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Use of Physiologically Based Pharmacokinetic Modeling to Evaluate the Impact of Chronic Kidney Disease on CYP3A4-Mediated Metabolism of Saxagliptin

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Abstract

We characterized the impact of chronic kidney disease (CKD) on the cytochrome P450 (CYP) 3A4–mediated metabolism of saxagliptin to its metabolite, 5-hydroxysaxagliptin, using a physiologically based pharmacokinetic (PBPK) model. A PBPK model of saxagliptin and its CYP3A4 metabolite, 5-hydroxysaxagliptin, was constructed and validated for oral doses ranging from 5 to 100 mg. The observed ratios of area under the plasma concentration–time curve (AUC) and maximum plasma concentration (C_{max}) between healthy subjects and subjects with CKD were compared with those predicted using PBPK model simulations. Simulations were performed with virtual CKD populations having decreased CYP3A4 activity (ie, 64%-75% of the healthy subjects' CYP3A4 abundance) and preserved CYP3A4 activity (ie, 100% of the healthy subjects' CYP3A4 abundance). We found that simulations using decreased CYP3A4 activity generally overpredicted the ratios of saxagliptin AUC and C_{max} in CKD compared with those using preserved CYP3A4 activity. Similarly, simulations using decreased CYP3A4 activity underpredicted the ratio of 5-hydroxysaxagliptin AUC in moderate and severe CKD compared with simulations using preserved CYP3A4 activity. These findings suggest that decreased CYP3A4 activity in CKD underpredicts saxagliptin clearance compared with that observed clinically. Preserving CYP3A4 activity in CKD more closely estimates saxagliptin clearance and 5-hydroxysaxagliptin exposure changes observed in vivo. Our findings suggest that there is no clinically meaningful impact of CKD on the metabolism of saxagliptin by CYP3A4. Since saxagliptin is not a highly sensitive substrate and validated probe for CYP3A4, this work represents a case study of a CYP3A4 substrate-metabolite pair and is not a generalization for all CYP3A4 substrates.

Keywords

chronic kidney disease, CYP3A, CYP3A4, cytochrome P450 3A4, nonrenal clearance, physiologically based pharmacokinetic modeling

Patients with chronic kidney disease (CKD) typically have complex therapeutic regimens and high medication burdens. The combination of polypharmacy and kidney disease–associated changes in pharmacokinetics (PK) results in an increased risk of drug-related adverse events and drug-drug interactions (DDIs) in these patients.[1–4] Therefore, understanding altered drug clearance in CKD is critical in optimizing therapy and improving outcomes.

It is well established that CKD affects both renal clearance (CL_R) and nonrenal clearance (CL_{NR}). Changes in CL_{NR} are thought to be mediated by uremic solutes, either by downregulation of protein and gene expression or by direct inhibition of drug-metabolizing enzymes and transporters.[5–7] The impact of CKD on CL_{NR} is pathway specific; not all drug-metabolizing enzymes or transporters are impacted to the same degree by CKD.[8,9] Therefore, elucidating the effect of CKD on individual clearance pathways can be challenging, particularly due to overlapping substrate specificity of metabolizing enzymes and transporters of interest.

Saxagliptin is an oral dipeptidyl peptidase-4 inhibitor that is eliminated via renal and metabolic routes. Of the administered dose of saxagliptin, ≈25% is eliminated renally and 50% is metabolized by

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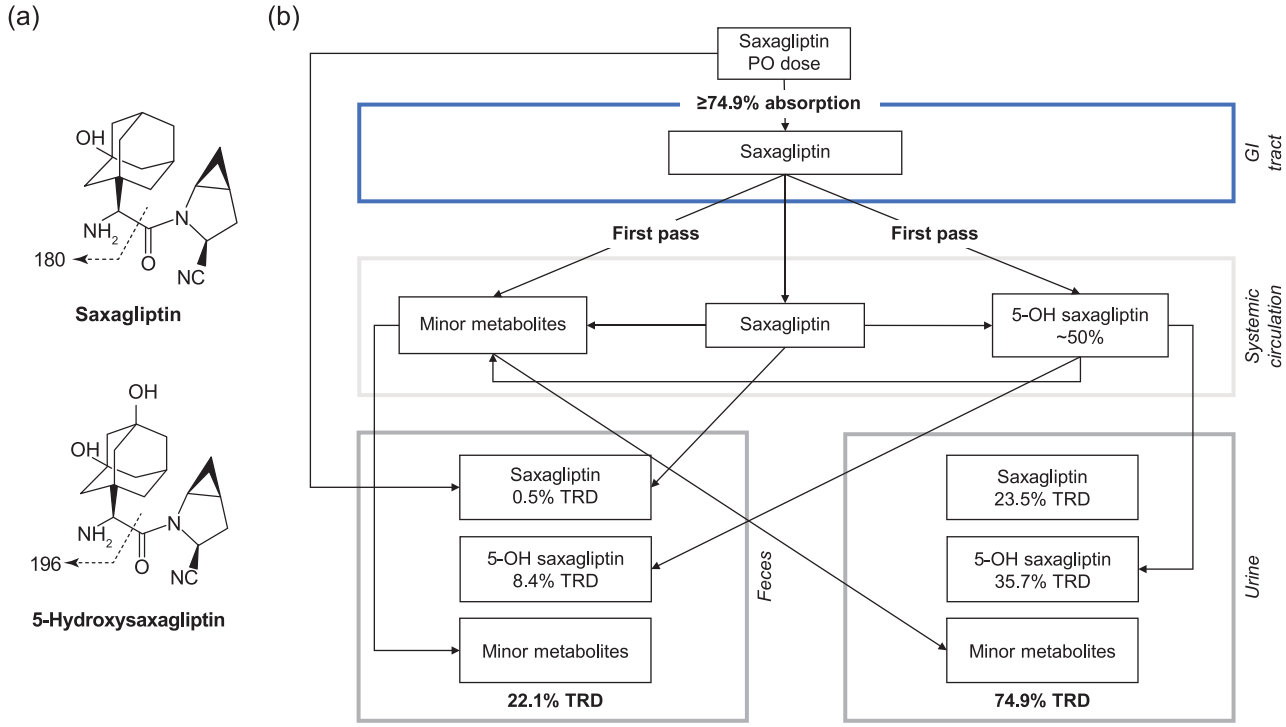

Figure 1. Biotransformation of saxagliptin and 5-hydroxysaxagliptin. Chemical structures of saxagliptin and 5-hydroxysaxagliptin (a). Saxagliptin is extensively absorbed following oral administration, with approximately 75% of the total dose being recovered in the urine. Both renal clearance and metabolism play a role in saxagliptin disposition. Approximately 50% of saxagliptin is oxidized by CYP3A4/5 to 5-hydroxysaxagliptin, which is the major species circulating in the plasma. 5-Hydroxysaxagliptin is majorly renally excreted (≈36% of the total dose is eliminated in the urine as 5-hydroxysaxagliptin), with some additional elimination in the feces (8.4% of the total dose is eliminated in the feces as 5-hydroxysaxagliptin) (b). Adapted with permission from Su et al.[18] CYP3A4, cytochrome P450 3A4; GI, gastrointestinal; PO, orally; TRD, total radioactive dose.

cytochrome P450 (CYP) 3A4. Saxagliptin is metabolized by CYP3A4 to its major active metabolite, 5-hydroxysaxagliptin, which predominantly undergoes renal excretion (Figure 1). Neither saxagliptin nor 5-hydroxysaxagliptin is a prominent substrate for the known clinically relevant drug transporters.[10] Patients with CKD exhibit increased exposure to both saxagliptin and 5-hydroxysaxagliptin, leading to a recommended dose decrease from 2.5 or 5 mg once daily in patients with normal kidney function to 2.5 mg daily in patients with creatinine clearance <50 mL/min.[11] The extent to which potential CKD-related changes to CL_R and CL_{NR} (ie, CYP3A4 metabolism) of saxagliptin contribute to this phenotype is unknown.

Physiologically based pharmacokinetic (PBPK) modeling is a useful tool to mechanistically describe drug PK. The effect of CKD on individual clearance pathways can be evaluated using PBPK models by altering the relevant physiological (system) parameters of the model that describe CL_R (ie, glomerular filtration rate [GFR]) and CL_{NR} (ie, relative abundance of nonrenal transporters and metabolic enzymes).[12–15] In the current study, we employed PBPK modeling to evaluate the impact of CKD on the metabolism of saxagliptin to 5-hydroxysaxagliptin by CYP3A4.

Methods

Physiologically Based Pharmacokinetic Modeling and Simulations

PBPK modeling and simulations were performed using Simcyp V19R1 (Certara, Sheffield, UK). All simulations were performed in 100 subjects (10 trials with 10 subjects per trial). Simulations were conducted using the same study conditions as those in the clinical trial (age range, sex proportion, and dosing regimen). Unless otherwise noted, simulations were performed using the Sim–Healthy Subject population. The workflow of model development and validation is described in Figure 2. Summaries of clinical studies used to develop and validate the model and virtual trial parameters are presented in Tables S1 and S2.

Model Development and Validation in Healthy Subjects

A PBPK model was developed for saxagliptin, including CYP3A4-mediated metabolism to 5-hydroxysaxagliptin. A training data set of low- (10 mg), medium- (30 mg), and high-dose (75 mg) oral, single-dose PK data was used for parameter estimation.[16] The final model parameters are described in Table 1.

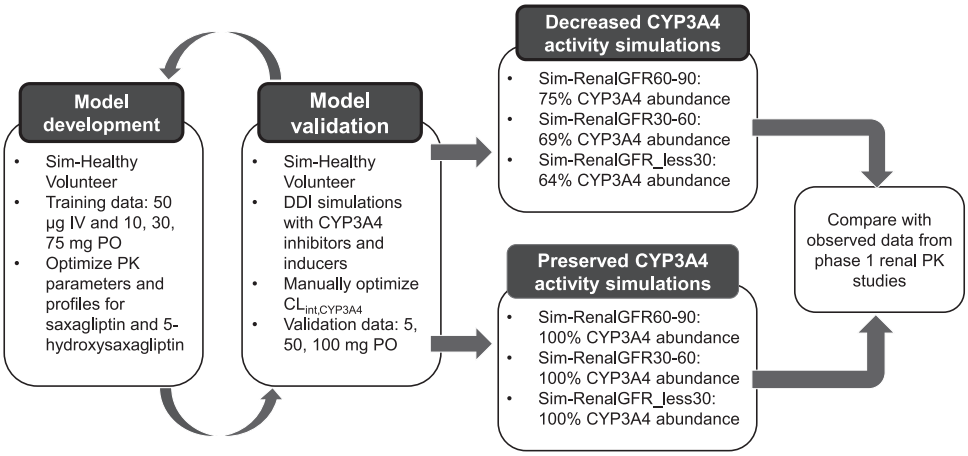

Figure 2. Overview of modeling and simulation workflow. The model for saxagliptin and 5-hydroxysaxagliptin was built using in vitro and clinical PK data. A training data set of concentration-time profiles following low (10 mg), medium (30 mg), and high (75 mg) oral, single doses of saxagliptin was used for parameter estimation and optimization. DDI simulations with CYP3A4 inhibitors (ketoconazole and diltiazem) and inducers (rifampicin) were used to optimize and validate $CL_{int,CYP3A4}$. The model was validated in healthy subjects following low (5 mg), medium (50 mg), and high (100 mg) oral, single doses of saxagliptin. The validated model was applied to simulations using virtual CKD populations with decreased CYP3A4 activity (64%-75% CYP3A4 abundance) and preserved CYP3A4 activity (100% CYP3A4 abundance). CKD, chronic kidney disease; $CL_{int,CYP3A4}$, CYP3A4-mediated metabolic clearance of saxagliptin; CYP3A4, cytochrome P450 3A4; DDI, drug-drug interaction; GFR, glomerular filtration rate; IV, intravenous; PK, pharmacokinetic; PO, orally.

An advanced dissolution, absorption, and metabolism model was used to mechanistically simulate saxagliptin absorption. A full PBPK model was developed to conduct a fully mechanistic assessment. The partition coefficient scalar value for saxagliptin was manually optimized to recover the observed volume of distribution at steady state (V_{ss}).[21] The V_{ss} of 5-hydroxysaxagliptin is unknown and was therefore determined by parameter estimation. The reported CL_R values of saxagliptin and 5-hydroxysaxagliptin from clinical studies were used in the model.[11,16,22,23]

The CYP3A4-mediated metabolic clearance of saxagliptin ($CL_{int,CYP3A4}$) was initially obtained from in vitro data[22] and optimized by parameter estimation. DDI studies with CYP3A4 inhibitors (ketoconazole and diltiazem)[24] and inducers (rifampicin)[25] were used to manually adjust $CL_{int,CYP3A4}$ to recover ratios of the maximum plasma concentration (C_{max}) and the area under the plasma concentration–time curve (AUC; C_{max} ratio [$C_{max}R$] and AUC ratio [AUCR]) observed in clinical DDI studies. Prevalidated PBPK models within the Simcyp repository were used for perpetrator drugs (ketoconazole, diltiazem and its desmethyl metabolite, and rifampicin) in DDI simulations. The final model was validated for $CL_{int,CYP3A4}$ with CYP3A4-modulating DDI studies,[24,25] and for PK parameters and concentration-time profiles with low- (5 mg), medium- (50 mg), and high-dose (100 mg) oral, single-dose PK data.[16]

Assessment of the Goodness of Fit of the Model

The goodness of fit during model validation was assessed by visual inspection of the concentration vs time plots and comparison of the predicted and observed PK parameters. For validation of the PK parameters (C_{max} and AUC), the predicted and observed geometric means were compared by determining the ratio (R) of the predicted value to the observed value (R = predicted geometric mean/observed geometric mean). Good fit was defined as a value being within ± 20% of the observed value (ie, R = 0.80−1.25).

For validation of the PK parameter ratios for DDI studies ($C_{max}R$ and AUCR), the predicted and observed ratios were compared using Guest limits.[26] Instead of the conventional fixed 2-fold prediction limits as acceptance criteria, this method employs stringent variable prediction limits, which reduces bias toward successful prediction at lower interaction levels (AUCR or $C_{max}R$ <2) by narrowing the boundary of acceptable prediction as the PK ratios approach 1. Additionally, this method incorporates the impact of clinical PK variability. The predictability was considered acceptable if the mean predicted AUCR or $C_{max}R$ fell between the upper and lower limits described in Equations 1 to 3, in which the observed ratio ($Ratio_{obs}$) is taken into account and the intraindividual variability parameter, δ = 1.25, corresponds to the conventional PK variability of 20% used in bioequivalence assessments.

$$Guest\ limit = \frac{\sigma + 2\,(Ratio_{obs} - 1)}{R_{obs}} \quad (1)$$

$$Upper\ limit = Ratio_{obs} \times Guest\ limit \quad (2)$$

$$Lower\ limit = \frac{Ratio_{obs}}{Guest\ limit} \quad (3)$$

Table 1. Saxagliptin and 5-Hydroxysaxagliptin PBPK Model Parameters

	Saxagliptin	5-Hydroxysaxagliptin
Physicochemical properties		
Molecular weight (g/mol)	315.4[10]	331.4[a]
Compound type	Monoprotic base	Monoprotic base
LogP	0.607[b]	−1.50[c]
ECCS	Class 2—Metabolism	Class 4—Renal
pKa	7.3[b]	7.6[d]
fu	1[16]	1[16]
B/P	0.83[e]	0.83[e]
Absorption		
Absorption model	ADAM	First order
fa	0.994	…
ka (h)	2.240	…
$P_{eff,man}$ (10^{-4} cm/s)	5.130[f,g]	0.025
S_o (mg/mL)	8.910[f,g]	…
PSA (A^2)	…	11[a]
HBD	…	3[a]
Distribution		
Distribution model	Full PBPK	Full PBPK
Kp scalar	2.03[h]	0.24[f,g]
V_{ss} (L/kg)	1.558	0.242
V_{ss} prediction method	Method 2 (Rodgers)	Method 2 (Rodgers)
Elimination		
Elimination model	Enzyme kinetics	Enzyme kinetics
CL_{int}, CYP3A4 (μL/min/pmol protein)	0.07[i]	…
CL_{int}, HLM (μL/min/mg protein)	3.41[f,g]	…
CL_R (L/h)	10.37[j]	4.540[j]
Additional systemic clearance (L/h)	…	3.250[g]

ADAM, advanced dissolution, absorption, and metabolism; AUCR, ratio of the area under the plasma concentration–time curve; B/P, blood-to-plasma ratio; CL_{int}, intrinsic clearance; CL_R, renal clearance; $C_{max}R$, ratio of the maximum plasma concentration; CYP3A4, cytochrome P450 3A4; ECCS, extended clearance classification system; fa, fraction absorbed; fu, fraction unbound; HBD, number of hydrogen bond donors; HLM, human liver microsomes; ka, absorption rate constant; Kp, partition coefficient; PBPK model, physiologically based pharmacokinetic model; $P_{eff,man}$, effective permeability in man; pKa, acid dissociation constant; PSA, polar surface area; S_o, intrinsic solubility; V_{ss}, volume of distribution at steady state.

[a] 17.

[b] 18.

[c] 19.

[d] 20.

[e] Unpublished data (AstraZeneca).

[f,g] Parameter estimation using saxagliptin and 5-hydroxysaxagliptin concentration-time profiles following [f] 10 mg, 30 mg, and 75 mg or [g] 10 mg and 30 mg single oral doses of saxagliptin.[16]

[h] Manually optimized to recover V_{ss} observed in vivo.[21]

[i] Manually optimized to recover AUCR and $C_{max}R$ from drug-drug interaction studies.[24,25]

[j] Calculated as a weighted average of reported CL_R.[11,16,23]

Pharmacokinetic Data of Subjects With CKD

Subjects from a previously published renal PK study of saxagliptin[11] were classified into CKD groups based on estimated GFR (eGFR) calculated using the Chronic Kidney Disease Epidemiology Collaboration equation[27] as follows: normal kidney function (eGFR ≥90 mL/min/1.73 m^2), mild CKD (eGFR 60-89 mL/min/1.73 m^2), moderate CKD (eGFR 30-59 mL/min/1.73 m^2), and severe CKD (eGFR <30 mL/min/1.73 m^2). Since the methods used for the assessment of kidney function and classification of kidney function groups differed from the original renal PK study, several patients were reclassified into new kidney function groups, including a substantial decrease in the number of patients in the normal kidney function group. Therefore, additional subjects with normal kidney function were included from the healthy controls of a hepatic impairment study and a single-ascending-dose study.[11] The $C_{max}R$ and AUCR for each CKD group were calculated by dividing the geometric means of AUC and C_{max} by those of the normal kidney function group. Analyses were completed using Prism 8.0 (GraphPad Software, San Diego, California).

Predicted PK in Patients With CKD

Simcyp does not have a mild CKD population in its population library. Therefore, a mild CKD population (Sim-RenalGFR60-90) was built from the existing Sim-RenalGFR30-60 population by adapting from mild

CKD population as described by Heimbach et al,[12] namely, by

1. Decreasing serum creatinine cutoff values by 50 μmol/L,
2. Increasing the GFR cap to <90 mL/min and >60 mL/min,
3. Retaining non-CYP3A4 liver CYP abundances to the levels of the Sim–Healthy Subject population, and
4. Changing liver CYP3A4 abundance to 103 pmol/mg protein (75% of the Sim–Healthy Subject population).

This assumes that subjects with mild CKD have similar kidney size parameters to subjects with moderate CKD, lower serum creatinine and higher GFR than subjects with moderate CKD, and CYP3A4 abundance that reflects a linear increase from severe to moderate to mild CKD (87.3, 95.2, and 103 pmol/mg protein, respectively).

Two sets of CKD simulations were performed: "decreased CYP3A4 activity simulations," where CYP3A4 activity was assumed to be decreased in CKD per the default Simcyp CKD populations, and "preserved CYP3A4 activity simulations," where CYP3A4 activity was modified in the Simcyp CKD populations to restore CYP3A4 abundance to that of the healthy controls (Figure 2). Decreased CYP3A4 activity simulations were performed using the Sim-RenalGFR60-90, Sim-RenalGFR30-60, and Sim-RenalGFR_less30 populations for mild, moderate, and severe CKD, respectively. The liver CYP3A4 abundances (pmol/mg protein) in populations with decreased CYP3A4 activity were 103 (Sim-RenalGFR60-90), 95.2 (Sim-RenalGFR30-60), and 87.3 (Sim-RenalGFR_less30), corresponding to 75%, 69%, and 64% CYP3A4 abundance, respectively, compared with Sim–Healthy Subjects. Preserved CYP3A4 activity simulations were performed by increasing the liver CYP3A4 abundance of the Sim-RenalGFR60-90, Sim-RenalGFR30-60, and Sim-RenalGFR_less30 populations to 137 pmol/mg protein, corresponding to 100% CYP3A4 relative abundance compared with Sim–Healthy Subjects. The predicted vs observed PK ratios in the CKD populations were compared by determining the ratio of the predicted value to the observed value (R = predicted ratio/observed ratio) to determine which virtual population better fit the observed data.

Midazolam PK in Patients With CKD

Although saxagliptin metabolism is almost entirely dependent on CYP3A4 activity, it is not a validated CYP3A4 probe drug. Therefore, analogous simulations were performed for midazolam, a well-known CYP3A4 probe, to validate the results observed for saxagliptin and 5-hydroxysaxagliptin. Midazolam PK in patients with CKD was extracted from the literature.[28] Simulations with virtual CKD populations with decreased and preserved CYP3A4 activity described previously (in the Predicted PK in Patients With CKD section) were performed using the prevalidated PBPK model of midazolam available within the Simcyp repository. The ratios of predicted and observed values for midazolam unbound AUC (AUC_u) and unbound C_{max} ($C_{max,u}$) were compared between simulations using decreased and preserved CYP3A4 activity.

Results

Model Validation in Healthy Subjects

The predicted concentration-time profiles (Figure 3) and PK parameters (Table 2) of saxagliptin and 5-hydroxysaxagliptin corresponded well with the observed oral, single-dose PK data. Saxagliptin C_{max} and AUC were well predicted at all dose levels (R = 0.88-1.18). 5-Hydroxysaxagliptin C_{max} and AUC were well predicted at the 5 and 50-mg doses (R = 0.81-1.07) and slightly overpredicted at the 100-mg dose (R = 1.43-1.46) but were still within 50% of the observed values. Most of the observed data points for saxagliptin and 5-hydroxysaxagliptin were within the 5th and 95th percentiles of the predicted concentration-time curve across all doses.

The predicted and observed C_{max}R and AUCR from DDI trials and corresponding prediction limits using the Guest method are described in Figure 4 and Table 3. Simulations with ketoconazole, diltiazem, and rifampicin predicted AUCR and C_{max}R well for both saxagliptin and 5-hydroxysaxagliptin. The predicted PK ratios were within the Guest limits[26] for all DDI scenarios except for 5-hydroxysaxagliptin with rifampicin, where the predicted AUCR was slightly outside of the Guest limits (predicted AUCR = 1.45, Guest limit = 0.81-1.31).

Observed and Predicted PK in Patients With CKD

The predicted concentration-time profiles and PK parameters of saxagliptin and 5-hydroxysaxagliptin for decreased and preserved CYP3A4 activity simulations are presented in Figures S1 and S2, and Table S3. Comparisons between observed and predicted CKD AUCR and C_{max}R are described in Table 4.

Decreased CYP3A4 activity simulations overpredicted the AUCR and C_{max}R of saxagliptin in mild and moderate CKD (R = 1.19-1.46) compared with preserved CYP3A4 activity simulations (R = 1.06-1.23). Both decreased and preserved CYP3A4 activity simulations predicted saxagliptin PK ratios in severe CKD by ± 20% of the observed values, with decreased

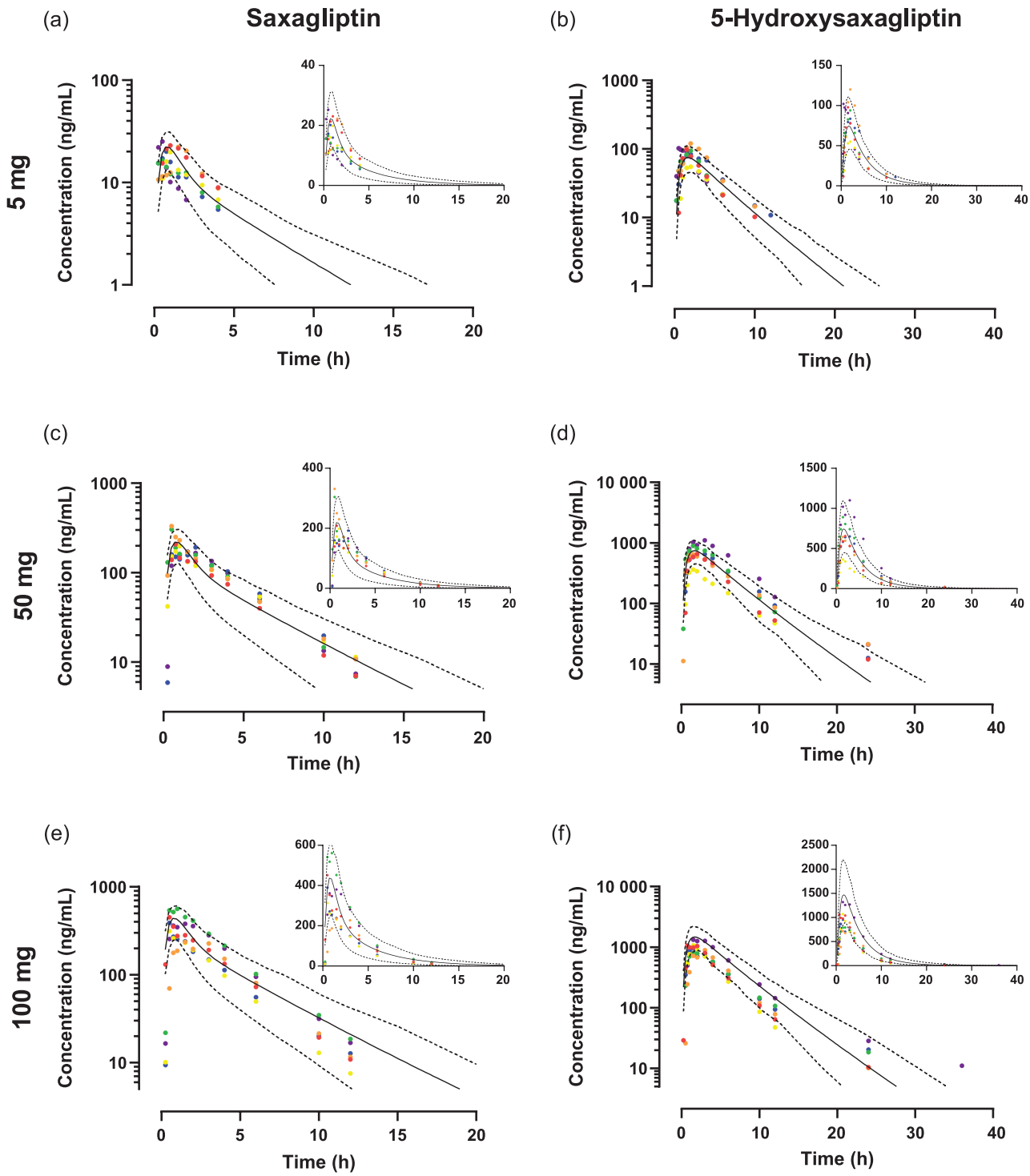

Figure 3. Recovery of observed saxagliptin and 5-hydroxysaxagliptin PK profiles in healthy subjects. Simulated plasma concentration–time profiles of saxagliptin (a, c, e) and 5-hydroxysaxagliptin (b, d, f) in healthy subjects following 5 mg (a, b), 50 mg (c, d), or 100 mg (e, f) single oral doses of saxagliptin. Solid lines are simulated mean values; dotted lines are simulated 5th and 95th percentiles; and data points are observed values. Linear scale plots are shown as insets. Clinical trial designs for simulations are described in Table S2 under SAD. PK, pharmacokinetic; SAD, single ascending dose.

Table 2. Recovery of Observed Saxagliptin and 5-Hydroxysaxagliptin PK Parameters in Healthy Volunteers

		C_{max}, ng/mL			AUC, ng • h/mL		
		Observed[a]	Simulated[b]	Ratio[c]	Observed[a]	Simulated[b]	Ratio[c]
5 mg (PO)	Saxagliptin	21 (14)	21.99 (21.01-23.02)	1.05	63 (23)	74.11 (69.60-78.90)	1.18
	5-Hydroxysaxagliptin	89 (24)	72.53 (69.06-76.18)	0.81	417 (25)	419.07 (400.60-438.40)	1.00
50 mg (PO)	Saxagliptin	227 (28)	217.27 (207.40-227.61)	0.96	829 (10)	730.57 (685.42-778.70)	0.88
	5-Hydroxysaxagliptin	679 (35)	724.19 (689.61-760.51)	1.07	4257 (39)	4161.83 (3982.42-4349.42)	0.98
100 mg (PO)	Saxagliptin	375 (29)	433.54 (413.82-454.19)	1.16	1364 (30)	1465.13 (1374.22-1562.02)	1.07
	5-Hydroxysaxagliptin	1001 (18)	1428.92 (1357.80-1503.77)	1.43	5619 (26)	8217.11 (7841.92-8610.24)	1.46

AUC, area under the plasma concentration–time curve; C_{max}, maximum plasma concentration; CV, coefficient of variation; PK, pharmacokinetic; PO, orally.
[a] Reported[16] as geometric mean (% CV).
[b] Reported as geometric mean (95%CI).
[c] Reported as predicted geometric mean/observed geometric mean.

Table 3. Recovery of Observed Saxagliptin and 5-Hydroxysaxagliptin PK Parameters in Drug-Drug Interaction Studies

		C_{max}R			AUCR		
		Observed[a]	Guest Limits[b]	Simulated[a]	Observed[a]	Guest Limits[b]	Simulated[a]
Ketoconazole	Saxagliptin	1.62 (1.47-1.80)	1.05-2.49	1.65 (1.60-1.70)	2.45 (2.30-2.60)	1.45-4.15	2.45 (2.35-2.55)
	5-Hydroxysaxagliptin	0.05 (0.05-0.06)	0.03-0.10	0.06 (0.05-0.06)	0.12 (0.10-0.13)	0.06-0.23	0.11 (0.10-0.12)
Diltiazem	Saxagliptin	1.63 (1.40-1.90)	1.06-2.51	1.42 (1.39-1.45)	2.09 (1.97-2.23)	1.27-3.43	1.83 (1.77-1.89)
	5-Hydroxysaxagliptin	0.57 (0.50-0.64)	0.36-0.90	0.38 (0.35-0.40)	0.66 (0.61-0.71)	0.44-0.99	0.46 (0.44-0.49)
Rifampicin	Saxagliptin	0.47 (0.38-0.57)	0.29-0.77	0.43 (0.40-0.45)	0.24 (0.21-0.27)	0.13-0.44	0.32 (0.30-0.34)
	5-Hydroxysaxagliptin	1.39 (1.23-1.56)	0.95-2.03	1.78 (1.72-1.84)	1.03 (0.97-1.09)	0.81-1.31	1.45 (1.41-1.48)

AUCR, ratio of the area under the plasma concentration–time curve; C_{max}R, ratio of the maximum plasma concentration; PK, pharmacokinetic.
[a] Reported[20,21] as the ratio of geometric means (95%CI).
[b] Reported as the lower limit of simulated geometric mean ratio with 20% variability, upper limit of simulated geometric mean ratio with 20% variability.

CYP3A4 activity simulations predicting the AUCR better (R = 1.08 vs R = 0.81) and preserved CYP3A4 activity simulations predicting the C_{max}R better (R = 1.20 vs R = 1.03).

Although both sets of simulations overpredicted 5-hydroxysaxagliptin AUCR and C_{max}R in mild CKD, decreased CYP3A4 activity simulations predicted 5-hydroxysaxagliptin AUCR and C_{max}R better (AUCR = 1.25, C_{max}R = 1.28) than that predicted by preserved CYP3A4 activity simulations (AUCR = 1.39, C_{max}R = 1.51). In moderate CKD, preserved CYP3A4 activity simulations predicted 5-hydroxysaxagliptin AUCR better (R = 0.94) than that predicted by decreased CYP3A4 activity simulations (R = 0.83). Both decreased and preserved CYP3A4 simulations underpredicted AUCR in severe CKD (R = 0.47-0.54), and only the AUCR from preserved CYP3A4 activity simulations was within 2-fold of the observed value.

Midazolam PK in Patients With CKD

Comparisons of observed and predicted AUC_u and $C_{max,u}$ of midazolam in patients with CKD are described in Table S4. Normal kidney function simulations were within ± 20% of the observed values. Similar to that in saxagliptin, mild CKD simulations with both decreased and preserved CYP3A4 activity overpredicted AUC_u and $C_{max,u}$, although the preserved CYP3A4 activity simulations predicted closer to the observed values (decreased CYP3A4 activity R = 1.63-2.44 vs preserved CYP3A4 activity R = 1.43-1.91). In moderate CKD, midazolam AUC_u was predicted to ± 20% by both simulations with decreased (R = 1.24) and preserved (R = 0.91) CYP3A4 activity, but $C_{max,u}$ was predicted more closely to the observed value with preserved CYP3A4 simulations (R = 1.36). In severe CKD, midazolam AUC_u and $C_{max,u}$ were overpredicted in simulations using decreased CYP3A4 activity (R = 1.50 and 1.46, respectively) and better predicted in simulations using preserved CYP3A4 activity (R = 1.01 and 1.20, respectively).

Discussion

It is well established that CKD affects the CL_{NR} of drugs in a pathway-specific manner.[5–7] CYP3A4 is one of the most important metabolizing enzymes involved in the metabolism of many currently available drugs.[29] However, there is no general consensus regarding the

Table 4. Recovery of Observed Saxagliptin and 5-Hydroxysaxagliptin PK Ratios in Patients With Kidney Impairment

		Saxagliptin					5-Hydroxysaxagliptin				
		Observed[a]	Predicted				Observed[a]	Predicted			
			Decreased CYP3A4 Activity Simulations[a]	R[b]	Preserved CYP3A4 Activity Simulations[a]	R[b]		Decreased CYP3A4 Activity Simulations[a]	R[b]	Preserved CYP3A4 Activity Simulations[a]	R[b]
Mild kidney impairment	AUCR	1.30 (1.09-1.55)	1.90 (1.78-2.02)	1.46	1.60 (1.50-1.71)	1.23	1.02 (0.89-1.18)	1.27 (1.21-1.33)	1.25	1.42 (1.36-1.49)	1.39
	$C_{max}R$	1.14 (0.91-1.42)	1.52 (1.45-1.59)	1.33	1.38 (1.31-1.45)	1.21	0.82 (0.64-1.06)	1.05 (0.99-1.11)	1.28	1.24 (1.18-1.31)	1.51
Moderate kidney impairment	AUCR	1.63 (1.34-2.00)	2.17 (2.03-2.33)	1.33	1.74 (1.62-1.87)	1.07	1.89 (1.58-2.25)	1.56 (1.48-1.64)	0.83	1.78 (1.70-1.98)	0.94
	$C_{max}R$	1.19 (0.99-1.44)	1.42 (1.35-1.49)	1.19	1.26 (1.20-1.33)	1.06	0.97 (0.78-1.22)	0.92 (0.87-0.97)	0.95	1.14 (1.08-1.20)	1.18
Severe kidney impairment	AUCR	2.29 (1.80-2.92)	2.48 (2.30-2.68)	1.08	1.86 (1.72-2.01)	0.81	4.17 (3.54-4.91)	1.94 (1.84-2.05)	0.47	2.27 (2.16-2.39)	0.54
	$C_{max}R$	1.20 (0.91-1.57)	1.44 (1.37-1.52)	1.20	1.24 (1.17-1.32)	1.03	1.12 (0.91-1.36)	0.98 (0.92-1.04)	0.88	1.27 (1.21-1.34)	1.13

AUCR, ratio of the area under the plasma concentration–time curve; $C_{max}R$, ratio of the maximum plasma concentration; CYP3A4, cytochrome P450 3A4; PK, pharmacokinetic.
[a]Reported[11] as geometric mean (95%CI) as described in the Methods section.
[b]Reported as predicted geometric mean/observed geometric mean.

Figure 4. Validation of saxagliptin $CL_{int, CYP3A4}$ by DDI studies. Simulated and observed AUCR (a) and $C_{max}R$ (b) of saxagliptin (orange) and 5-hydroxysaxagliptin (blue) and corresponding Guest limits following DDI simulations with ketoconazole (circles), diltiazem (triangles), and rifampicin (squares). Solid lines are Guest limits; dotted lines are traditional 2-fold limits; dashed line is perfect ratio prediction (ie, observed ratio = predicted ratio); and data points are plots of observed and simulated ratios from DDI studies and simulations. AUCR, ratio of the area under the plasma concentration–time curve; CL_{int}, intrinsic clearance; $C_{max}R$, ratio of the maximum plasma concentration; CYP3A4, cytochrome P450 3A4; DDI, drug-drug interaction.

impact of CKD on CYP3A4 activity. In the current study, we used saxagliptin and its 5-hydroxy metabolite as a case study of a CYP3A4 substrate-metabolite pair and performed PBPK modeling and simulation to evaluate potential changes to CYP3A4 metabolism of saxagliptin in CKD, based on observed clinical renal PK data. PBPK modeling and simulation presents a unique methodology to assess pathway-specific changes to the PK of specific drugs in special populations, such as CKD, due to its ability to manipulate systems (physiologic) parameters within a population to best recover the observed PK. To the best of our knowledge, this is the first report to assess the impact

of CKD quantitatively and mechanistically on the CYP3A4-mediated metabolism of saxagliptin to 5-hydroxysaxagliptin.

A PBPK model for saxagliptin and 5-hydroxysaxagliptin was developed and validated. The model accurately recovered the single-dose PK profiles and parameters for saxagliptin by ± 20% of the observed values across a wide range of doses (5-100 mg). The model also recovered the observed ratios of saxagliptin C_{max} and AUC within the Guest prediction limits after the administration of CYP3A4 inhibitors and inducers. These findings indicate that the model accurately recapitulates saxagliptin disposition, including the extent of CYP3A4-mediated metabolism that is observed in vivo.

The major CYP3A4-mediated metabolite of saxagliptin, 5-hydroxysaxagliptin, was included in the model as an additional assessment of CYP3A4 activity. The model could accurately recover the observed single-dose PK profiles and parameters for 5-hydroxysaxagliptin by ± 20% at low and medium saxagliptin doses (5-50 mg) and by ± 50% at high saxagliptin doses (100 mg). The model could also recover the observed ratios of 5-hydroxysaxagliptin C_{max} and AUC within the Guest prediction limits of the observed ratios after administration of several CYP3A4 modulators. This finding provides additional support that the model of saxagliptin closely simulates the extent of its metabolism by CYP3A4.

The ratios of saxagliptin C_{max} and AUC in patients with CKD and normal kidney function were compared using decreased and preserved CYP3A4 activity simulations to quantitatively assess the CYP3A4 phenotypes in patients with CKD receiving saxagliptin. Decreased CYP3A4 activity simulations generally overpredicted the ratios of saxagliptin C_{max} and AUC across all levels of kidney function compared with preserved CYP3A4 activity simulations. Therefore, when decreased CYP3A4 activity is assumed in patients with CKD, saxagliptin clearance is underpredicted and exposure is overpredicted compared with the healthy controls. Furthermore, when CYP3A4 activity is restored to that of the healthy controls, the clearance and exposure of saxagliptin in CKD compared with healthy controls is predicted with greater accuracy, as indicated by the predicted AUCR and C_{max}R being closer to the corresponding values observed in vivo. Only the AUCR was better predicted by decreased CYP3A4 activity simulations in subjects with severe CKD, but the prediction of preserved CYP3A4 activity simulations was still within ± 20% of the observed value. These data suggest that metabolism of saxagliptin by CYP3A4 is not decreased to a clinically significant degree in patients with CKD or even in those with severe CKD.

Decreased CYP3A4 activity simulations underpredicted the ratios of 5-hydroxysaxagliptin AUC in moderate and severe CKD compared with preserved CYP3A4 activity simulations. Therefore, when decreased CYP3A4 activity is assumed in moderate and severe CKD, exposure to 5-hydroxysaxagliptin is underpredicted. When CYP3A4 activity is restored to that of the healthy controls, exposure to 5-hydroxysaxagliptin is predicted with greater accuracy, and the predicted ratios of C_{max} and AUC are closer to those observed in vivo. These data further support the conclusion that the metabolism of saxagliptin by CYP3A4 is not appreciably decreased even in patients with severe CKD.

There are conflicting reports on the impact of CKD on CYP3A4 activity. Preclinical reports have demonstrated the downregulation of CYP3A mRNA and protein expression in rodent models of CKD, suggesting decreased activity.[30–35] Similarly, a PBPK model of the CYP3A4 substrate sildenafil and its metabolite UK103320 recapitulated the observed AUCR of sildenafil in severe CKD by incorporating a 55% decrease in CYP3A4 activity[36] (notably, limitations of the model include potential unaccounted for transport pathway[s] for sildenafil[37] and incomplete characterization of UK103320 clearance pathway[s]). Conversely, a meta-analysis of pooled clinical renal PK data of multiple CYP3A4 substrate drugs demonstrated no clear or consistent relationship between kidney function and the clearance of CYP3A4 substrates, suggesting that CYP3A4 activity is not consistently decreased in CKD.[9] Likewise, a clinical study of orally administered midazolam (a nontransported CYP3A4 probe) showed similar PK in patients with end-stage kidney disease and in healthy controls,[38] although these results have not been fully recapitulated in studies involving intravenous midazolam.[39]

Recently, a clinical study reported a comprehensive comparative analysis of midazolam PK in multiple CKD populations.[28] Similar to our findings with saxagliptin, CYP3A4-mediated clearance of midazolam was not altered to a clinically relevant degree in any stage of CKD. We conducted PBPK modeling of midazolam with decreased and preserved CYP3A4 activity simulations using the validated midazolam PBPK model within the Simcyp repository and observed that preserved CYP3A4 simulations of midazolam predicted PK in all CKD populations better than that with simulations with decreased CYP3A4 (Table S4). These findings corroborate the observations from our simulations of saxagliptin and 5-hydroxysaxagliptin PK in which preserved CYP3A4 activity better predicts PK in CKD patients.

Saxagliptin clearance is dependent on CYP3A4 metabolism and kidney function, with about one-half

and one-quarter of the administered drug undergoing elimination by these pathways, respectively. Saxagliptin is not bound to plasma proteins[16] and is therefore unaffected by potential changes in drug-protein binding observed in CKD.[40] These factors make saxagliptin an attractive case study for assessing the impact of potential CKD-related changes to CYP3A4 on the clearance of an individual drug. The saxagliptin PBPK model presented here offers several advantages as a case study for evaluating CYP3A4 activity in CKD, including (1) incorporation of data from a CYP3A4-generated metabolite with primarily renal clearance; (2) an absence of known transport activity for saxagliptin or 5-hydroxysaxagliptin, which minimizes the impact of overlapping substrate specificity on the conclusions of the study; and (3) the quantitative/mechanistic nature of the PBPK analysis.

The current study has a few limitations. First, it was assumed that the CYP3A metabolism of saxagliptin was mediated by CYP3A4 with negligible CYP3A5 involvement. This assumption is reasonable given that (1) the catalytic efficiency of CYP3A4 is 4-fold higher than that of CYP3A5, and that (2) the *CYP3A5*3* variant likely has no effect on the exposure of saxagliptin or 5-hydroxysaxagliptin.[21] Second, due to a lack of in vitro 5-hydroxysaxagliptin data, it was assumed that the unbound fraction and blood-to-plasma ratio were the same as those for saxagliptin. Third, although 5-hydroxysaxagliptin predominantly (>50%) undergoes CL_R, it does exhibit CL_{NR}, for which the contributing pathways are not fully characterized. Therefore, the major conclusions of our study are made only on the basis of saxagliptin data, with metabolite data acting as supporting evidence. Although neither saxagliptin nor 5-hydroxysaxagliptin is a known substrate of major clinically relevant drug transporters,[10] the role of unknown transporters in their disposition cannot be definitively ruled out. Since Simcyp does not contain a mild CKD population in its virtual patient population library, a population was constructed with assumptions regarding renal physiology parameters, hepatic non-CYP3A4 abundances, and a linear increase in CYP3A4 activity from severe to mild CKD. These assumptions likely represent an oversimplification of the mild CKD population and may not reflect the true physiology observed in these patients. Finally, saxagliptin is not a highly sensitive substrate for CYP3A4 and is not a formally validated CYP3A4 phenotypic probe. Therefore, the major conclusions of the work represent a case study of a CYP3A4 substrate-metabolite pair, and not necessarily a generalization for all CYP3A4 substrates.

In conclusion, our findings have important implications in the fields of clinical and quantitative pharmacology and drug development. First, we have presented a mechanistic assessment of the impact of CKD on the CYP3A4 metabolism of saxagliptin and showed that such an effect is likely to be minimal. Second, our findings that simulations with preserved CYP3A4 activity better predict saxagliptin PK in patients with CKD further support previous findings that patients with CKD exhibit preserved CYP3A4 activity that is comparable with healthy controls over a wide range of kidney function. Third, we presented a parent-metabolite case study to demonstrate the unique ability of PBPK modeling to incorporate altered physiological (system) parameters into simulations, making it an ideal tool for characterizing altered PK of drugs in patients with CKD more efficiently and mechanistically than traditional clinical renal PK studies.[12] Furthermore, due to its mechanistic nature, PBPK modeling can be used to probe disease-related changes to drug clearance in a pathway-specific manner, even for drugs with multiple routes of elimination, such as saxagliptin. Understanding the degree to which specific CL_{NR} pathways, including CYP3A4, for individual drugs are affected by CKD is essential to inform dose adjustments in patients with CKD. Such an analysis supports prospective application of PBPK modeling of drugs primarily metabolized by CYP3A4, and/or renally excreted drugs can be applied to predict PK and recommend dose adjustment in subjects with CKD in lieu of clinical trials addressing the PK changes.

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Conflicts of Interest

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Author Contributions

M.A.B., W.T., D.W.B., T.D.N., and P.S. wrote the manuscript, designed the research, and analyzed the data; M.A.B. and P.S. performed the research; M.A.B., D.W.B., and P.S. contributed new reagents/analytical tools.

Data Availability Statement

Data underlying the findings described in this article may be obtained in accordance with AstraZeneca's data-sharing

policy described at https://astrazenecagrouptrials.pharmacm.com/ST/Submission/Disclosure.

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Supplemental Information

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