minute and 5–10 mmHg, respectively, and with a significant fraction of subjects with a maximum increase in sBP of 10% or higher. Although a PK-PD model of vital signs for duloxetine is not available, reported data suggest that effects on vital signs are minimal.[14] The high PK variability, thus, resulted in an inability to define a dosing regimen that has a superior balance of target engagement and effect on vital signs as compared with duloxetine, even with genotype-based dosing. Therefore, the chance of LY2878735 to improve upon the risk–benefit profile of duloxetine is small, leading the development team to recommend termination of the clinical development of LY2878735 for chronic pain indications. CYP2D6 is a highly polymorphic gene, with new discoveries arising on a yearly basis. Over 100

allelic variants have been discovered to date, resulting in a broad range of functional variations.[22,23] This complex variability is unlikely to be adequately described with a four-bin categorization enzymatic capacity system. It is inevitable that a range of functions will remain within some or all of such categories, perhaps with the exception of the category associated with a complete loss of activity (PM). We have noticed that the interindividual variability in PM subgroup was too small to be estimated. Nonetheless, the exploratory nature of our early clinical studies and the small sample size are important limitations that can only be addressed with further clinical work.

The work presented above highlights the utility of PK-PD modeling in the learning phases of drug development. The models were driven by specific development questions that are difficult, if not impossible, to answer with traditional data analysis methods. Multiple confounding factors hindered the utility of simple statistical methods: the two studies from which the data were obtained cover disparate designs; CYP2D6 genotype proportions were imbalanced across treatments and studies; not all biomarkers were measured in all subjects (e.g., SERT occupancy assessment was evaluated in different subjects than those from whom rich DHPG data was collected); and underlying diurnal variation confounded drug effect for vital signs. These confounding factors are only aggravated by the high between-subject variability, thus hindering the ability to compare the pharmacologic response at different dose levels, discerning the therapeutic window, and understanding the impact of CYP2D6 on the PK of LY2878735. Population PK-PD models allowed for the synthesis of information and disentanglement of different attributes of LY2878735 and design discrepancies, and significantly enhanced the efficiency to the early development research. The models, however, do rely on few assumptions that allow for dealing with the confounding factors, which include the stability of PK/PD relationships over time and upon multiple dose administration, the similarity of subject pools in factors other than the confounding ones, and the dose independence of CYP2D6 effect on clearance. Based on the experience with duloxetine, and careful model evaluation, we believe that these assumptions are reasonable.

The model-based comparisons to duloxetine are indirect due to the absence of duloxetine as a comparator in our studies. This should be taken into consideration when comparing LY2878735 to duloxetine in any of the PK or PD measures. The simulations generated from the models are also considered "*in numero*" experiments to address questions yet to be answered empirically. As such, they contribute to early assessment of the technical probability of success for the novel therapeutic agent, and should not be interpreted as definitive confirmation of drug properties.

Several improvements to this integrated pharmacometric analysis can be envisioned for future applications. First, the application of a quantitative weighting and scaling model, like a clinical utility index,[24] could reduce benefit–risk assessment complexity and aid dose selection optimization. Second, the inclusion of an active comparator in the study would allow for direct rather than indirect comparison. Third, more intensive sampling of vital signs could help better characterize the time course and drug effects. Lastly, joint (multivariate) modeling of the PD and vital sign variables would allow for better estimation of the relative potency and the therapeutic index.

In conclusion, these model-based analyses proved an efficient and effective way to synthesize complex, confounded clinical pharmacology information from early clinical data of LY2878735, thus helping elucidate the relative positioning of LY2878735 in the class of SNRIs with regard to relative SERT/NET potency, target engagement, and cardiovascular and drug interaction liabilities. CYP2D6 was identified as the major clearance pathway of LY2878735, resulting in large exposure variability, indicating that CYP2D6 genotyping is insufficient to drive a competitive benefit–risk balance for LY2878735, providing an early yet compelling basis for the termination of its current scope of development.

METHODS

Clinical studies

Study design. Two incomplete crossover design studies were conducted in healthy volunteers using similar enrollment criteria and study endpoints. The first, SNAA, was a typical first-in-man single ascending dose study. The second study, SNAB, was a two-part study, the first of which followed a typical multiple ascending dose design, and the second evaluated SERT occupancy using positron electron tomography. LY2878735 dosing was oral, mainly with food with the exception of one period to explore food effect. In SNAB, dosing was once daily for up to 10 days. A total of 57 subjects participated in the studies; 55 males and 2 females between 18 and 65 years of age. PM of CYP2D6 were specifically excluded from SNAA study, whereas SNAB study preferentially recruited CYP2D6 PM, resulting in 7 (25%) such subjects participating. Dense sampling was implemented for PK, *ex vivo* NET and SERT uptake inhibition, plasma NE and DHPG, and vitals (BP and HR). Sparser sampling was implemented for SERT occupancy due to limitations of radiation exposure. Both the studies were reviewed and approved by the local research ethics committees and internal review boards, and conducted according to the principles expressed in the Declaration of Helsinki. Details of study designs, genotyping methodology, and measured variables can be found in the **Supplementary Methods** online. Data from both the studies were pooled for analysis.

Population PK-PD model development

PK model. To characterize the plasma concentration vs. time profile following administration of LY2878735 after single or multiple oral dosing, a variety of compartmental models were evaluated. CYP2D6 metabolizer and food status were the only covariates evaluated for effect on PK parameters.

PK-PD models for SERT and NET uptake inhibition biomarkers. The time course of DHPG plasma levels, the relationship between SERT occupancy and LY2878735 plasma concentration, and the relationships between *ex vivo* NE and 5-HT uptake inhibition and plasma concentrations were described using population PK-PD models.

An indirect response model structure was used to describe the plasma DHPG concentration vs. time profiles following the administration of single or multiple oral doses of LY2878735. For this model, individual-predicted plasma drug concentrations were used as the driving function. This model structure is identical to a previously described model for duloxetine.[18]

Direct simple or sigmoidal E_{max} model structures were used to describe the relationships between observed drug concentrations and SERT occupancy, *ex vivo* NE uptake inhibition and *ex vivo* 5-HT uptake inhibition following LY2878735 administration. A logistic transformation was applied to the E_{max} parameter to ensure that the model estimates and the predictions of E_{max} are appropriately bounded at a maximum of 100%.

PK-PD models of vital signs. The time course of HR, sBP, and dBP were described using separate population PK-PD models. Due to the rhythmic nature of their time courses, an oscillating cosine function was used to characterize the underlying baseline vital sign patterns without treatment.

$$\text{Base} = \text{Mean}\left(1 + \text{AMP}\cdot\cos\left[2\pi\left(\frac{\text{MCLK} - \text{Peak}}{24}\right)\right]\right)$$

where AMP and MCLK represent the amplitude and time using the 24-h military clock, respectively. The individual-predicted plasma LY2878735 concentrations were used as the driving functions of the fractional effect on vital sign baseline through a sigmoidal E_{max} function. For all population PK and PK-PD analyses, candidate models were evaluated using the computer program NONMEM Version 7.1.2, implementing the first-order conditional estimation method with interaction.[25] Interindividual variability for each parameter was modeled using an exponential or additive error model. Residual variability was modeled using either additive or proportional error structures. For each model, NONMEM computed the minimum value of the objective function. For nested models, a change in the minimum value of the objective function of $\leq$10.83 (α = 0.001, degree of freedom = 1) was used to define statistical significance for inclusion of a parameter to the model using a likelihood ratio test. Akaike's information criterion was considered for non-nested models. The NONMEM codes for select biomarker and vital sign models are provided in **Supplementary Methods** online.

Model evaluation was based upon examination of population parameter estimates and their precision, and examination of standard goodness-of-fit diagnostics plots and simulation-based visual predictive check.[26]

Model-based simulations. Simulations based on the final PK-PD models associated with different virtual LY2878735 dosing regimens were performed. A broad range of clinically relevant LY2878735 doses was considered (0.1–100 mg), extending beyond doses tested in the aforementioned studies. A total of 1,000 subjects were simulated with a predefined prevalence of CYP2D6 metabolizer status[27] for each LY2878735 dosing scenario at steady state. Simulated time courses of PD biomarkers and vital signs at different dose levels of LY2878735 were constructed to evaluate the therapeutic index between target engagement biomarkers (SERT and DHPG) and safety parameters (BP and HR) in comparison to duloxetine, when applicable. Final estimates from previously reported PK,[28] SERT occupancy,[17] and DHPG[18,19] models for duloxetine at steady state (60 mg once daily oral dosing) were the basis of similar simulations for comparison. A more detailed description of the data analyses and simulation methodology is provided under **Supplementary Methods** online.

Acknowledgments. This research was funded by Eli Lilly and Company. The authors thank Vish S. Watkins and John P. Polzer for their contributions to the design and interpretation of the clinical studies upon which this analysis was conducted, as well as Michelle Eli for coordinating the clinical studies and collecting information needed for writing this manuscript.

Author contributions. E.R., M.R.M., J.S.S.-L., J.W.M. wrote manuscript. E.R., M.R.M., J.S.S.-L., J.W.M. designed research. M.R.M., S.A. Van W., C.M.R. analyzed data.

Conflict of interest. Drs Raddad, Sloan-Lancaster, and Miller are full-time employees at Eli Lilly and Company. Drs Rubino, Melhem, and Mr Van Wart are employees of the Institute for Clinical Pharmacodynamics and were contracted by Eli Lilly and Company to perform the analyses.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE OF THE TOPIC?

✓ Utilizing pharmacokinetic-pharmacodynamic modeling to inform development decisions during Phase 1 is infrequently demonstrated. Serotonin norepinephrine reuptake inhibitors (SNRIs) inhibit serotonin and norepinephrine transporters, with demonstrated benefits in depression and pain. LY2878735, a novel SNRI with balanced transporter affinity, is in development for chronic pain indications.

WHAT QUESTION THIS STUDY ADDRESSED?

✓ We utilized modeling to characterize the potential balance of LY2878735 in humans, and evaluate the therapeutic window between target engagement and adverse effects on heart rate and blood pressure.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE

✓ Although its affinity balance is confirmed *in vivo*, LY2878735 is associated with a narrow therapeutic window, which is aggravated by highly variable CYP2D6-dependent pharmacokinetics. Simulations suggest that even with a CYP2D6-based dosing paradigm, a therapeutic index superior to current SNRIs is not achievable, justifying termination of its development.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS

✓ Decision-focused model-based integration of pharmacokinetic and pharmacodynamic information illustrated in this report can stimulate more pervasive utilization of pharmacometrics in early clinical studies to elucidate differentiating features of novel therapeutic agents.

1. Chien, J.Y., Friedrich, S., Heathman, M.A., de Alwis, D.P. & Sinha, V. Pharmacokinetics/Pharmacodynamics and the stages of drug development: role of modeling and simulation. *AAPS J.* **7**, E544–E559 (2005).
2. Gobburu, J.V. & Marroum, P.J. Utilisation of pharmacokinetic-pharmacodynamic modelling and simulation in regulatory decision-making. *Clin. Pharmacokinet.* **40**, 883–892 (2001).
3. Bhattaram, V.A. *et al.* Impact of pharmacometrics on drug approval and labeling decisions: a survey of 42 new drug applications. *AAPS J.* **7**, E503–E512 (2005).
4. Bhattaram, V.A. *et al.* Impact of pharmacometric reviews on new drug approval and labeling decisions–a survey of 31 new drug applications submitted between 2005 and 2006. *Clin. Pharmacol. Ther.* **81**, 213–221 (2007).
5. Wang, Y. *et al.* Leveraging prior quantitative knowledge to guide drug development decisions and regulatory science recommendations: impact of FDA pharmacometrics during 2004-2006. *J. Clin. Pharmacol.* **48**, 146–156 (2008).
6. Lee, J.Y. *et al.* Impact of pharmacometric analyses on new drug approval and labelling decisions: a review of 198 submissions between 2000 and 2008. *Clin. Pharmacokinet.* **50**, 627–635 (2011).
7. Aarons, L., Karlsson, M.O., Mentré, F., Rombout, F., Steimer, J.L. & van Peer, A.; COST B15 Experts. Role of modelling and simulation in Phase I drug development. *Eur. J. Pharm. Sci.* **13**, 115–122 (2001).
8. Stahl, S.M., Grady, M.M., Moret, C. & Briley, M. SNRIs: their pharmacology, clinical efficacy, and tolerability in comparison with other classes of antidepressants. *CNS Spectr.* **10**, 732–747 (2005).
9. Wong, D.T., Bymaster, F.P. & Engleman, E.A. Prozac (fluoxetine, Lilly 110140), the first selective serotonin uptake inhibitor and an antidepressant drug: twenty years since its first publication. *Life Sci.* **57**, 411–441 (1995).
10. Beasley, C.M. Jr, Holman, S.L. & Potvin, J.H. Fluoxetine compared with imipramine in the treatment of inpatient depression. A multicenter trial. *Ann. Clin. Psychiatry* **5**, 199–207 (1993).
11. Burrows, G.D., Maguire, K.P. & Norman, T.R. Antidepressant efficacy and tolerability of the selective norepinephrine reuptake inhibitor reboxetine: a review. *J. Clin. Psychiatry* **59** (suppl. 14), 4–7 (1998).
12. Nelson, J.C. A review of the efficacy of serotonergic and noradrenergic reuptake inhibitors for treatment of major depression. *Biol. Psychiatry* **46**, 1301–1308 (1999).
13. Savella (milnacipran) [package insert]. New York, NY: Forest Pharmaceuticals, Inc., 2011.
14. Cymbalta (duloxetine) [package insert]. Indianapolis, IN: Eli Lilly & Co., 2012.
15. Koch, S. *et al.* Comparison of effects of dual transporter inhibitors on monoamine transporters and extracellular levels in rats. *Neuropharmacology* **45**, 935–944 (2003).
16. Wang, J., Liu, X. & Mullins, C.D. Treatment adherence and persistence with duloxetine, venlafaxine XR, and escitalopram among patients with major depressive disorder and chronic pain-related diseases. *Curr. Med. Res. Opin.* **27**, 1303–1313 (2011).
17. Takano, A. *et al.* A dose-finding study of duloxetine based on serotonin transporter occupancy. *Psychopharmacology (Berl.)* **185**, 395–399 (2006).
18. Quinlan, B.S., Knadler, M.P., Pangallo, B., Chappell, J. & Lobo, E. Pharmacokinetic/pharmacodynamic modeling of the effects of duloxetine on 3,4-dihydroxyphenylglycol (DHPG) in plasma and cerebrospinal fluid in healthy subjects. Poster presented at the American Conference on Pharmacometrics (ACoP) Annual Meeting, 4–7 October 2009; Mashantucket, CT.
19. Lobo, E. *et al.* Pharmacokinetic-pharmacodynamic modeling of inhibition of serotonin and norepinephrine uptake by duloxetine and escitalopram in healthy subjects. Abstract PI-57. Poster presented at the 110th Annual Meeting of the American Society for Clinical Pharmacology and Therapeutics (ASCPT), 18–21 March 2009; Washington, DC.
20. Vaishnavi, S.N., Nemeroff, C.B., Plott, S.J., Rao, S.G., Kranzler, J. & Owens, M.J. Milnacipran: a comparative analysis of human monoamine uptake and transporter binding affinity. *Biol. Psychiatry* **55**, 320–322 (2004).
21. Knadler, M.P., Lobo, E., Chappell, J. & Bergstrom, R. Duloxetine: clinical pharmacokinetics and drug interactions. *Clin. Pharmacokinet.* **50**, 281–294 (2011).
22. Streetman, D.S., Bertino, J.S. Jr & Nafziger, A.N. Phenotyping of drug-metabolizing enzymes in adults: a review of in-vivo cytochrome P450 phenotyping probes. *Pharmacogenetics* **10**, 187–216 (2000).
23. The Human Cytochrome P450 (CYP) Allele Nomenclature Committee. The human cytochrome P450 (CYP) allele nomenclature database [database online]. Karolinska Institutet: Stockholm, Sweden. <http://www.cypalleles.ki.se/>. Accessed 4 June 2013.
24. Poland, B. *et al.* The clinical utility index as a practical multiattribute approach to drug development decisions. *Clin. Pharmacol. Ther.* **86**, 105–108 (2009).
25. Bauer, R.J. *NONMEM Users Guides*, NONMEM 7, Version 7.1.2 (ICON Development Solutions, Ellicott City, MD, 2010).
26. Post, T.M., Freijer, J.I., Ploeger, B.A. & Danhof, M. Extensions to the visual predictive check to facilitate model performance evaluation. *J. Pharmacokinet. Pharmacodyn.* **35**, 185–202 (2008).
27. Zanger, U.M., Raimundo, S. & Eichelbaum, M. Cytochrome P450 2D6: overview and update on pharmacology, genetics, biochemistry. *Naunyn Schmiedebergs Arch. Pharmacol.* **369**, 23–37 (2004).
28. Lobo, E.D., Quinlan, T., O'Brien, L., Knadler, M.P. & Heathman, M. Population pharmacokinetics of orally administered duloxetine in patients: implications for dosing recommendation. *Clin. Pharmacokinet.* **48**, 189–197 (2009).