**Application of a PBPK Model of Rivaroxaban to Prospective Simulations of Drug-Drug-Disease Interactions with Protein Kinase Inhibitors in CA-VTE**

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The data that support the findings of this study are available from the corresponding author upon reasonable request. Some data may not be made available because of privacy or ethical restrictions.

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**Keywords:**

Rivaroxaban, Erlotinib, Nilotinib, Drug-drug-disease interactions, Physiologically-based pharmacokinetic

# Abbreviations

|  |  |
| --- | --- |
| AUC | Area under the plasma concentration-time curve |
| AF | Atrial fibrillation |
| BCA | Bicinchoninic acid |
| CA-VTE | Cancer-associated venous thromboembolism |
| CI | Confidence interval |
| CKD | Chronic kidney disease |
| CL | Total clearance |
| CL/F | Apparent oral clearance |
| CLR | Renal clearance |
| CLuint,met | *In vitro* unbound metabolic intrinsic clearance |
| CLuint,T | *In vitro* unbound transporter-mediated intrinsic clearance |
| Cmax | Peak plasma concentration |
| DDDIs | Drug-drug-disease interactions |
| DDIs | Drug-drug interactions |
| E3S | Estrone 3-Sulfate |
| GFR | Glomerular filtration rate |
| HBSS | Hank’s balanced salt solution |
| HEK | Human embryonic kidney |
| HEPES | 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid |
| HSA | Human serum albumin |
| IC50 | Half-maximal inhibitory concentration |
| INH | Intact nephron hypothesis |
| Jmax | *In vitro* maximal rate of transporter-mediated uptake |
| *Ki* | Inhibition constant for the enzyme-inhibitor/transporter-inhibitor complex |
| *KI* | Inactivator concentration yielding an inactivation rate at half of *kinact* |
| *kinact* | The theoretical maximum inactivation rate constant at infinite inactivator concentration |
| Km,u | Unbound Michaelis-Menten constant |
| OCT | Organic cation transporter |
| OAT3 | Organic anion transporter 3 |
| PBPK | Physiologically-based pharmacokinetic |
| P-gp | P-glycoprotein |
| PKIs | Protein kinase inhibitors |
| RAF | Relative activity factor |
| REF | Relative expression factor |

**Summary:**

**What is already known**

* Rivaroxaban is emerging as an attractive alternative for the prophylaxis and treatment of CA-VTE.
* Rivaroxaban is susceptible to drug-drug-disease interactions (DDDIs) attributed to impairment of its multiple clearance pathways.

**What this study adds**

* Erlotinib and nilotinib irreversibly inhibit CYP3A4/CYP2J2-mediated metabolism and reversibly inhibit OAT3-mediated uptake of rivaroxaban.
* Physiologically-based pharmacokinetic model is established to prospectively investigate DDDIs implicating rivaroxaban in CA-VTE.

**Clinical significance**

* Our model facilitates rivaroxaban dose adjustment to ensure its efficacious and safe use in CA-VTE.

**Abstract**

**Background and Purpose**

Rivaroxaban is emerging as a viable anticoagulant for the pharmacological management of cancer associated venous thromboembolism (CA-VTE). Being eliminated via CYP3A4/2J2-mediated metabolism and organic anion transporter 3 (OAT3)/P-glycoprotein-mediated renal secretion, rivaroxaban is susceptible to drug-drug interactions (DDIs) with protein kinase inhibitors (PKIs), erlotinib and nilotinib. Physiologically based pharmacokinetic (PBPK) modelling was applied to interrogate the DDIs for dose adjustment of rivaroxaban in CA-VTE.

**Experimental Approach**

The inhibitory potencies of erlotinib and nilotinib on CYP3A4/2J2-mediated metabolism of rivaroxaban were characterized. Using prototypical OAT3 inhibitor ketoconazole, *in vitro* OAT3 inhibition assays were optimized to ascertain the *in vivo* relevance of derived inhibitory constants (*Ki*). DDIs between rivaroxaban and erlotinib or nilotinib were investigated using iteratively verified PBPK model.

**Key Results**

Mechanism-based inactivation (MBI) of CYP3A4-mediated rivaroxaban metabolism by both PKIs and MBI of CYP2J2 by erlotinib were established. The importance of substrate specificity and nonspecific binding to derive OAT3-inhibitory *Ki* values of ketoconazole and nilotinib for the accurate prediction of DDIs was illustrated. When simulated rivaroxaban exposure variations with concomitant erlotinib and nilotinib therapy were evaluated using published dose-exposure equivalence metrics and bleeding risk analyses, dose reductions from 20 mg to 15 mg and 10 mg in normal and mild renal dysfunction, respectively, were warranted.

**Conclusion and Implications**

We established the PBPK-DDI platform to prospectively interrogate and manage clinically relevant interactions between rivaroxaban and PKIs in patients with underlying renal impairment. Rational dose adjustments were proposed, attesting to the capacity of PBPK modelling in facilitating precision medicine.

**Introduction**

Cancer-associated venous thromboembolism (CA-VTE) is a prevalent occurrence in patients with malignancies. Cancer patients have an intrinsically elevated risk of recurrent VTE and anticoagulant-induced bleeding complications.1 Unsurprisingly, VTE has been reported to be the second most prevalent cause of death in cancer, second only to disease progression.2,3 While parenteral anticoagulants are recommended as the therapeutic mainstay for the prevention and treatment of CA-VTE, direct oral anticoagulants (DOACs) have recently emerged as an attractive alternative options, with the landmark CASSINI4 and SELECT-D5 trials providing the first positive evidence of the efficacy of rivaroxaban, a Factor Xa inhibitor, in the primary prophylaxis and treatment of CA-VTE respectively.

Approximately two-thirds of the systemically absorbed dose of rivaroxaban undergoes metabolic degradation in the liver, with contributions from cytochrome P450 (P450) enzymes (CYP3A4/3A5 and CYP2J2) as well as P450-independent mechanisms.6 The remaining third of the absorbed dose is eliminated as unchanged drug in the urine largely via P-glycoprotein (P-gp) and breast cancer resistance protein-mediated secretion.6,7 We have recently uncovered the additional significance of basolateral organic anion transporter 3 (OAT3)-mediated proximal tubular uptake in mediating the renal elimination of rivaroxaban.8 This inevitably increases rivaroxaban’s susceptibility to drug-drug-disease interactions (DDDIs) attributed to simultaneous impairment of its multiple clearance pathways.9

Inherent in the setting of rivaroxaban therapy is the presence of narrow therapeutic indices with steep dose exposure relationships towards major bleeding.10 Outcomes from the SELECT-D trial revealed a higher risk of clinically relevant nonmajor bleeding with rivaroxaban versus dalteparin treatment.5 This was despite a strict exclusion criterion where the concomitant use of strong CYP3A4/P-glycoprotein (P-gp) inducers or inhibitors was disallowed. Updated recommendations by major clinical guidelines regarding the use of DOACs were thus qualified with the statement that the choice of rivaroxaban was only acceptable if there were no concomitant risk factors for bleeding and/or drug interactions.11 However, with polypharmacy prevalent in anticancer management, patients are likely to receive therapies which may impair the reported elimination pathways of rivaroxaban.11 A case in point would be protein kinase inhibitors (PKIs) such as erlotinib and nilotinib, which are orally administered, chronically dosed and are known to exhibit class effects as dual enzyme/transporter inhibitors.12–14 Both erlotinib and nilotinib have been reported to inhibit CYP3A415,16 and P-gp, with nilotinib additionally inhibiting OAT3.17 Renal impairment, a common comorbidity in the elderly cancer population, has also been verified as a covariate for increased rivaroxaban exposure and bleeding risks.18 While rivaroxaban was initially poised to become a cornerstone of anticoagulant therapy in CA-VTE, its clinical utility remains restricted by limited knowledge of the nature and extent of DDDIs between rivaroxaban and anticancer therapies. The lack of specific dosing recommendations in the presence of anticipated exposure variations creates a compelling case for precision dosing guided by physiologically-based pharmacokinetic (PBPK) modelling.

Parameterized with a combination of drug and system-specific input parameters, PBPK models represent a quantitative mechanistic framework by which *a priori* simulations of drug exposure can be performed and have proven to be a panacea to current challenges faced in extrapolation beyond the tested trial population. Nevertheless, application of PBPK modelling for prospective DDDI simulations warrants that the PBPK-DDDI platform be qualified as “fit for purpose”.19 Our previous PBPK model of rivaroxaban has mechanistically delineated its hepatic and renal elimination pathways for application as a victim substrate.8 Additionally, successful recapitulation of the magnitude of enzyme-mediated drug-drug interactions (DDIs) with prototypical CYP3A4/2J2 inhibitors (verapamil and ketoconazole) also attests to the *in vivo* relevance and robustness of the *in vitro* derived enzymatic inhibitory parameters. However, simulations overpredicted the renal elimination of rivaroxaban in the presence of concomitant ketoconazole therapy. Subsequent sensitivity analysis attributed this observation to an underprediction in the characterized inhibitor potency of ketoconazole on the OAT3-mediated transport of rivaroxaban.8 Indeed, this phenomenon is consistent with difficulties faced by previous efforts in recapitulating transporter-mediated DDI.20,21 We hypothesize that this *in vitro-in vivo* disconnect could be a result of substrate-dependent differences in inhibitory parameters22,23 and an underestimation of inhibitory potential attributed to experimental design.24,25

To confidently apply a PBPK-DDDI platform to the prospective simulation of DDDIs between erlotinib/nilotinib and rivaroxaban, we aim to first characterize the *in vitro* inhibitory parameters of erlotinib and nilotinib against rivaroxaban’s enzyme-mediated elimination pathways. Using ketoconazole as a prototypical OAT3 inhibitor, exploratory ­*in vitro* transporter inhibition experiments would be performed to accurately recapitulate the extent of OAT3-mediated DDI between rivaroxaban and ketoconazole. A similar workflow can subsequently be applied to quantify the inhibition of OAT3-mediated rivaroxaban uptake by nilotinib. Lastly, knowledge of complex and dynamic alterations in the underlying processes governing renal physiology across the various stages of renal dysfunction would be incorporated to allow prediction of the augmented effects of chronic kidney disease on rivaroxaban’s disposition in the presence of the putative PKIs.26,27 We envision that this systematic approach to PBPK-guided precision dosing of rivaroxaban would minimize the extent of inadvertent increases in the systemic exposure of rivaroxaban in the presence of DDDIs while still ensuring that anticoagulant efficacy is preserved, extending the utility of rivaroxaban across the spectrum of patients within the CA-VTE population.

**Methods**

**PBPK Modelling Workflow.** All PBPK modelling and simulations were conducted using a population-based absorption, distribution, metabolism and excretion simulator (version 18, Simcyp®, Sheffield, UK). A stepwise approach was applied to qualify the PBPK-DDDI platform for prospective DDDI simulations between rivaroxaban and erlotinib or nilotinib (**Figure 1**). Both PKIs have been reported to perpetrate mechanism-based inactivation (MBI) of CYP3A4, while their ability to elicit MBI of CYP2J2 is unknown. Hence, MBI and reversible inhibition experiments were first performed to investigate and quantify their inhibitory potencies on the CYP3A4/2J2-mediated metabolism of rivaroxaban. By applying a similar inhibition protocol as that defined for ketoconazole, the capacity of nilotinib to inhibit OAT3-mediated rivaroxaban uptake was evaluated.

**Investigating MBI and Reversible Inhibition of P450 Metabolism of Rivaroxaban by PKIs.** Chemicals and reagents used for all *in vitro* experiments are detailed in the **Supplementary Methods**. To investigate and quantify the inhibitory potency of erlotinib or nilotinib on the CYP3A4/2J2-mediated metabolism of rivaroxaban, MBI and reversible inhibition experiments were performed according to procedures previously outlined by Cheong *et al*.8 Additional details on the range of inhibitor concentrations utilized, calculation of inactivation kinetic parameters (*KI*and *kinact*) as well as reversible inhibition parameters (IC50 and *Ki*) are detailed in the **Supplementary Methods**.

**Investigating Inhibition of *In Vitro* OAT3-Mediated Uptake of E3S and Rivaroxaban by Ketoconazole and Nilotinib in Both Protein-Free and HSA-Containing Buffer.** Both wild type and hOAT3-transfected human embryonic kidney (HEK293) cell lines used in this study were obtained from Dr. Kathleen Giacomini (University of California, San Francisco, San Francisco, CA). HEK293 cell culture methods are highlighted in the **Supplementary Methods**. Transport and inhibition studies were conducted in HEK293 cells as described previously, with some modifications.28,29 Briefly, cells were washed once with transport buffer (HBSS supplemented with 20 mM HEPES (pH 7.4) or HBSS with 20 mM HEPES (pH 7.4) containing 5% w/v human serum albumin (HSA)). Stock solutions of probe substrate (E3S – 1 µM) and test substrate (rivaroxaban – 1 µM) as well as inhibitors (ketoconazole or nilotinib) were dissolved in DMSO and co-incubated in transport buffer (either HBSS or HBSS containing 5% w/v HSA). Final organic content of transport buffer was ≤1% v/v. 100 μL of transport buffer was used to initiate the uptake experiment, which was allowed to proceed for 4 min for IC50 experiments. Following removal of the uptake solution, cells were washed twice with ice-cold DPBS and lysed with 0.2 N NaOH containing the respective internal standards (pregnenolone sulfate for E3S and testosterone-2,3,4-13C3 for rivaroxaban) for 10 min at 4°C. 80 μL of lysate solution was removed and transferred into Eppendorf tubes containing 80 μL of 0.2 N HCL and 20 μL of ACN. Following thorough mixing and centrifugation at 14,000 *g* for 15 min at 4°C, the supernatant was removed for liquid chromatography tandem mass spectrometry quantification of drug amount in cellular lysate (**Supplementary Methods**). The total protein concentration in each well was measured using a BCA protein assay kit.

*Calculation of transport inhibitory kinetic parameter (IC50).* Concentration-dependent diminution of total uptake measured in OAT3-transfected cells across inhibitor concentrations was first fitted to log (inhibitor) vs. response variable slope (four parameters) analysis as presented in **Equation 1** to establish the IC50 of ketoconazole or nilotinib against OAT3-mediated E3S or rivaroxaban uptake.

|  |  |
| --- | --- |
|  | (**1**) |

where min is the minimum inhibitory effect, max is the maximum inhibitory effect, I is the inhibitor concentration, and Hill slope is the Hill coefficient. The derived IC50 value would guide the choice of inhibitor concentrations to be utilized in subsequent *Ki* determinations. In the presence of 5% w/v HSA, the apparent IC50 value obtained would be corrected for the fraction unbound in plasma of the putative inhibitor(s) to establish the effective unbound inhibitor concentrations (IC50,u) attenuating OAT3-mediated uptake.

**Determining the Mode of Inhibition of Ketoconazole and Nilotinib against *In Vitro* OAT3-mediated E3S and Rivaroxaban Uptake.** Prior to the conduct of *Ki* experiments to identify the inhibition modality which could eventually be used to correct the IC50 values, concentration-dependent uptake of both E3S (1 – 50 µM E3S) and rivaroxaban (0.5 – 20 µM) was first assessed to determine the range of substrate concentrations that would be used for subsequent inhibition experiments. The uptake protocol was similar to that of the IC50 experiments except that uptake was only performed in protein-free HBSS buffer for a duration of 1 min for E3S and 2 min for rivaroxaban (previously determined to be within the range of linear uptake).

*Calculation of transporter uptake kinetic parameters (Jmax and Km).*Cellular uptake of E3S and rivaroxaban in both HEK wild type and OAT3-transfected cells was normalized for incubation time and total protein content in each well. OAT3-mediated uptake was subsequently calculated by subtracting the passive uptake measured in wild type cells from total uptake quantified in OAT3-transfected cells. Concentration-dependent OAT3-mediated uptake data was eventually fitted via nonlinear regression to the Michaelis−Menten equation (**Equation 2**) to calculate kinetic parameters.

|  |  |
| --- | --- |
|  | (**2**) |

where represent the uptake rate of the substrate (pmol/min/mg protein), the unbound substrate concentration in the incubation buffer (µM) (fraction unbound is assumed to be 1 in the protein-free HBSS buffer), maximum uptake rate (pmol/min/mg protein) and the unbound Michaelis-Menten constant (µM) respectively.

*Calculation of transport inhibitory kinetic parameter (Ki).* Michaelis-Menten curves generated at various inhibitor concentrations were transformed to Dixon plots to discern the mode of reversible inhibition by ketoconazole on OAT3-mediated E3S and rivaroxaban uptake. The derived inhibition modality dictated the selection of appropriate equations to determine the value via non-linear regression analyses for competitive (**Equation 3a**) and non-competitive inhibition (**Equation 3b**).30,31

|  |  |
| --- | --- |
|  | (**3a**) |
|  |  |
|  | (**3b**) |

**Application of PBPK Modelling for DDI or DDDI Simulations with Ketoconazole, Erlotinib and Nilotinib.**

*PBPK models of Inhibitors.*In the construction of PBPK-DDI models for retrospective DDI simulations, the verified compound file of ketoconazole provided in Simcyp® (version 18) was used. For subsequent prospective DDI simulations, although compound files of erlotinib and nilotinib were not available in the Simcyp simulator, PBPK models of erlotinib32,33 and nilotinib34 have previously been established and verified. Final model parameters for erlotinib and nilotinib are summarized in **Table S1**, with specific modifications being highlighted in the **Supplementary Methods.**

Chronic dosing regimens of erlotinib and nilotinib achieve *in vivo* steady state concentrations in approximately 7 days35 and 8 days36 respectively. As such, prospective DDI simulations were conducted with trial subjects receiving PKIs under clinically relevant dosage regimens (erlotinib 150 mg once daily (OD) and nilotinib 400 mg twice daily (BID)) chronically before a single dose of rivaroxaban (20 mg) was administered together with the PKI on day 9 for erlotinib37 and day 15 for nilotinib.36 However, while rivaroxaban is indicated to be administered under fed conditions, both erlotinib and nilotinib are to be taken on an empty stomach at least one hour before or two hours after the ingestion of food.38,39 Hence, to ensure that our trial design conforms to the stipulated dosing guidelines, both inhibitors were administered one hour prior to the rivaroxaban dose.

*DDI simulations.* Derived *in vitro* inhibitory parameters were subsequently incorporated into PBPK-DDI models to predict the extent of interactions between rivaroxaban and ketoconazole as well as rivaroxaban and erlotinib or nilotinib (**Supplementary Methods**).

*Development of renal impairment populations for DDDI simulations.* As outlined in the **Supplementary Methods**, upon verification of the predictive capabilities of the mechanistic kidney model in healthy subjects, system-dependent parameters within the model were subsequently modified to examine the utility of the developed model of rivaroxaban in recapitulating exposure changes across the spectrum of renal impairment.18

**Assessing the Clinical Significance of the Predicted DDDIs.** Systematic dose adjustments to mitigate the predicted DDIs were guided by the dual objectives of preserving anticoagulant efficacy (i.e. minimizing thromboembolic complications in CA-VTE) while managing bleeding risks associated with augmented rivaroxaban exposure.

*Dose Exposure Equivalence.* A unique characteristic of rivaroxaban is the close pharmacokinetic/pharmacodynamic correlation. For patients with atrial fibrillation (AF) and moderate renal impairment, previous population PK simulations showed that dose reduction to 15 mg OD would result in approximately similar drug exposure to patients with AF and normal or mildly impaired renal function receiving 20 mg OD, falling within the predefined range of 0.70-1.43 for drug exposure equivalence.41 This reduced dose was adapted for the ROCKET AF trial comparing rivaroxaban to warfarin where it was established that both safety and efficacy measures did not differ between mild and moderate renal impairment subgroups.42 Hence, simulated AUC fold changes of rivaroxaban in the presence of erlotinib or nilotinib were first evaluated using this prespecified dose-exposure equivalence statistic of 0.70-1.43 to determine if dose modifications would be warranted.

*Bleeding Risk Analyses.* Based on reported outcomes from an exposure-response analysis, Ismail *et al* utilized an exponential regression model to depict the relationship between risk of major bleeding and steady-state AUC0-24h as shown in **Equation 4**.10

|  |  |
| --- | --- |
|  | (**4**) |

According to published analyses, a 10 mg dose of rivaroxaban yields a major bleeding risk of 4.5% when used concurrently with a combined P-gp and strong CYP450 inhibitor.43 As a result, ketoconazole, a combined P-gp and strong CYP inhibitor is contraindicated with all doses of rivaroxaban.43,44 Hence, it follows that the objective of any dose modifications should entail reducing the bleeding risk to under 4.5%. To determine if dose adjustments would be required for rivaroxaban in the presence of erlotinib or nilotinib, simulated AUC0-24h of rivaroxaban were incorporated into **Equation 4** for calculation of major bleeding risks.

**Results**

**Consideration of OAT3 Inhibition Enabled Successful recapitulation of Transporter-mediated DDIs between Rivaroxaban and Ketoconazole.** Despite incorporation of *in vitro* CYP3A4/2J2 and P-gp inhibitory parameters, the modelled plasma concentration-time profile in the presence of ketoconazole evidently demonstrated an underestimation of the clinical DDI magnitude between rivaroxaban and ketoconazole (blue solid line in **Figure 2A**). Moreover, the PBPK-DDI model predicted minimal reductions in CLR in comparison to the 36% reduction in CLR (2.5 L/h to 1.6 L/h) reported by Mueck *et al.,*44 suggesting that the nature and potency of transporter-mediated interactions between rivaroxaban and ketoconazole have not been adequately elucidated (**Table 1**).

The IC50 value obtained when using rivaroxaban as substrate (0.58 µM) was approximately 12-fold lower compared to the IC50 value obtained when E3S was utilized (6.90 µM), highlighting the differential sensitivity of our test substrates to OAT3 inhibition (**Figure 2B**). Concentrations of ketoconazole spanning the IC50 were chosen for subsequent *Ki* experiments. The intersection of all lines at the same point on the x-axis of the Dixon plots (**Figures 2C** and **2E**) demonstrated that irrespective of the substrate utilized, ketoconazole was a non-competitive inhibitor of OAT3-mediated basolateral uptake. *Ki* values obtained based on non-linear regression analyses (**Figures 2D and 2F** for E3S and rivaroxaban respectively) corroborated IC50 determinations based on the identified non-competitive mode of inhibition and further verified that ketoconazole was a more potent inhibitor of rivaroxaban active uptake compared to E3S (*Ki* of 0.93 µM and 14.96 µM respectively; **Table 2**).

Inhibition of OAT3-mediated uptake of rivaroxaban by ketoconazole was further investigated in the presence of 5% HSA, approximating the physiological albumin concentrations *in vivo*.45,46 Adjustment of the apparent IC50 value of ketoconazole determined in the presence of 5% HSA (**Figure 2G**) for the reported fraction unbound of ketoconazole in plasma revealed an approximate 9-fold decrease in the IC50 as compared to that measured in protein-free buffer (0.037 µM versus 0.58 µM in **Table 2**).

Collectively, considerations of substrate specificity and differential inhibitory potential of ketoconazole in protein-free versus protein containing buffer culminated in an *in vitro* *Ki,u* value47 that accurately recapitulated the extent of transporter-mediated DDI between rivaroxaban and ketoconazole (**Table 1**; blue dashed line in **Figure 2H**).

**Prospective Predictions of Enzyme- and/or Transporter-Mediated DDDIs between Rivaroxaban and Erlotinib or Nilotinib**

*In vitro experiments affirmed the presence of MBI and/or reversible inhibition of CYP3A4- and/or CYP2J2-mediated metabolism of rivaroxaban by erlotinib and nilotinib.* Using rivaroxaban as the probe substrate, MBI of CYP3A4-mediated metabolism by erlotinib and nilotinib was demonstrated (**Figures S1A-D**). Preliminary studies in the presence of positive control (dronedarone) demonstrated that erlotinib (**Figure S1E**), but not nilotinib produced time-dependent inactivation of CYP2J2 **(Figure S1F)**. Hence, the MBI potency of erlotinib on CYP2J2-mediated metabolism of rivaroxaban was further characterized (**Figure S1G** and **S1H**). Quantification of the reversible inhibitory potencies of erlotinib and nilotinib on CYP3A4-mediated rivaroxaban metabolism was also performed (**Figures S2A** and **S2C** respectively). R1 ratios calculated based on derived IC50 values of 20.24 µM (erlotinib) and 6.05 µM (nilotinib) were below the FDA recommended threshold of 1.02 (1.014 and 1.019 for erlotinib and nilotinib respectively) (**Table 2**). Hence, *Ki* determinations were not conducted.48 Conversely, reversible inhibition of CYP2J2 by erlotinib (**Figure S2B**) and nilotinib (**Figure S2D**) yielded IC50 values of 1.81 µM and 0.30 µM. Calculated R1 ratios exceeded 1.02, hence warranting further elucidation of *Ki* via enzyme kinetics experiments. Michaelis-Menten curves were derived in the presence of varying concentrations of the putative inhibitors (erlotinib and nilotinib). Lineweaver-Burk transformations (**Figures S2E** and **S2G**) verified that erlotinib and nilotinib competitively inhibit CYP2J2 and subsequent non-linear regression analyses yielded *Ki* values of 0.76 µM and 0.12 µM respectively (**Figures S2F** and **S2H**). Derived *in vitro* inhibitory parameters from MBI and reversible inhibition assays are summarized in **Table 2**.

*PBPK models of erlotinib and nilotinib recapitulated clinically observed PK profiles.* Modified PBPK models of erlotinib and nilotinib were first systematically verified against in vivo single oral PK profiles using the “Sim-Cancer” population within the Simcyp simulator (**Figures 3A -** **3C**).36,37,49 Nevertheless, given that potential DDDI scenarios would likely implicate chronic dosing of both the perpetrator drug (either erlotinib or nilotinib) as well as the victim substrate (rivaroxaban), robust quantification of DDDI magnitude also remains contingent on accurate characterization of the plasma-concentration time profiles of erlotinib and nilotinib upon multiple dosing. The potential of both PKIs to cause MBI of CYP3A4 has been established and with CYP3A4 being the main enzyme governing the metabolic elimination of both erlotinib (~70%)50 and nilotinib (~80%),34 auto-inactivation of oral clearance is likely to occur upon multiple dosing. In this study, incorporation of *in vitro* MBI parameters against CYP3A4 (**Table 2**) was pivotal in facilitating recapitulation of the observed non-linear accumulation kinetics governing the PK of erlotinib (**Table S2**, **Figures 3A** to **3B**). In the case of nilotinib, correcting the derived MBI parameters for potential non-specific binding in the *in vitro* incubation as defined by Burns et al (fuinc = 0.43)51 resulted in overestimations of the systemic exposure upon multiple dosing (black dashed lines in **Figures 3C** to **3D**). In contrast, utilizing fuinc = 1 demonstrated increased congruency with the clinical profiles reported by both Tanaka et al. (**Figure 3C**) and Larson et al. (**Table S2**; **Figure 3D**).36,52

*Inhibition of OAT3-mediated uptake of rivaroxaban by nilotinib.* The inhibitory potential of nilotinib on OAT3-mediated transport was subsequently evaluated in a similar manner as that described for ketoconazole**.** Quantifying the attenuation of OAT3-mediated rivaroxaban uptake in the presence of nilotinib produced a leftward shift in the IC50 curve (**Figure 4A**), yielding a measured IC50 value (0.062 µM) that was 0.012-fold that obtained when E3S was utilized as the probe substrate (5.12 µM) (**Table 2**). Further investigations via Dixon plots substantiated a non-competitive versus competitive mode of inhibition when E3S (**Figure 4B**) and rivaroxaban (**Figure 4D**) were used as the probe substrates respectively. Considering both the competitive nature of inhibition nilotinib displays against OAT3-mediated rivaroxaban uptake and the fraction unbound of nilotinib in plasma, the apparent IC50 derived in the presence of 5% w/v HSA (**Figure 4F**) was corrected to obtain a Ki,u of 0.0098 µM (**Table 2**), which was eventually incorporated into the PBPK-DDI model for prospective simulations of transporter-mediated DDIs between rivaroxaban and nilotinib.

*PKIs demonstrated augmented systemic exposure of rivaroxaban, necessitating consideration of rational dose adjustments.* Simulations of a single 20 mg dose of rivaroxaban performed using the default “Sim-Cancer” in place of the default healthy “NEurCaucasian” populations yielded comparable plasma concentration-time profiles, establishing that demographic and physiological changes incorporated within the oncology population did not significantly impact the PK of rivaroxaban monotherapy (**Figure S3A**). Similarly, given that renal dysfunction has been shown to minimally affect the PK of erlotinib37 and nilotinib,53 simulations of the steady state exposure of erlotinib (**Figure S3B**) and nilotinib (**Figure S3C**) using the developed mild renal impairment populations verified the recapitulation of clinical outcomes within the observed range of interindividual variabilities. In contrast, simulations using the developed moderate renal impairment population markedly overestimated the exposure of erlotinib (**Figure S3B**) and nilotinib (**Figure S3C**). Consequently, calculation of AUC fold changes in the presence of PKIs across patients with normal renal function and mild renal impairment were derived upon comparison with the geometric mean AUC0-INF following a single rivaroxaban dose of 20 mg administered in the fed state to the “Sim-Cancer” population.

A forest plot facilitating quantitative comparison of the simulated fold changes in rivaroxaban’s systemic exposure in the presence of DDDIs is presented in **Figure 5A**. Clinical metrics applied to rationalize the predicted AUC ratios are as follows. First, as denoted by the red dotted line in **Figure 5A**, the use of rivaroxaban is currently contraindicated with strong CYP3A4 and P-gp inhibitors such as ketoconazole and ritonavir, where concomitant administration has been shown to yield 2.58-fold and 2.53-fold increases in AUC over that observed in healthy controls.44 Conversely, the blue dotted line demarcates a reported AUC fold change of 1.52 in patients with moderate renal impairment (GFR = 30 - 49 mL/min), where a dose reduction of rivaroxaban from 20 mg to 15 mg once daily is currently recommended.

From our simulations, we demonstrated how co-administration of 20 mg rivaroxaban (Day 8) with erlotinib 150 mg OD (Days 1-8, based on the trial design defined by Togahsi *et al.*37) generated geometric mean rivaroxaban AUC fold changes of 1.52 and 1.84 in patients with normal renal function and mild renal impairment, respectively (**Figure 5A**). In the presence of nilotinib as the putative inhibitor (400 mg BID, Days 1-15, based on the trial design defined by Tanaka *et al.*36), predicted magnitude of elevations in rivaroxaban exposure (20 mg OD, Day 15) surpassed that observed with erlotinib across the spectrum of renal function (**Figure 5A**). This was expected considering the added dimension of OAT3 inhibition by nilotinib, culminating in simultaneous impairment of both hepatic and renal clearance pathways governing rivaroxaban’s elimination.

Corresponding major bleeding risks were calculated to be 5.07% and 6.02% in the presence of erlotinib, as well as 5.83% and 6.81% with nilotinib co-administration, in normal renal function and mild renal impairment respectively (**Figure 5B**), exceeding the defined threshold of 4.5% (red solid line) reflecting the risk of major bleeding associated with concomitant rivaroxaban and ketoconazole therapy.

Collectively, there is a clear consensus that dose adjustments are warranted. Using our verified PBPK model and considering the linear PK of rivaroxaban under fed conditions in doses up to 20 mg, we demonstrated how decreasing the dose of rivaroxaban to 15 mg in normal renal function and 10 mg in mild renal impairment would sufficiently reduce major bleeding risks to below 4.5% (**Figure 5B** and **5C**). Simulated AUC fold changes also fell within the predefined dose-exposure equivalence range of 0.70-1.43 (grey zone in **Figure 5A**), plausibly preserving the anticoagulant efficacy of rivaroxaban while minimizing hematologic toxicities.

**Discussion**

The higher probability of hematologic toxicities observed with rivaroxaban in comparison to low molecular weight heparins constitutes an important consideration that may diminish the widespread adoption of rivaroxaban in CA-VTE where patients are intrinsically predisposed to increased risk of bleeding.5,54 Incidentally, PKIs could perpetrate significant DDIs with rivaroxaban.13,55,56 Here, we performed PBPK-guided prospective predictions of potential DDDIs in CA-VTE that provides the basis for eventual dose adjustments of rivaroxaban.

We adopted multiple cycles of “predict, learn and confirm” to ensure adequate verification of our PBPK models (**Figure 1**).19,57 For the PBPK model of rivaroxaban, we characterized the relative contributions of its metabolic and excretion pathways in order to apply it as a victim substrate in DDDI simulations.19 It is important to note that retrospective DDDI simulations could be confounded by parameter non-identifiability, where multiple parameter permutations culminate in the same outcome. For instance, Grillo *et al.* estimated the fractional CYP3A4 and CYP2J2 metabolism in the liver to be 0.37 and 0.29 respectively in their PBPK analysis, and utilizing similar estimates, we previously demonstrated how the enzyme-mediated DDI between rivaroxaban and ketoconazole (CYP3A4/2J2 inhibitor) was adequately recapitulated.8,9 Conversely, Otsuka *et al.* adjusted the fractional CYP3A4/2J2 metabolism to recapitulate the extent of DDI between rivaroxaban and fluconazole,58 where neither OAT3 interaction nor demonstrated the clinically-observed reduction in CLR of rivaroxaban in the presence of fluconazole was considered.44 As our preliminary data demonstrated fluconazole is an inhibitor of OAT3-mediated uptake of rivaroxaban(**Figure S4**), future PBPK modelling of this DDI should be performed with the consideration of OAT3 interaction.

Parameter non-identifiability also arises when there is incomplete deconvolution of the underlying mechanisms contributing to the elimination processes. Here, we provided mechanistic credibility to the estimation of the OAT3-mediated CLuint,T of rivaroxaban via a bottom-up approach. In OAT3-transfected HEK cells, experimental CLuint,T was initially derived to be 3.5 µL/min/mg protein [Jmax = 31.0 pmol/min/mg (95% CI: 20.8, 72.8), Km = 8.91 µM (95% CI: 3.01, 40)] (**Figure S5A**). Considering the number of HEK cells per measured protein (5.62 million cells/mg) and the differential OAT3 abundance between the transfected system in this study (0.37±0.06 pmol/mg membrane protein – **Table S3**) versus that reported in the kidney cortex by Cheung *et al.* (median = 26.71 pmol/mg membrane protein) yielded a relative expression factor (REF) of 73.26±10.71,59 and the eventual CLuint,T was determined to be 45.96 µL/min/106 cells. The convergence of clinical (43 µL/min/106 cells)8 and our experimental findings reinforced OAT3 as the key basolateral transporter mediating the active renal secretion of rivaroxaban, exemplifying a comprehensive approach to overcome such parameter non-identifiability.

Establishing confidence in a PBPK-DDI platform could be hampered by poor *in vitro*-*in vivo* correlationsin transport inhibitory parameters.60,61 OAT3 has been reported to accept larger and more cationic/zwitterionic ligands.62 Such variabilities in the transporter-ligand interaction could yield substrate-dependent *Ki* values. Here, we highlighted how substrate substitution (rivaroxaban versus E3S) would result in underprediction of the extent of DDI between rivaroxaban and ketoconazole or nilotinib (**Table 2**). While reversible competitive inhibition was being assumed in most transport inhibition assays,63 we further demonstrated that ketoconazole is a non-competitive inhibitor of OAT3 (**Figures 2C** and **2E**), ratifying how understanding the mode of inhibition is essential for accurate conversion of experimental IC50 to *Ki* values for incorporation into the PBPK-DDI model.

Additionally, marked differences in experimentally derived inhibitory potencies in the absence and presence of proteins have been alluded to in previous studies. Conventionally, IC50/*Ki* values against transporters are measured *in vitro* in protein free buffers such as HBSS (fuinc = 1). Translation of these values to the *in vivo* setting would result in an attenuation of the intrinsic transporter-mediated clearance of the test substrate (CLint,T-inhibitor), as presented in **Equation 5**. Overestimations of IC50 and corresponding underestimations of DDI risk utilizing this approach have been reported.64

|  |  |
| --- | --- |
|  | (**5**) |

By considering plasma protein binding in our *in vitro* assay and upon application of **Equation 6**, comparing the relative ratios of CLint,T-inhibitor and CLint,T, exposure was predicted to increase by 1.5-fold, demonstrating a closer correlation with the clinically observed fold change.

|  |  |
| --- | --- |
|  | (**6**) |

Baik and Huang provided a theoretical basis for the greater *in vivo* relevance of inhibitory parameters determined in plasma for highly protein bound drugs using the concept of a transporter-induced protein binding shift (TIPBS).65 Alternatively, Kikuchi *et al.* and Taskar *et al.* posit that *in vitro* “free” inhibitor concentrations may not be equivalent to 1 due to poor aqueous solubility or high nonspecific binding within the *in vitro* system.24,63 Consequently, the presence of *in vitro* albumin is expected to mitigate the confounding effects postulated by these authors. As both ketoconazole and nilotinib are highly protein bound, our experiments were conducted in the presence of 5% HSA, simulating a physiological environment. Consistently, the experiments conducted in 5% HSA yielded a further 16-fold increase in inhibitor potency in the case of ketoconazole and a 6-fold increase in the case of nilotinib (**Table 2**).

Upon systematic verification of the PBPK-DDDI platform, prospective explorations of potential DDDIs of rivaroxaban with erlotinib or nilotinib across the spectrum of renal function was performed. For rivaroxaban in moderate renal impairment, apart from the INH, a reduction in CLuint,OAT3 by 27% (REF = 0.73) as proposed by Hsueh *et al.* to reflect the additional inhibition of OAT activity by uremic solutes enabled accurate recapitulation of the PK of rivaroxaban (**Table S4**). Conversely, in severe renal impairment, application of a REF of 0.41 resulted in a simulated fold change in CLR of 0.11, surpassing the reported clinical reduction of 0.21-fold (**Table S4**). In view of the wide range of potential REFs (0.02-1) that were derived from analyses of 18 drugs to quantify the magnitude of disproportionate deterioration of active secretion over GFR, application of the median REF of 0.41 is expectedly non-optimal in all scenarios.66 Outcomes from a sensitivity analysis (**Figure S5B**) substantiated the need for an increase in REF in severe renal impairment to meet the success criteria highlighted in **Table S4**. For the putative inhibitors, elevations in inhibitor exposures accompanying increasing severities of renal impairment as highlighted **in Figures S3B** and **3C** arose as a result of other physiological changes synonymous with renal dysfunction (e.g. reductions in hepatic CYP expression).27 The inability to extend the DDDI investigations to patients with moderate and severe renal impairment remains a key limitation of the current platform. Nevertheless, our insights pave way for further clinical DDDI studies designed to validate the clinical relevance of the PBPK-guided outcomes.

When rivaroxaban was co-administered with erlotinib, simulated geometric mean AUC and CL ratios were 1.52 and 0.66 in comparison with 1.39 and 0.72 that was reported clinically with verapamil (**Figure 5A**).67 This amplification of interaction potential was in line with our *in vitro* data where erlotinib was shown to perpetrate MBI of CYP2J2 in addition to CYP3A4 (**Figure S1G**). Conversely, both verapamil and norverapamil did not cause MBI of CYP2J2.8 Traditionally, drugs that are primarily eliminated by a single pathway are expected to be considerably more sensitive to DDIs compared to drugs undergoing multi-pathway clearance, which implies that the parallel CYP3A4 and CYP2J2-mediated pathways of rivaroxaban elimination might reduce rivaroxaban’s susceptibility to DDIs.68 Paradoxically, we demonstrated how simultaneous impairment of the multiple hepatic elimination pathways of rivaroxaban could represent an alternative mechanism perpetrating clinically significant DDIs.

With the concomitant administration of erlotinib or nilotinib, decreasing the dose of rivaroxaban to 15 mg and 10 mg in normal renal function and mild renal impairment respectively would sufficiently mitigate the risks of bleeding to below 4.5% (**Figures 5B** and **5C**). As highlighted in **Figure 5A**, AUC-fold changes continued to fall within the range of dose-exposure equivalence. Ismail *et al.* previously showed that for 90% of simulated patients to have a bleeding risk below 4.5%, the dose of rivaroxaban in the presence of verapamil should be reduced to 10 mg regardless of renal impairment.10 However, our simulations showed how dose reductions to 10 mg of rivaroxaban produced an AUC fold change of 0.70 (90% CI: 0.66 to 0.79), which falls outside the acceptable dose equivalence range (**Figure S6**). In contrast, a rivaroxaban dose of 15 mg yielded an AUC ratio of 1.03 (90% CI: 0.96 to 1.14), falling within the range of dose-exposure equivalence. Evidently, it is imperative that any attempts to mitigate bleeding risks via systematic dose adjustments must also be accompanied by holistic considerations of potential implications on anticoagulant efficacy. With the availability of both 15 mg and 10 mg formulations, dose reductions should be carefully calibrated based on individual patient’s bleeding risk profile.

In conclusion, we presented a case study of rivaroxaban in CA-VTE, where the safety of anticoagulation therapy could be undermined with administration of the conventional 20 mg daily dose due to the presence of DDDIs. We established the utility of applying a verified PBPK-DDI platform to the prospective interrogation and management of clinically relevant, albeit untested interactions between rivaroxaban and PKIs, in patients with underlying renal impairment. Rational dose adjustments were proposed, attesting to the capacity of PBPK modelling in facilitating precision medicine.

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**Authorship Contributions**

|  |  |
| --- | --- |
| *Participated in research design:* | Cheong, Ng, Chin, Wang and Chan |
| *Conducted experiments:* | Cheong, Ng, Chin and Wang |
| *Performed data analysis:* | Cheong, Ng, Chin, Wang and Chan |
| *Wrote or contributed to the writing of the manuscript:* | Cheong, Ng, Chin, Wang and Chan |

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**Figure Legends**

**Figure 1.** Physiologically-based pharmacokinetic (PBPK) modelling workflow detailing the iterative processes involved in the development, verification and prospective application of PBPK-drug-drug-disease interaction (PBPK-DDDI) platforms. Previous incongruencies between the simulated and observed *in vivo* transporter-mediated DDI between ketoconazole (inhibitor) and rivaroxaban (victim substrate) prompted modification of *in vitro* transporter inhibition experimental protocols in attempts to bridge the *in vitro in vivo* disconnect. Successful recapitulation of the reported enzyme- and transporter-mediated DDI between rivaroxaban and ketoconazole would instill confidence in the accuracy and relevance of *in vitro* inhibitory parameters subsequently derived for prospective DDI simulations with erlotinib and nilotinib as clinically relevant perpetrator drugs in cancer-associated venous thromboembolism.

**Figure 2.** Examining the implications of experimental design on the inhibitory potential of ketoconazole on the organic anion transporter (OAT)3-mediated basolateral uptake of rivaroxaban and the eventual magnitude of DDI between ketoconazole and rivaroxaban.

1. Consideration of the inhibitory effects of ketoconazole on CYP3A4/2J2-mediated metabolism and P-glycoprotein-mediated efflux of rivaroxaban (blue solid line) underestimated the observed DDI magnitude (open symbols), suggesting that the nature and potency of transporter-mediated interactions between rivaroxaban and ketoconazole have not been adequately elucidated. (**B**)represents concentration-dependent diminutions of total E3S or rivaroxaban uptake into OAT3-transfected HEK cells in the presence of ketoconazole, measured in protein-free transport buffer. Reversible inhibition experiments were subsequently performed in the presence of multiple substrate and inhibitor (ketoconazole) concentrations, with Dixon plots utilized to discern the mode of inhibition when (**C**) E3S or (**E**) rivaroxaban was utilized as the probe substrate. The intersection of lines on the x-axis was diagnostic of non-competitive inhibition and guided application of the appropriate non-linear regression model (**Equation 3b**) to obtain the inhibitory constants (*Ki*) for ketoconazole on (**D**) E3S and (**F**) rivaroxaban. (**G**) shows the apparent IC50 value of ketoconazole on OAT3-mediated transport of rivaroxaban determined in the presence of 5% w/v human serum albumin, which will be corrected for the fraction unbound in plasma to obtain IC50,u. (**H**) As demonstrated by the blue dashed line, incorporation of the IC50,u value (corrected to *Ki,u* via the Cheng Prusoff equation) enabled accurate recapitulation of the clinically observed DDI (open symbols). Each point in (**B-G**) represents the mean ± S.D. of three independent experiments. Abbreviations: E3S – estrone 3-sulfate; HEK – human embryonic kidney

**Figure 3.** Simulated PK profiles of erlotinib and nilotinib. Simulated mean (solid line) plasma concentration-time profiles of (**A-B**) erlotinib and (**C-D**) nilotinib after single and/or multiple oral dosing. Incorporation of enzyme inactivation parameters for erlotinib and nilotinib against CYP3A4 (**Table 2**) was essential to recapitulate observed accumulation upon multiple dosing. Dashed lines in (**C-D**) represent overestimations of the systemic exposure of nilotinib when potential non-specific binding in the *in vitro* incubation was considered. Open symbols represent the mean or mean ± SD of observed clinical data.

**Figure 4.** Systematic characterization of *in vitro* inhibitory parameters describing inhibition of OAT3-mediated transport of E3S and rivaroxaban by nilotinib.

1. IC50 curves in (**A**) illustrate the differential inhibitory potencies of nilotinib on OAT3-mediated uptake of E3S and rivaroxaban. Subsequent reversible inhibition experiments performed at multiple substrate and nilotinib concentrations yielded Dixon plots characteristic of **(B)** non-competitive inhibition and (**D**) competitive inhibition when E3S and rivaroxaban were used as probe substrates respectively. *Ki*values obtained based on non-linear regression analyses was applied to further verify the relative inhibitory potencies of ketoconazole against OAT3-mediated (**C**) E3S versus (**E**) rivaroxaban uptake. The apparent IC50 value describing inhibition of OAT3-mediated uptake of rivaroxaban by nilotinib in the presence 5% w/v human serum albumin obtained in (**F**) was corrected to *Ki,u* by considering the competitive mode of inhibition and the plasma fraction unbound of nilotinib. Each point represents the mean ± S.D. of biological triplicates.

**Figure 5.** Quantitative assessment of the influence of potential drug-drug-disease interactions (DDDIs) with erlotinib or nilotinib on rivaroxaban’s pharmacokinetics and systemic exposure as well as associated major bleeding risks. (**A**) Geometric mean (90% confidence interval) AUC0-INF fold changes of rivaroxaban when co-administered with erlotinib or nilotinib across the spectrum of renal function were simulated and compared with clinically observed AUC ratio thresholds corresponding to either a recommendation for dose reduction (blue dotted line) or avoidance of therapy (red dotted line). Rational dose adjustments were designed to fall within the pre-defined range of dose exposure equivalence (gray region). Additionally, an exponential regression model depicting the relationship between major bleeding risks and AUC0-24h was also applied to evaluate the predicted systemic exposures of rivaroxaban arising from various DDDI scenarios with (**B**) erlotinib and (**C**) nilotinib. The 4.5% boundary (red solid line) indicates the major bleeding risk attributed to concomitant ketoconazole and rivaroxaban therapy, a clinically contraindicated combination. Abbreviation: AUC – area under the plasma concentration-time curve

**Supplemental Figure Legends**

**Figure S1.** Mechanism-based inactivation of CYP3A4 and CYP2J2-mediated metabolism of rivaroxaban by erlotinib and nilotinib.

Semi-logarithmic plots demonstrate time- and concentration-dependent inactivation of CYP3A4 by (**A**)erlotinib and (**C**) nilotinib. In (**B**)and(**D**), the relationship between *kobs* values determined from (**A**) and (**C**) and inactivator concentrations were further investigated via nonlinear regression analysis. In (**E**) and (**F**), potential time-dependent inactivation of CYP2J2-mediated metabolism of rivaroxaban by erlotinib and nilotinib was also investigated. Attenuated CYP2J2 activity across pre-incubation times was observed with erlotinib and dronedarone (positive control), but not with nilotinib. (**G**) and (**H**) presents the subsequent MBI characterization of erlotinib against the CYP2J2-mediated metabolism of rivaroxaban. Each point in (**A-H**) represents the mean ± S.D. of triplicate determinations.

**Figure S2.** Reversible inhibition of CYP3A4 and CYP2J2-mediated metabolism of rivaroxaban by erlotinib and nilotinib.

IC50 curves illustrate the concentration-dependent reversible inhibition of CYP3A4 and CYP2J2 by (**A-B**) erlotinib and (**C-D**) nilotinib respectively. Further enzyme kineticexperiments were conducted to determine inhibitory potencies (*Ki*) of (**E**) erlotinib and (**G**) nilotinib on CYP2J2-mediated rivaroxaban metabolism. Data were transformed to Lineweaver-Burk plots in (**F**) and **(H)** to discern the mode of inhibition. Each point in (**A-D, E and G**) represents the mean ± S.D. of triplicate determinations.

**Figure S3.** Examining the influence of virtual population changes on the plasma concentration-time profiles of rivaroxaban, erlotinib and nilotinib. Given that prospective DDI simulations were to be performed using the “Sim-Cancer” population in patients with normal renal function, potential differences in the pharmacokinetics of rivaroxaban between the “Sim-Cancer” and “NEurCaucasian” populations were first investigated in (**A**). The effects of renal impairment on the magnitude of DDIs observed between rivaroxaban and erlotinib or nilotinib would subsequently be investigated and we established how the steady-state exposures of the perpetrator drugs, (**B**) erlotinib and (**C**) nilotinib were not significantly affected when the developed “mild renal impairment” population was utilized (red solid line) but were significantly augmented when the “moderate renal impairment” population was adopted (blue solid line).

**Figure S4.** Concentration-dependent diminutions of total rivaroxaban uptake into OAT3-transfected HEK cells in the presence of fluconazole, measured in protein-free transport buffer and in the presence of 5% w/v human serum albumin.

**Figure S5.** Development of the mechanistic kidney model (MechKiM) for simulation of rivaroxaban PK in healthy and renal impaired patients.

(**A**) Estimates of the *in vitro* OAT3-mediated CLuint,T were obtained via a bottom-up approach where concentration-dependent rivaroxaban uptake was investigated in wild type and OAT3-transfected HEK cells over . Each point in represents the mean ± SD of at least two independent experiments with triplicate determinations. In (**B**) severe renal impairment, sensitivity analysis was performed to gain insights into the magnitude of deterioration in OAT3-mediated CLuint,T (REF) required for the predicted/observed CLR to fall within the predefined success criteria defined in **Table S4** (denoted by the grey shaded area).

**Figure S6.** Evaluating the effects of dose adjustments on the simulated AUC fold changes of rivaroxaban in the presence of verapamil and underlying renal impairment.