Physiologically Based Biopharmaceutics Modeling of regional and colon absorption in humans

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Abstract

Colon absorption is a key determinant for successful development of extended release and colon targeted drug products. This is the first systematic evaluation of the ability to predict in vivo regional differences in absorption and the extent of colon absorption in humans using mechanistic physiologically based biopharmaceutics modeling (PBBM). A new dataset, consisting of 19 drugs with a wide range of biopharmaceutics properties and extent of colon absorption in humans, was established. Mechanistic predictions of the extent of absorption and plasma exposure after oral, or jejunal and direct colon administration were performed in GastroPlus and GI-Sim using an *a priori* approach. Two new colon models developed in GI-Sim, were also evaluated to assess if the prediction performance could be improved. Both GastroPlus and GI-Sim met the pre-defined criteria for accurate predictions of regional and colon absorption for high permeability drugs irrespective of formulation type, while the prediction performance was poor for low permeability drugs. For solutions, the two new GI-Sim colon models improved the colon absorption prediction performance for the low permeability drugs while maintaining the accurate prediction performance for the high permeability drugs. In contrast, the prediction performance decreased for non-solutions using the two new colon models. In conclusion, PBBM can be used with sufficient accuracy to predict regional and colon absorption in humans for high permeability drugs in candidate selection as well as early design and development of extended release or colon targeted drug products. The prediction performance of the current models needs to be improved to allow high accuracy predictions for commercial drug product applications including highly accurate predictions of the entire plasma concentration-time profiles as well as for low permeability drugs.

Key words: physiologically based biopharmaceutics modeling; PBPK; PBBM; colon absorption; regional absorption; relative bioavailability; permeability; solubility; extended release; colon targeting

1. Introduction

Physiologically Based Biopharmaceutics Modeling (PBBM) or Physiologically Based Pharmacokinetic (PBPK) analysis for biopharmaceutics applications has become increasingly popular in recent years to predict the rate and extent of absorption as well as plasma exposure during oral drug product design and development[1-7]. Several different commercial (e.g. GastroPlus, Simcyp), open source (e.g. PK-Sim) and inhouse (e.g. GI-Sim) PBBM software exist, all sharing the ability to take the combined effects of gastrointestinal physiology, the physicochemical and biopharmaceutics properties of a drug as well as formulation aspects into account in the prediction of clinical performance of a drug[8-12]. Applications of PBBM span over both early and commercial drug product development, where examples of early applications include human dose predictions and predictions of the impact of drug substance (particle size, salts/polymorphs etc.) and physiology (e.g. gastric pH and transit time) on absorption while commercial drug product applications include virtual bioequivalence (BE) trials, establishing in vitro – in vivo relationships/correlations (IVIVR/IVIVC) and justification of clinically relevant dissolution and particle size specifications [2, 5, 13-16]. Altogether, by providing the ability to link in vitro drug product and clinical performance, PBBM has the potential to enable patient focused product quality specifications and accelerate the drug development process [2, 3, 5, 7, 16].

To establish confidence in the use of PBBM in drug product development, systemic evaluations of the prediction performance of several models have been performed and case studies as well as workshop reports have been published[2, 3, 5, 7, 11, 16-24]. In addition, in 2020 FDA issued a draft guidance on the use of PBBM[1]. However, the majority of the studies so far have focused on predictions of immediate release (IR) formulations where absorption mainly occurs in the

proximal small intestine. In contrast, significantly less attention has been paid to absorption predictions of modified/extended release (MR/ER) or colon targeted formulations, where the need to model regional intestinal differences in absorption provides an additional dimension of complexity. Assessment and prediction of colon absorption is especially important for successful ER formulation development since they often are designed to release the drug content for a time period exceeding that of the small intestinal transit time, resulting in that the majority of drug release and absorption will occur in the colon[25-27]. In addition, the environment in the colon provides a formidable absorption barrier where the small surface area and tight junctions in the epithelial cell layer impact the permeability/membrane transport while the low water content, lack of bile salts as well as the irregular motility pattern may restrict dissolution of a drug in the colon[26, 28-32]. Furthermore, drugs may undergo bacteria-mediated luminal degradation in the colon[33-35]. Human regional absorption studies using various intubation or remote controlled capsule techniques to estimate the relative bioavailability in the colon ($Frel_{colon}$) have provided significant insight towards an understanding of the factors affecting colon absorption and enabled recent development of in vivo predictive in vitro methods to assess the extent of colon absorption as well as approaches to calculate the colon permeability in humans [26, 28, 33, 34, 36-41]. Despite this, there are only a few case reports available where modeling of colon absorption have been attempted, with various degree of success, and no systematic evaluation of the ability to predict colon absorption in humans has been published [17, 42-45]. Moreover, in silico modeling of colon absorption has been recognized as challenging and is a gap in the current biopharmaceutics modeling and simulation capability[46, 47].

To bring insight into the ability of current PBBM tools to predict the rate and extent of colon absorption as well as the plasma exposure of ER and colon targeted formulations, the objective

of this work was to evaluate how well human regional and colon absorption was predicted by GastroPlus and GI-Sim after establishing a dataset based on available human regional absorption and biopredictive in vitro data. In addition, the aim was also to develop new colon models in GI-Sim and evaluate if the prediction performance could be further improved.

2. Material & Methods

2.1 Model drug selection and dataset establishment

The starting point for the selection of the model drugs for this evaluation was a previously published dataset of 42 compounds where clinical regional absorption data, including pharmacokinetic data as well as the relative bioavailability and estimated fraction absorbed after colon administration to human subjects, was available[26]. The original dataset was further expanded based on recent publications and additional internal data. To allow an absorption focused evaluation regarding prediction of regional and colon absorption after dosing to different regions of the gastrointestinal tract and to reduce the uncertainty in the predictions related to the distribution and elimination of a drug, all compounds lacking clinical intravenous pharmacokinetic data were excluded from the evaluation. The only exception to this was AZD5904, where the high permeability, dose linear exposure and low hepatic extraction ratio provided sufficient confidence to estimate the pharmacokinetic parameters from oral solution data. Compounds identified as potential substrates of small intestinal gutwall metabolism, which could result in overestimation of the observed $Frel_{colon}$ were also excluded except for budesonide and ticagrelor where the extent of small intestinal gutwall metabolism were appropriately accounted for[48-50]. To ensure that the developed *a priori* mechanistic absorption models were

as accurate as possible, only compounds with reliable biopharmaceutics and physicochemical input parameters were included. The data were mainly gathered from internal measurements at AstraZeneca using established methods or, when not available, from previously published reports (Table 1). The human effective permeability (Peff) values for the model drugs used in the predictions were either the clinically measured Peff values or estimated using measured in vitro intrinsic Caco-2 apparent permeability (Papp) values in combination with a previously established Caco-2 Papp – human Peff correlation[11, 51, 52]. In vitro buffer solubility was used as input in the modeling of colon absorption, while biorelevant solubility measured in fasted simulated small intestinal fluid (FaSSIF) was also used in the predictions of oral or jejunal administration of the model drugs. In the absence of biorelevant solubility data, it was assumed that no distribution into micelles or other colloidal structures (CS) occurred and that the biorelevant and buffer solubility was the same. Missing particle size data was handled by assuming a mean particle radius of 10 µm as previously described [11, 18]. Molar density (ρ) was calculated by $\rho = M_W/V_M$, where M_W is the molecular weight and V_M is the molar volume. The diffusion coefficient in water (D) was estimated by Stoke-Einstein's equation (Eq. 1):

$$D = \frac{k*T}{6*\pi*\eta*r} \qquad \text{(Eq. 1)}$$

where k is the Boltzmann's constant, T is the absolute temperature, η is the viscosity of water and r is the molecule radius. Missing data on ρ and D were handled by using default values of 1.2 g/mL and 0.75 x 10^{-9} m^2/s, respectively as previously described[11, 18] (Table 1).

The systemic pharmacokinetic parameters were estimated by compartmental modelling of the mean plasma concentration-time profiles after intravenous (oral for AZD5904) administration using the PK Plus module in GastroPlus (version 9.0.0007) (Table 1). All plasma concentration

data were obtained from either internal AstraZeneca studies or previously published work. The first pass liver extraction ratio (E_H) was either obtained directly from literature or estimated by (Eq. 2):

$$E_H = \frac{CL_H}{Q_H * B/P} \quad \text{(Eq. 2)}$$

where CL_H is the hepatic clearance, Q_H the hepatic blood flow (1350 ml/min) and B/P is the blood to plasma concentration ratio. CL_H was assumed to be equal to non-renal clearance and was calculated by $CL = CL_H + CL_R$, where CL_R is the renal clearance.

The final data set used in the evaluation consisted of 19 compounds with a total of 24 colon administrations (17 as solutions and 7 as non-solutions, i.e. as suspension, powder or granules) (Tables 1-2). The dose volume for each colon administration as well as reference to each regional absorption study is presented in Table 6. The selected model compounds covered all Biopharmaceutics Classification System (BCS) classes and the range in estimated human Peff and buffer solubility was 0.03-8 $x10^{-4}$ cm/s and 0.0002-43 mg/ml, respectively. Furthermore, the $Frel_{colon}$ and the estimated fraction absorbed in the colon ($Fabs_{colon}$) for the model compounds ranged between 5-100% and 3-100%, respectively.

2.2 Evaluation of current human colon models

2.2.1. Modeling strategy

The established dataset was used to evaluate how well the human fasted state colon models in GI-Sim (version 5.6) and GastroPlus (v9.0.0007) could predict the observed $Frel_{colon}$, the estimated $Fabs_{colon}$ and the plasma exposure, primarily AUC_{0-t}, for the model drugs, which all

had been administered both orally (or to the jejunum) and directly to the colon in humans. This was achieved by developing mechanistic physiologically based biopharmaceutics models for each model drug allowing prediction of both the oral or jejunal reference and the direct colon administration for the different types of formulations according to an *a priori* approach. No fitting to observations was allowed in the absorption modeling, while the systemic pharmacokinetic input parameters were obtained by compartmental modelling of intravenous data to ensure as accurate systemic pharmacokinetic input as possible to allow an absorption focused evaluation.

2.2.2. Investigated absorption models

2.2.2.1 GI-Sim

The gastric and small intestinal (SI) regions of GI-Sim have been thoroughly described and evaluated previously[11]. Briefly, this human physiologically based biopharmaceutics model consists of seven gastrointestinal (GI) compartments: the stomach (1) and the SI (2−7) where each compartment, described by a defined volume and area, was considered ideal except for a thin aqueous boundary layer (ABL) lining the intestinal wall of the SI (Table 2). The pH-dependent solubility of a compound was described by the Henderson−Hasselbalch equation and the dissolution rate by Fick's law with the Nielsen stirring model. Micelles and other colloidal structures were included in the SI compartments into which dissolved uncharged molecules can partition[11].

The colon part of the current GI-Sim consists of two compartments, the proximal (8) and distal colon (9), which has been described but not evaluated previously (Table 2)[11]. The majority of

equations for the gastric/SI compartments are also valid for the colon compartments. However, since the absolute majority of the bile salts are reabsorbed in the distal SI resulting in remaining low concentrations, below CMC, in the colon the micellar volume fraction were set to 0 in the colon compartments and only buffer solubility was used as a solubility input in the colon absorption predictions[29]. In addition, in the evaluation of the current version of GI-Sim there was no ABL present in colon compartments and it was assumed that the colon Peff was the same as the jejunal/SI Peff.

The rate of absorption, $F_{absorption}$, of free dissolved molecules across the intestinal membrane from each compartment was calculated by (Eq. 3):

$$F_{absorption} = P_{eff} \times C_b \times SA \quad \text{(Eq. 3)}$$

where C_b is the concentration in the bulk and SA is the surface area available for absorption.

In the colon absorption predictions, direct administration to the colon was simulated using a "Dose-to-colon" functionality where the gastric and SI compartments were excluded and the drug was administered directly to the proximal colon at the specified dose, formulation and dose volume. Solutions were modeled by selecting the solution formulation option while non-solutions, i.e. suspensions, powders or granulates, were modeled using the suspension formulation type in GI-Sim.

2.2.2.2 GastroPlus

GastroPlus (Simulations Plus, Inc., Lancaster, CA) is based on the advanced compartmental absorption and transit (ACAT) model and has been thoroughly described previously[8, 53]. The

204 “immediate release solution” or “immediate release suspension” formulation options were used
205 for the solutions and non-solutions, respectively. The human fasted physiology in GastroPlus has
206 9 compartments; stomach (1), duodenum (2), jejunum 1 (3), jejunum 2 (4), ileum 1 (5), ileum 2
207 (6), ileum 3 (7), caecum (8) ascending colon (9) and is summarized in Table 3. To simulate
208 administration directly to colon, the transit times in compartment 1-7 were set to 0.01 minutes
209 and the % fluid in SI was set to 0.1. Oral and proximal SI reference administrations were
210 simulated by using default settings or by setting the transit time in compartment 1 to 0.01
211 minutes, respectively. Since the absolute majority of the bile salts are reabsorbed in the distal SI
212 resulting in remaining low concentrations below CMC, in the colon, it was assumed that this
213 would not affect the solubility in the colon and only buffer solubility was used as solubility input
214 in the colon absorption predictions[29].

215

216 2.3 Development and evaluation of new human colon models in GI-Sim

217 2.3.1. Model development and evaluation strategy

218 Two new human fasted colon models were developed in GI-Sim. The overall goal was to define
219 new colon models, which better reflected the current physiological understanding of the colon
220 and at the same time evaluate the impact of the volume available for dissolution in the colon. The
221 objective was also to utilize the recent approach where the human in vivo colon effective
222 permeability was estimated from human regional absorption studies to enable inclusion of a
223 colon specific Peff ($Peff_{colon}$) input in the modeling of colon absorption[41]. This is in contrast to
224 the initial evaluation of the current versions of GI-Sim and GastroPlus where it was assumed that

the Peff remained unchanged throughout the small and large intestine. Detailed description of the development of the new models is presented below (sections 2.3.2. – 2.3.4.).

The new GI-Sim colon models using the predicted $Peff_{colon}$ as permeability input were evaluated in the same way as the initial GI-Sim colon model regarding how well human colon absorption was predicted. In the evaluation of the new colon models in GI-Sim, the dataset was slightly refined where budesonide and ximelagatran were excluded since they undergo bacterial-mediated degradation in the colon[33] while hydrochlorothiazide, cimetidine and furosemide were excluded due to lack of in vivo plasma concentration-time data.

2.3.2. Colon model structure and transit time

A colon model structure consisting of three compartments were adopted to describe the large intestine where the luminal content was assumed to be sufficiently semi-viscous to facilitate drug dissolution/release, diffusion and absorption. In proximal to distal direction, these compartments represent the three main anatomical regions of the colon: 1) ascending colon including caecum (AC), 2) transverse colon (TC) and 3) the descending colon (DC). These compartments were added to the gastric and SI model structure previously described for GI-Sim (section 2.2.2.1.)[11].

The specific transit times selected for the three colon compartments were estimated based on the mean distribution of radiolabeled pellets (diameter 0.4-0.6 mm) over a time period of 48 hours. The pellets were measured in the different regions of the colon, represented by the three different model compartments, after oral administration[54]. Transit rate constants were estimated by non-linear regression of the model to all data simultaneously assuming a continuous proximal-to-

distal first order flow of pellets from one compartment to the next (Figure 1S, Supporting Information). The estimated transit times for AC, TC and DC were 12.9, 13.2 and 5.50 hours, respectively, which corresponds to a total transit time of 31.5 hours through these regions of the colon (Table 4). This estimated colon transit time corresponded well with previous reported values[25, 31, 55, 56].

2.3.3. Volume, surface area and pH

The mean total colon water volume accessible for luminal drug distribution, including water in the viscous luminal content and bacteria, has been reported to be > 500 ml and homogenously distributed throughout the colon with 203±75, 199±79 and 159±85 ml present in the AC, TC and TC, respectively in healthy fasted subjects[57]. On the other hand, the free water volume in the colon has been reported to be significantly lower (13±12 mL, range 1–44 mL) and distributed as scattered fluid filled pockets along the length of the colon[30]. Assuming the same regional distribution as for the total colon water volume, the free water volumes in the AC, TC and DC would be 4.7 ml, 4.6 ml and 3.7 ml, respectively. The differences in water volumes were assigned to two models, Model 1 and Model 2, representing the high and low water volume scenario, respectively (Table 4). Furthermore, the specified volumes were used to calculate the epithelial surface area available for absorption for each model. In accordance with the calculation of published values on colon permeability this was performed by assuming that specified volumes have the geometric shape of a cylinder and by adopting a mean colon radius of 2 cm[41]. Regional differences in diameter were accounted for by adopting a radius of 2.5 cm, 2.1 cm and 1.7 cm for the AC, TC and DC, respectively in accordance to reported

measurements[58]. No further adjustments of the surface area, e.g. amplifications due to folds or villi, were applied under the assumption that any regional anatomical differences are negligible in this respect. The pH in each colon compartment was set to reflect the regional mean values reported from a recent meta-analysis, where the pH in the AC, TC and DC were estimated to 6.28, 6.33 and 7.10, respectively[59]. Each compartment was considered ideal and without presence of micelles or other colloidal structures. The final physiology parameters for the two new colon models in GI-Sim are summarized in Table 4.

2.3.4. Modeling of colon permeability and rate of absorption

Modulation of the effective surface area available for absorption has previously been applied as a model strategy to account for the observed regional differences in the rate of absorption between colon and the small intestine[8, 45, 56]. However, this approach assumes a linear correlation between the small intestinal and colon Peff, but recent learnings have demonstrated that there is only a weak linear correlation between the two parameters ($r2 < 0.5$) (Figure 2S, Supporting Information)[41]. This indicates that observed regional differences in the absorption rate cannot be adequately explained just by assigning different effective surface area in the different model compartments and therefore an alternative strategy was selected where the $Peff_{colon}$ was predicted for the model drugs and subsequently used as input in the evaluation of the new colon models.

To investigate if the correlation between the small intestinal and colon Peff could be improved, a partial least square (PLS) regression analysis was performed based on a limited number of well-defined molecular descriptors. Compounds with available human in vivo jejunal and colon Peff values measured using the same methodology were included in the analysis[28, 41]. In case of

multiple measurements of the same parameter were available, an average was calculated and used in the analysis. Cyclosporine was excluded due to high uncertainty in the $Peff_{colon}$ measurement and theophylline was included under the assumption that the distal small intestinal Peff was representative for the jejunal Peff. In addition to the jejunal Peff, the following molecular descriptors were assessed in the PLS: MW, PSA, HBA, HBD, Ring, Ringsystems, BalabanIndex, WienerIndex, logd6.5, logd7.2, logd7.4, MolecularPolarizability, Vol, Rotbond, Max proj Area, Min proj Area, Strongest Acid pKa, Strongest base pKa, ASAhydrophobic, ASAnegative, ASAPlus, ASAPolar, averagePolarizability, hmoPiEnergy , wienerPolarity, topologicalPolarSurfaceArea, vanDerWaalsSurfaceArea, NonpolarArea. Molecular structures were obtained as SMILES-strings from chemicalize.org and all molecular descriptors were calculated using Chemaxon Excel*[60].

A step-wise variable selection was performed to remove descriptors with low influence on the model. The final correlation model obtained by PLS regression analysis included jejunal logPeff, polar surface area (PSA), number of hydrogen bound donors (HBD) and number of rotating bonds (Rotbond) as described by (Eq. 4):

$$\text{Colon logPeff} = 0.44 \times \text{jejunal logPeff} - 0.077 \times \text{Rotbond} - 0.24 \times \text{HBD} - 0.0097 \times \text{PSA} + 1.05$$

This was used to establish a correlation between the above predicted $Peff_{colon}$ and the estimated human in vivo $Peff_{colon}$, to evaluate the validity of the approach, which was considered to be adequate (AAFE=1.8, AFE=1.00) (Figure 3S, Supporting Information). The predicted $Peff_{colon}$ at pH 6.5 for the model drugs used in the evaluation of the new GI-Sim colon models are summarized in Table 5.

2.4. Model prediction performance assessment.

The ability of the models to predict the extent of absorption in the colon of the model drugs administered as solutions was evaluated based on the prediction of the mean $AUC_{0\text{-}t}$, the relative bioavailability after administration to the colon ($Frel_{colon}$) in comparison to reference oral or jejunal administration ($Frel_{colon} = AUC_{colon}/AUC_{reference}$) and the estimated fraction absorbed in the colon ($Fabs_{colon}$), where the observed $Fabs_{colon}$ was estimated by $Fabs_{colon} = Fabs_{oral} \times Frel_{colon}$ as described previously[26]. For non-solution formulations only $AUC_{0\text{-}t}$ and $Frel_{colon}$ were used to evaluate the prediction performance due to lack of relevant $Fabs_{oral}$ data. The absolute average fold error (AAFE) was used to assess the overall predictive accuracy for each parameter (Eq. 5):

$$AAFE = 10^{\Sigma|\log(\frac{predicted}{observed})|/n} \quad \text{(Eq. 5)}$$

To assess the tendency for over- or underprediction, the average fold error (AFE) was used (Eq. 5):

$$AFE = 10^{\Sigma\log(\frac{predicted}{observed})/n} \quad \text{(Eq. 6)}$$

For visualization purposes the average absolute prediction error (AAPE%) was also calculated (Eq. 7):

$$AAPE(\%) = \frac{100}{n}\sum\left|\frac{Obs_i - Pred_i}{Obs_i}\right| \quad \text{(Eq. 7)}$$

Furthermore, visual inspection of the predicted plasma concentration-time profiles in relation to the corresponding observed plasma concentration-time profiles as well as the ability to qualitatively predict observed regional differences in absorption was used as a qualitative measure of the prediction performance.

A perfect accurate model with no systematic trends for over- or underprediction would have AAFE and AFE values of 1. AFE values below 1 indicate a trend for underprediction whereas values above 1 indicate overprediction. Two levels of acceptance criteria for the PBBM colon absorption prediction performance were defined prior to the evaluation:

1) AAFE < 1.25 and AFE between 0.8-1.25 was considered to be highly accurate and reflecting a prediction performance sufficient to predict regional and colon absorption from a commercial drug product applications perspective.
2) AAFE between 1.25-2 and AFE within [0.5-0.8] or [1.25-2] was considered to be accurate and reflecting a prediction performance sufficient to predict regional and colon absorption from a candidate drug selection and early drug product design and development perspective.

The above criteria were considered to be justified given the *a priori* modelling approach used where model development was based on independent parallel studies and is in accordance with previous investigations[6, 61]. Predictions with AAFE values > 2 and AFE values within [0-0.5] or above 2 were considered to be poor.

3. Results

3.1. Colon absorption prediction performance evaluation of the current models in GI-Sim and GastroPlus

The current versions of GI-Sim and GastroPlus were evaluated regarding how well they could predict the regional and colon absorption estimated after oral/jejunal reference and direct

354 administration to the colon in human fasted subjects where the $Frel_{colon}$, the $Fabs_{colon}$ and the
355 AUC_{0-t} were the primary parameters of interest. The observed and predicted mean plasma
356 concentration-time profiles after oral/jejunal and colon administration are shown in Figures 1 and
357 2 for GI-Sim and GastroPlus, respectively. The observed and predicted primary colon absorption
358 parameters are summarized in Table 6 and the overall predictive performance of the current
359 models are summarized in Table 7 and Figure 3.

360 For the colon absorption predictions when the model drugs had been administered to the colon as
361 solutions, neither of the models met the pre-defined criteria for highly accurate predictions while
362 both models were on the borderline to meet the criteria for accurate predictions (Table 7). For
363 both models, a prediction error slightly above 2-fold for the colon AUC_{0-t} predictions (AAFE >
364 2) was shown while AAFE was < 2 for the $Frel_{colon}$ and $Fabs_{colon}$ predictions (Table 7). The AFE
365 values did not indicate any significant trends for over- or under predictions (Table 7). For both
366 models, the overall prediction performance for the solution formulations was clearly attributed to
367 poor accuracy in the prediction of the drugs with low permeability while the predictions for the
368 high permeability drugs met the criteria for accurate predictions (AAFE < 2). The AAFE range
369 in the predictions of $Frel_{colon}$, $Fabs_{colon}$ and colon AUC_{0-t} for solutions of model drugs with high
370 permeability was 1.43-1.80 and 1.24-1.56 for GastroPlus and GI-Sim, respectively with no
371 significant trend for over- or underpredictions based on the AFE values (Table 7, Figures 3 and
372 4). Furthermore, >90% of the predictions for the high permeability drugs were within 2-fold
373 prediction error (Figures 3 and 4). In contrast, for the low permeability drugs the AAFE range in
374 the predictions of $Frel_{colon}$, $Fabs_{colon}$ and colon AUC_{0-t} was 2.51-3.15 and 2.62-3.96 for
375 GastroPlus and GI-Sim, respectively (Table 7). Interestingly, based on the AFE values there was
376 a clear difference in the prediction performance between the two models where GastroPlus

377 underpredicted while GI-Sim overpredicted the colon absorption significantly for the low
378 permeability drugs (Table 7, Figures 3 and 4).

379 All of the model drugs used in the colon absorption predictions when the model drugs had been
380 administered to the colon as non-solutions, i.e. as suspensions, powder or granulate, were high
381 permeability drugs. In the predictions of the non-solutions, both models met the criteria for
382 accurate predictions (AAFE < 2) where the AAFE range for $Frel_{colon}$ and colon AUC_{0-t} was 1.40-
383 1.99 and 1.56-1.99 in GastroPlus and GI-Sim, respectively with no significant trend for over- or
384 underpredictions based on the AFE values (Table 7, Figures 3 and 4).

385 Based on visual inspection, the predicted and observed plasma profiles generally agreed well for
386 both the solutions and non-solutions of the high permeability model drugs irrespective of
387 software and the identified under- and overpredictions for the low permeability model drugs
388 were clearly visible in the predicted plasma profiles (Figures 1 and 2). Qualitatively, the regional
389 differences in absorption were generally adequately captured in the predictions irrespective of
390 formulation or permeability of the model drugs (Figures 1 and 2). The prediction performance
391 for Cmax was comparable to that of AUC_{0-t} while tmax was less well predicted (Figure 3).

392

393 3.2. Colon absorption prediction performance evaluation of the new colon models
394 in GI-Sim

395 The two new GI-Sim colon models were evaluated in the same way as the original GI-Sim model
396 and GastroPlus regarding the ability to predict the regional and colon absorption estimated after
397 oral/jejunal reference and direct administration to the colon in human fasted subjects. The
398 observed and predicted mean plasma concentration-time profiles after colon administration using

Model 1 with high water volume in comparison with the original GI-Sim model are shown in Figure 5. The observed and predicted primary colon absorption parameters are summarized in Table 8 and the overall predictive performance of both models is summarized in Table 19 and Figure 6. Since both new GI-Sim colon models displayed the same prediction performance for the solutions and as the prediction performance of Model 2 was very poor for the non-solution formulations only the results for Model 1 is presented in the Figures 5 and 6.

For the colon absorption predictions when the model drugs had been administered to the colon as solutions, both new GI-Sim colon models displayed a slower absorption rate, which resulted in lower predicted values of $Frel_{colon}$, $Fabs_{colon}$ and AUC_{0-t} as well as an improved prediction performance for the majority of the low permeability model drugs while the corresponding colon absorption predictions remained mainly unchanged for the high permeability model drugs (Table 8, Figures 5 and 6). As a result, both new colon models met the pre-defined criteria for accurate predictions (AAFE < 2) with no observed trend for over- or underprediction (Table 9). The AAFE range in the predictions of $Frel_{colon}$, $Fabs_{colon}$ and colon AUC_{0-t} was 1.31-1.76 and 1.95-2.16 for the high and low permeability model drugs, respectively with no significant trend for over- or underpredictions based on the AFE values (Table 9). In contrast, the predictions for the non-solutions did not meet the criteria for accurate predictions for neither of the new colon models due to significant underpredictions where the AAFE range for $Frel_{colon}$ and colon AUC_{0-t} for Model 1 with a high water volume in the colon was 2.70-3.25 while the prediction error was approximately 20-fold for Model 2 with the low water volume in the colon (Table 9, Figure 5).

4. Discussion

The main objective of this work was to perform the first systematic evaluation of the ability to predict regional differences in absorption and the extent of colon absorption in humans by using PBBM. Availability of PBBMs demonstrating accurate mechanistic predictions of regional and colon absorption in humans would have a significant impact on the development time and cost for ER and colon targeted drug products since the selection of candidate drugs, decision to initiate development or not as well as definition of target release profiles and in vitro dissolution methods would be greatly facilitated. Accurate predictions would also reduce the need for clinical relative bioavailability studies. In the evaluation of the usefulness of the current models to predict regional and colon absorption, both the purpose of the predictions and the stage of development needs to be taken into account. During candidate selection and early product design and development clinical in vivo data is not available, which precludes accurate estimation of the pharmacokinetic parameters as well as any type of parameter optimization. Therefore the prediction accuracy will depend on the general prediction performance of the model using default model settings and in vivo predictive input parameters. The main purpose of early predictions is to enable assessment of potential limitations in colon absorption with sufficient accuracy. Hence, even if quantitatively highly accurate predictions of the exposure after administration to the colon are not achieved, accurate predictions (AAFE:1.25-2) combined with the ability to qualitatively predict differences in regional absorption should be considered sufficient for this purpose. This is in line with the prediction performance criteria applied for predictions of other pharmacokinetic parameters at the same stage of development[61]. In contrast, during late stage commercial product development clinical data emerges and the applications require models that both can predict the pharmacokinetic parameters (AAFE <1.25)

443 and the entire plasma concentration-time profile with high accuracy. As a consequence, it is
444 appropriate to set different prediction performance criteria in relation to the stage of development
445 and the intended model use. The results in this investigation showed that, in relation to the pre-
446 defined prediction performance criteria, both GastroPlus and GI-Sim were sufficiently accurate
447 to be used to predict regional and colon absorption during candidate selection and early product
448 design and development for high permeability drugs.

449 Despite the encouraging results, it was also clearly demonstrated that both GI-Sim and
450 GastroPlus could be further improved since the highly accurate prediction performance criterium
451 was not met and the prediction accuracy for low permeability drugs was poor. There are several
452 different factors related to the dataset, lack of model functionalities and in vivo understanding as
453 well as methodology aspects, which likely contribute to the non-optimal prediction performance
454 observed in this evaluation. For example in both GI-Sim and GastroPlus it is currently assumed
455 that the effective permeability is the same in the colon as in the small intestine due to lack of
456 direct measurements of the in vivo $Peff_{colon}$ in humans. In addition, it is still unclear what volume
457 of fluid is available for drug dissolution in different regions of colon[29, 30, 57]. In this work the
458 default colon fluid volumes were used but there are some reports where lower colon fluid
459 volumes have been applied in attempts to improve predictions[21, 62, 63], however there is no
460 systematic evaluation reported using a larger dataset demonstrating that this improves the
461 prediction performance from a general perspective. Neither do the current colon models take
462 binding to fecal matter, physiologically correct colon motility or the mucus layer into account.
463 Regarding bacterial mediated degradation in the colon, GastroPlus can only take degradation rate
464 in relation to pH into account while GI-Sim has the possibility to include the luminal degradation
465 half-life in the colon. However, this functionality seems to be pre-mature since an attempt to

include luminal colon degradation data in the colon absorption predictions for budesonide and ximelagatran did not impact the predictions (data not shown)[33]. Additional physiological factors such as regional differences between the colon and the small intestine regarding the expression of efflux transporters and the tight junction may potentially also affect the rate and extent of colon absorption as well as the prediction performance in this investigation. However, it has previously been concluded that there is no indication that efflux-mediated transport limits colon absorption, which suggests that it is likely the intrinsic passive permeability that is the major determinant of the membrane transport in the colon[26]. This is further supported by recently established correlations between in vitro permeability and human colon absorption, where the in vitro assays mainly measure the passive drug transport[26, 37]. Furthermore, as the main source for the estimated Peff in this investigation was the Caco-2 model, which is of colonic origin, it is likely that the well-known effect of narrower tight junctions in the colon was appropriately accounted for in the predictions. Relating to the poor prediction performance for the low permeability drugs it may be argued that this is of lesser concern since compounds with low permeability are considered to be poor candidates for ER formulation development[26]. However, the demonstrated ability of a model to accurately predict the colon absorption for both low and high permeability drugs would provide a higher level of confidence of its usefulness. Interestingly, although the regional and colon absorption prediction performance of the low permeability drugs was poor for both models, there were significant differences between the models where GI-Sim and GastroPlus overpredicted and underpredicted the colon absorption, respectively. The reason for this difference in prediction performance can be attributed to the fact that the effective permeability in each compartment is calculated differently where the lipophilicity (LogD and logP) is taken into account in GastroPlus while GI-Sim only considers

the unionized fraction [8, 11]. Surprisingly, the colon absorption of the high permeability drug metoprolol, which is considered to have ideal colon absorption properties, was underpredicted by both GI-Sim and GastroPlus. As metoprolol is considered to be a divider between low and high permeability, the GastroPlus results could potentially be explained by that metoprolol behaves as a low permeability drug in the prediction, however the GI-Sim prediction results may instead imply that the colon Peff of metoprolol is actually higher than the jejunal Peff in vivo. This has previously been observed in in vitro excised tissue permeability investigations[37]. Furthermore, it is important to recognize that prediction of colon absorption is challenging, especially with a full *a priori* approach used in this investigation, where the majority of the drugs (>80%) in the dataset have some level of observed colon absorption limitation. Finally, is important to evaluate the key assumptions made in the model development and their potential impact on the prediction outcome. In this investigation, care was made to select a dataset with model drugs with suitable in vivo data together with biopredictive in vitro input parameters to allow an absorption focused evaluation. However, some revision of the dataset may be warranted in future investigations. For example, the rapid but incomplete colon absorption of fexofenadine where involvement of active uptake cannot be ruled out may not be an ideal model drug. Also, this investigation used an *a priori* modeling approach and as a consequence poor prediction of the oral reference administration, e.g. for theophylline, melagatran and ximelagatran in GI-Sim, may also affect the colon absorption predictions. Although out of scope for this work, it would be interesting to evaluate if the colon absorption prediction performance is improved by allowing the oral or jejunal reference pharmacokinetic parameters and effective permeability to be optimized, i.e., by using a middle-out approach to predict colon absorption. In addition, it was assumed in the initial

511 evaluation that the Peff was the same throughout the small intestine and the colon. However, this
512 assumption was changed in the development and evaluation of the new colon models in GI-Sim.

513 The ideal situation would be to have fully mechanistic colon models where *a priori* predictions
514 provide highly accurate results to allow applications for commercial drug product development
515 purposes. Consequently, the second aim of this work was to develop new colon models in GI-
516 Sim, which better reflected the current physiological understanding of the colon including the
517 introduction of a colon specific effective permeability as well as two different scenarios of the
518 volume available for dissolution in the colon and to evaluate if the prediction performance was
519 further improved by these implementations. Indeed, the results showed that for solutions, the
520 colon absorption predictions were improved for the low permeability drugs while the prediction
521 performance was maintained and the pre-defined criteria for accurate predictions was met with
522 the new colon models. Unfortunately, the prediction performance decreased for the non-solutions
523 using the two new colon models. For Model 1 with high water volume in the colon, the
524 decreased prediction performance could be attributed to ticagrelor where the estimated effective
525 permeability in the colon was more than 300-fold lower than the jejunal permeability while the
526 colon effective permeability was on average 6-fold lower for the rest of the model drugs. In
527 contrast, Model 2 with the low water volume in the colon resulted in underprediction of the
528 colon absorption in all cases. This suggests that it may be challenging to improve the prediction
529 performance of the model just by revising the physiology parameters of the model based on
530 emerging in vivo data since the new data is subject to individual interpretation. It is also evident
531 that it is challenging to define new colon models with a generic colon transit time due to the
532 large inter- and intraindividual day-to-day variability, the irregular motility pattern as well as
533 external factors, such as feeding, physical activity and sleep patterns, also affecting the colon

transit[31]. In addition, since the water content is reduced during the transit from proximal to distal colon and the liquid phase of the luminal content becomes increasingly more viscous it is also difficult to specify the volume of water available for dissolution and distribution in the colon lumen. Although out of scope for this investigation, further evaluation of Model 1 by applying it to predict the in vivo performance of extended release formulations could provide further insight into the usefulness of the model. Overall the results showed that more investigations are needed to enable development of colon models with higher prediction accuracy.

5. Conclusions

This is the first systematic evaluation of the ability to predict regional differences in absorption and the extent of colon absorption in humans. The investigation demonstrated that mechanistic physiologically based biopharmaceutics modeling can be successfully used to predict regional and colon absorption in humans for high permeability drugs, which in turn can be used in early design and development of extended release or colon targeted drug products as well as reduce product development time and costs. However, the results also clearly articulated that the prediction performance of the current models needs to be improved for low permeability drugs and before fully mechanistic modelling can be successfully used in predictions for commercial drug product applications. Refined colon models incorporating recent physiological understanding and new ways to estimate the colon permeability indeed demonstrated encouraging results in this regard. Finally, this report also provided a completely novel dataset, which can be used by the wider scientific community in the pursuit of improved mechanistic human colon models.

Supporting information

Additional figures providing scientific justification for the selected colon model structure, transit times and colon specific permeability implemented in the new GI-Sim colon models.

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Declaration of interest

The authors have no competing interests to declare. GI-Sim has been developed by AstraZeneca for internal and academic use. AstraZeneca has ongoing license agreements for GastroPlus.

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817 different regions of the gastro-intestinal tract using a remote controlled drug delivery device, European
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Tables

821 Table 1. Biopharmaceutics and physicochemical properties of the model compounds.

Compound	Mw (g/mol)	pK_a[a]	log$D_{7.4}$	ρ (g/ml)	Particle radius[b] (μm)	D (10^{-9}·m^2/s)	P_{eff} (10^{-4}·cm/s)	S_{buffer} (pH) (mg/ml)	S_{FaSSIF} (mg/ml)	BCS Class	CL (L/h)	V (L)	k12 (h^{-1})	k21 (h^{-1})	k13 (h^{-1})	k31 (h^{-1})	First Pass extraction (%)
Atenolol[64]	266.3	9.21 b	-1.65	1.1	-	0.72	0.2	13.3 (instrinsic)	13.3 [d]	III	10.5	18	2.3	0.8	n/a	n/a	0
AZD5904[65]	252.3	7.7 a 10.7 a	0.8 (pH 6.7)	1.25	10	0.762	2.035	0.31 (7.8)	0.31[d]	II	3.4	29.1	0.383	0.023	n/a	n/a	0
Budesonide[65]	430.54	neutral	3.3	1.2	-	0.633	5	0.026 (intrinsic)	0.045	II	78.4	28.5	7.3	1.4	n/a	n/a	87
Cimetidine[65]	252.3	6.76 b	0.35	1.15	-	0.77	0.26	24 (6.8)	24[d]	III	-	-	-	-	-	-	-
Dexloxiglumide[66]	461	4.48 a	1.56	1.2	10	0.75	3.98	0.533 (7.5)	0.533[d]	II	23.6	11.7	1.37	2.13	0.544	0.134	31
Fenofibrate[11]	360.8	neutral	5.2	1.18	0.1	0.663	7.7	0.0002 (6.5)	0.0014	II	0.75	6.8	0.565	1.420	0.128	0.083	0
Fexofenadine[11]	501.7	4.2 a 7.84 b	0.3	1.17	-	0.593	0.07	53 (6.5)	0.53	III	12.4	12.9	1.075	0.828	0.253	0.104	14
Furosemide[65]	330.75	9.87 a 3.51 a	1.18	1.35	-	0.714	0.05	2.25 (7.2)	2.25[d]	III	-	-	-	-	-	-	-
HCT[e][65]	297.7	9.78 a 8.53 a	-0.08	1.46	-	0.8	0.04	0.595 (intrinsic)	0.595[d]	III	-	-	-	-	-	-	-
Ketoprofen[64]	254.3	4.02 a	3.28 (logP)	1.14	-	0.762	2.04	0.118 (intrinsic)	0.118 [d]	II	4.43	2.9	1.171	1.937	0.297	0.189	6
Melagatran[65]	429.5	2 a 7 b 11.5 b	-1.3 (pH 9.7)	1.2	-	0.634	0.033	215 [c]	215[d]	III	8.4	9.4	1.23	1.34	n/a	n/a	2
Metoprolol[11]	267.4	9.18 b	1.74 (logP)	1.07	10	0.709	1.34	43 (6.5)	43[d]	I	48	67.2	5.59	1.271	n/a	n/a	53
Oseltamivir[65]	312.4	7.7 b	0.9	1.1	-	0.68	1.4	0.233 (7.4)	0.233[d]	III	155	45.3	1.435	0.862	0.756	0.020	60
Oxprenolol[65]	265.4	9.5 b	0.14	1.08	10	0.713	5.1	15 (6)	15[d]	I	32.2	16.8	10.63	5.05	n/a	n/a	36
Ranitidine[64]	351	7.62 b 2.22 b	-0.96	1.15	-	0.69	0.27	1.75 (7.4)	1.75[d]	III	38.8	22.5	3.62	1.5	n/a	n/a	9
Rofleponide[65]	468.5	neutral	2.59	1.21	10	0.614	6.55	0.049 (7.8)	0.049 [d]	I	155	49.1	11.14	2.267	n/a	n/a	95
Theophylline[64]	180.2	8.4 a	-0.12	1.25	-	0.853	7.2	1.8 (7.4)	1.8 [d]	I	2.2	4.2	4.21	1.11	n/a	n/a	2
Ticagrelor[65]	522.6	3.05 b	4.3	1.24	5	0.595	6.8	0.007 (7.4)	0.36	II	10.99	12.2	3.267	0.84	0.373	0.014	64 (oral)/30 (colon)
Ximelagatran[65]	474	4.5 b 5.2 b	0.9 (pH 7)	1.2	-	0.601	0.6	0.16 (intrinsic)	0.16[d]	III	8.4	9.4	1.23	1.34	n/a	n/a	56

822 [a] For pK_a values, a and b indicate acid and base, respectively.[b] Particle size is presented as mean particle radius.[c] Not stated. [d] Same value as S_{buffer} due to missing biorelevant
823 solubility data. [e]Hydrochlorthiazide. Systemic compartmental pharmacokinetic parameters not generated for furosemide, cimetidine and hydrochlorthiazide due to lack of reliable
824 plasma concentration-time profiles and pharmacokinetic parameters after oral and colon administration.
825

826 Table 2. Physiological parameters for a 70 kg human in the fasted state in GI-Sim.

GI-Compartment	Surface area (cm^2)	Volume (mL)	Transit time (minutes)	pH	Micellar volume fraction
Stomach	0	47	15	1.3	0
Duodenum	160	42	16	6.0	0.0002
Jejunum 1	580	150	56	6.2	0.0002
Jejunum 2	440	120	44	6.4	0.0002
Ileum 1	330	94	35	6.6	0.0002
Ileum 2	230	71	25	6.9	0.0002
Ileum 3	150	50	17	7.4	0.0002
Proximal Colon	28	47	250	6.4	0
Distal Colon	42	50	750	6.8	0

827

828 Table 3. Physiological parameters for a 70 kg human in the fasted state in GastroPlus.

GI-Compartment	Length (cm)	Radius (cm)	SEF[a]	Volume[b] (mL)	Transit time[b] (minutes)	pH	Bile salt (mM)
Stomach	28.29	9.67	1.000	46.56/**0.1**	15/**0**	1.3	0.0
Duodenum	14.13	1.53	4.235	41.56/**0.104**	15.6/**0.01**	6.0	2.800
Jejunum 1	58.40	1.45	3.949	154.2/**0.386**	57.6/**0.01**	6.2	2.330
Jejunum 2	58.40	1.29	3.489	122.3/**0.306**	44.4/**0.01**	6.4	2.030
Ileum 1	58.40	1.13	3.029	94.29/**0.236**	34.8/**0.01**	6.6	1.410
Ileum 2	58.40	0.98	2.569	70.53/**0.176**	25.2/**0.01**	6.9	1.160
Ileum 3	58.40	0.82	2.109	49.83/**0.125**	17.4/**0.01**	7.4	0.140
Caecum	13.19	3.39	1.790	47.49	251.4	6.4	0.0
Ascending Colon	27.65	2.41	2.480	50.33	754.2	6.8	0.0

829 [a] Surface area Enhancement Factor.

830 [b] Settings for mimicking colon administration marked in bold.

831

832

833 Table 4. Colon physiology parameters for a 70 kg human in the fasted state for the evaluated new
834 colon models in GI-Sim

GI-Compartment	**Surface area** (cm^2)		**Volume** (mL)		**Transit time** (minutes)	**pH**	**Micellar volume fraction**
	Model 1	**Model 2**	**Model 1**	**Model 2**			
AC	165	3.8	203	4.7	772	6.28	0
TC	189	4.4	199	4.6	792	6.33	0
DC	192	4.4	159	3.7	330	7.10	0

835 AC: Ascending Colon including caecum; TC: Transverse Colon; DC: Descending Colon
836

Table 5. The predicted colon Peff at pH 6.5 for the model drugs used in the evaluation of the new GI-Sim colon models. The corresponding Jejunal Peff values at pH 6.5 are included for comparison.

Compound	Jejunual P_{eff} ($\times 10^{-4}$ cm/s)	Colon P_{eff} at pH 6.5 ($\times 10^{-4}$ cm/s)
Atenolol	0.2	0.056
AZD5904	2.035	0.352
Dexloxiglumide	4	2.41
Fenofibrate	7.7	2.48
Fexofenadine	0.07	0.017
Ketoprofen	8.7	1.48
Melagatran	0.033	0.002
Metoprolol	1.34	0.407
Oseltamivir	1.4	0.213
Oxprenolol	5.1	0.739
Ranitidine	0.27	0.086
Rofleponide	6.55	0.523
Theophylline	7.2	3.29
Ticagrelor	6.8	0.021

Table 6. Observed and predicted mean human colon absorption parameters of the model drugs in relation to dose and formulation type for the current versions of GastroPlus (G^+) and GI-Sim (GS).

Compound	Colon Dose (mg)	Dose Volume (mL)	Colon Formulation	AUC_{0-t}[a] (µg*h/mL)	$AUC_{0-t,pred}$[b] (µg*h/mL)		$Frel_{colon}$[c]	$Frel_{colon,pred}$[d]		$Fabs_{colon}$[e]	$Fabs_{colon,pred}$[f]	
				obs	GS	G+	obs	GS	G+	obs	GS	G+
Atenolol[28]	10	12.65	solution	0.010	0.115	0.001	5	40	0.9	3	31	0.6
AZD5904[65]	15	31	solution	0.91	1.27	1.13	83	95	69	83	96	89
	75	30	granules	3.31	4.11	3.52	73	65	63	-	67	61
Budesonide[49]	3	6.2	solution	0.004	0.005	0.004	53	98	91	53	98	91
Cimetidine[67]	200	200	solution	-	na	na	-	na	na	19	64	75
Dexloxiglumide[26]	200	1	solution	4.6	5.63	5.71	75	100	99	62	98	99
	200	0	powder	4.7	2.75	5.60	95	100	97	-	51	98
Fenofibrate[68]	145	0[h]	suspension	9.87	0.61	5.79	16	1	10	-	1	7
Fexofenadine[69]	56	8	solution	0.515	0.937	0.401	44	100	43	13	25	11
Furosemide[67]	20	200	solution	-	na	na	-	na	na	28	17	18
HCT[g][67]	25	200	solution	-	na	na	-	na	na	19	14	3
Ketoprofen[28]	5	12.65	solution	0.736	1.055	0.915	88	99	96	88	100	98
Melagatran[65]	50	0.8	solution	0.0799	0.418	0.004	28	78	2	3	9	0.3
Metoprolol[36, 65]	19.5	6	solution	0.108	0.091	0.016	100	81	16	95	92	22
	19.5	6	powder	0.139	0.091	0.016	94	100	100	-	92	22
Oseltamivir[70]	150	0[h]	solution	0.189	0.327	0.248	83	97	75	67	95	73
Oxprenolol[26, 38]	80	5	solution	1.10	1.54	1.32	82	99	83	74	99	83
	80	5	powder	1.92	1.54	1.32	100	100	100	-	99	83
Ranitidine[71]	134	16	solution	0.269	1.682	0.075	15	79	5	9	60	3
Rofleponide[65]	6.4	8	solution	0.00057	0.0002	0.000	100	98	100	100	99	100
	6.4	0[h]	granules	0.00035	0.0002	0.000	100	98	100	-	99	100
Theophylline[72]	94	0.9	solution	14.2	37.7	33.8	81	97	59	81	100	93
Ticagrelor[50]	100	0.7	suspension	1.53	1.42	4.12	18	21	100	-	32	85
Ximelagatran[65]	50	2.5	solution	0.533	1.727	0.736	56	78	62	39	72	55

[a] Area under the curve between time zero and the last observed time point

[b] Predicted area under the curve between time zero and the last observed time point

[c] Relative bioavailability after administration to colon as compared to oral/duodenal administration

[d] Predicted relative bioavailability after administration to colon as compared to oral/duodenal administration

[e] Observed fraction absorbed after colon administration. Estimated by $Fabs_{oral}$ * $Frel_{colon}$

[f] Predicted fraction absorbed in colon

[g] Hydrochlorthiazide

[h] not stated. 0 mL used in the predictions

Table 7. Colon absorption prediction performance parameters for the current versions of GI-Sim and GastroPlus.

			AAFE[a]			AFE[b]			AAPE(%)[c]		
			AUC_{0-t}	$Frel_{colon}$	$Fabs_{colon}$	AUC_{0-t}	$Frel_{colon}$	$Fabs_{colon}$	AUC_{0-t}	$Frel_{colon}$	$Fabs_{colon}$
GastroPlus		**All**	2.29	1.87	2.05	0.61	0.62	0.71	52	36	57
	Solutions	**High Peff**	1.80	1.50	1.43	0.87	0.83	1.00	49	30	32
		Low Peff	3.15	2.51	2.82	0.39	0.41	0.53	57	44	80
	Non-solutions		1.99	1.40	n/a	0.71	1.20	n/a	57	75	n/a
GI-Sim		**All**	2.33	1.71	1.90	1.96	1.66	1.71	199	116	145
	Solutions	**High Peff**	1.56	1.24	1.26	1.16	1.17	1.25	51	26	29
		Low Peff	3.96	2.62	2.72	3.96	2.62	2.26	397	236	248
	Non-solutions		1.99	1.56	n/a	0.54	0.70	n/a	38	20	n/a

[a]Absolute Average Fold Error (AAFE), [b]Average Fold Error (AFE) and [c]Absolute Average Prediction Error (AAPE). n/a: Not Applicable

Table 8. Observed and predicted mean human colon absorption parameters of the model drugs for the new colon models in GI-Sim.

Compound	Colon Dose (mg)	Colon Formulation	AUC_{0-t}[a] (µg*h/mL)	$AUC_{0-t,pred}$[b] (µg*h/mL)		$Frel_{colon}$[c]	$Frel_{colon,pred}$[d]		$Fabs_{colon}$[e]	$Fabs_{colon,pred}$[f]	
			Obs	Model 1	Model 2	Obs	Model 1	Model 2	Obs	Model 1	Model 2
Atenolol[28]	10	solution	0.010	0.0202	0.0201	5	7	7	3	7	7
AZD5904[65]	15	solution	0.91	0.7947	0.794	83	59	59	83	88	88
	75	granules	3.31	3.698	0.2756	73	55	4	-	85	7
Dexloxiglumide[26]	200	solution	4.6	5.749	5.749	75	107	107	62	100	100
	200	powder	4.7	5.504	0.364	95	103	7	-	98	7
Fenofibrate[68]	145	suspension	9.87	1.125	0.045	16	2	0.07	-	2	0
Fexofenadine[69]	56	solution	0.515	0.5484	0.5475	44	59	59	13	15	15
Ketoprofen[28]	5	solution	0.736	1.042	1.041	88	98	98	88	98	98
Melagatran[65]	50	solution	0.0799	0.5518	0.5501	28	2	2	3	1	1
Metoprolol[36, 65]	19.5	solution	0.108	0.04063	0.04052	100	35	35	95	50	49
	19.5	powder	0.139	0.04061	0.03273	94	35	28	-	50	44
Oseltamivir[70]	150	solution	0.189	0.269	0.269	83	80	80	67	81	81
Oxprenolol[26, 38]	80	solution	1.10	1.457	1.456	82	94	94	74	97	97
	80	powder	1.92	1.457	1.295	100	94	83	-	97	91
Ranitidine[71]	134	solution	0.269	0.5518	0.5501	15	26	26	9	22	22
Rofleponide[65]	6.4	solution	0.00057	0.00018	0.00017	100	82	82	100	90	90
	6.4	granules	0.00035	0.00017	0.000044	100	82	21	-	90	26
Theophylline[72]	94	solution	14.2	31.95	31.94	81	82	82	81	100	100
Ticagrelor[50]	100	suspension	1.53	0.04277	0.00101	18	1	0.01	-	1	0

[a] Area under the curve between time zero and the last observed time point

[b] Predicted area under the curve between time zero and the last observed time point

[c] Relative bioavailability after administration to colon as compared to oral/duodenal administration

[d] Predicted relative bioavailability after administration to colon as compared to oral/duodenal administration

[e] Observed fraction absorbed after colon administration. Estimated by $Fabs_{oral}$ x $Frel_{colon}$

[f] Predicted fraction absorbed in colon

Table 9. Colon absorption prediction performance parameters for the new colon models in GI-Sim.

			AAFE[a]			AFE[b]			AAPE(%)[c]		
			AUC_{0-t}	$Frel_{colon}$	$Fabs_{colon}$	AUC_{0-t}	$Frel_{colon}$	$Fabs_{colon}$	AUC_{0-t}	$Frel_{colon}$	$Fabs_{colon}$
Model 1	**Solutions**	**All**	1.84	1.63	1.61	0.97	0.83	1.06	59	35	49
		High Peff	1.76	1.37	1.31	0.91	0.87	1.05	53	26	27
		Low Peff	1.95	2.08	2.16	1.07	0.77	1.06	67	49	50
	Non-solutions		3.25	2.70	n/a	0.33	0.38	n/a	52	43	n/a
Model 2	**Solutions**	**All**	1.84	1.63	1.61	0.97	0.83	1.06	58	35	49
		High Peff	1.77	1.37	1.31	0.91	0.87	1.05	53	26	27
		Low Peff	1.95	2.08	2.16	1.07	0.77	1.06	67	48	50
	Non-solutions		22.1	19.4	n/a	0.05	0.05	n/a	83	79	n/a

[a]Absolute Average Fold Error (AAFE), [b]Average Fold Error (AFE) and [c]Absolute Average Prediction Error (AAPE). n/a: Not Applicable

Figure legends

Figure 1. Observed and GI-Sim predicted mean plasma concentration-time profiles for the model drugs after oral/jejunal reference and colon administration in human subjects. Observed data is depicted with symbols and predicted data with solid lines. Oral/jejunal reference administration, colon administration of solutions and non-solutions are colored in blue, red and green, respectively. Oseltamivir excluded due to lack of available plasma concentration-time profile after colon administration.

Figure 2. Observed and GastroPlus predicted mean plasma concentration-time profiles for the model drugs after oral/jejunal reference and colon administration in human subjects. Observed data is depicted with symbols and predicted data with solid lines. Oral/jejunal reference administration, colon administration of solutions and non-solutions are colored in blue, red and green, respectively. Oseltamivir excluded due to lack of available plasma concentration-time profile after colon administration.

Figure 3. Colon absorption prediction performance of $Fabs_{colon}$, $Frel_{colon}$, AUC_{0-t}, Cmax and tmax for solutions (blue) and non-solutions (green) after direct administration to the colon in human subjects. GI-Sim and GastroPlus results are displayed in the left and right columns, respectively.

Figure 4. Accuracy of GastroPlus (blue) and GI-Sim (red) predicted $Fabs_{colon}$, $Frel_{colon}$ and AUC_{0-t} in relation to the effective permeability (Peff) used in the predictions for the model drugs

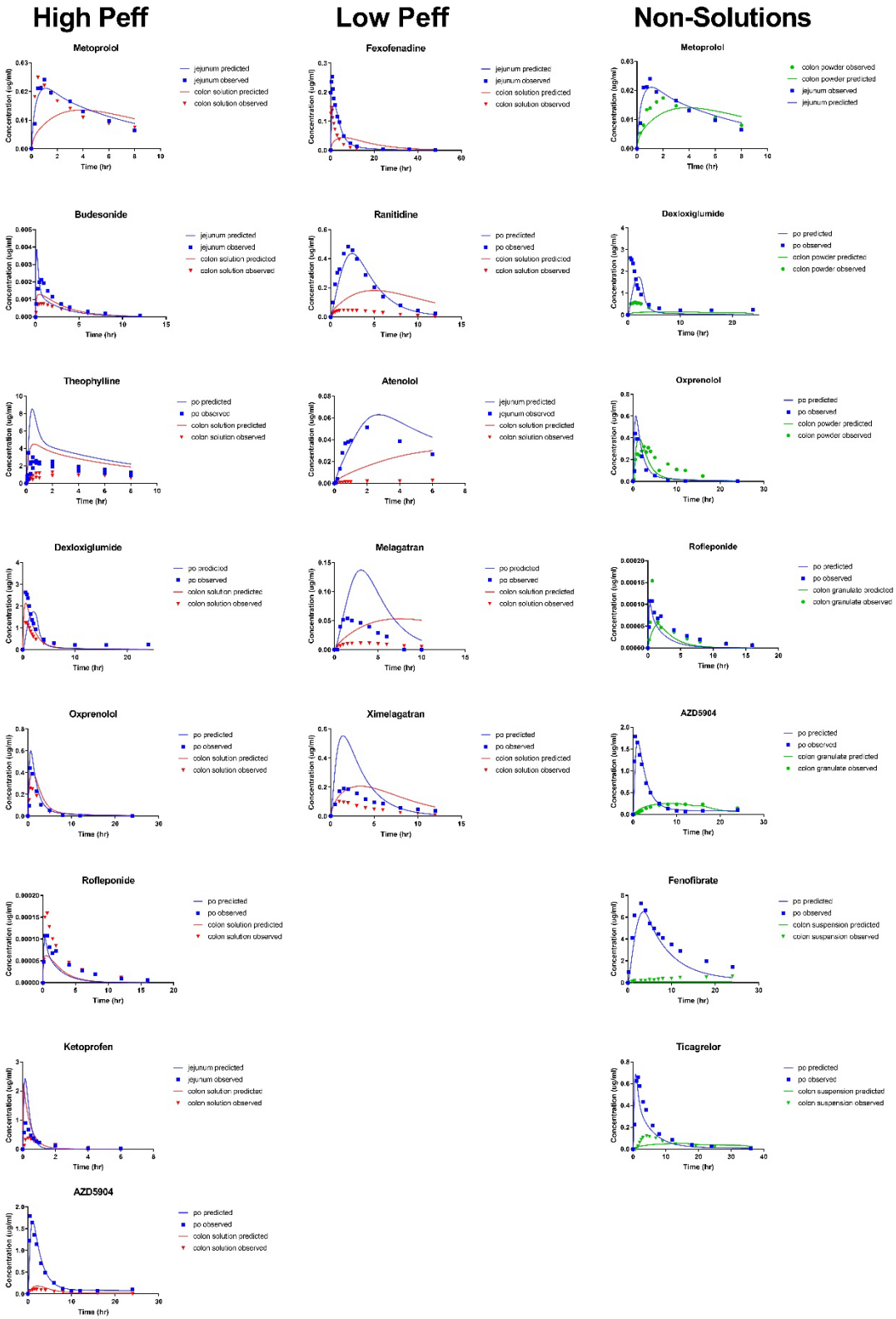
administered to the colon as solutions. The horizontal solid and dotted lines indicate a 2-fold deviation from the ideal prediction performance. The vertical dotted line corresponds to the Peff of the high permeability drug metoprolol as a divider of low and high permeability.

Figure 5. Observed and predicted mean plasma concentration-time profiles of the Orginal (dotted line) and the new GI-Sim colon Model 1 (High Volume) (solid line) for the model drugs after oral/jejunal reference and colon administration in human subjects. Observed data is depicted with symbols and predicted data with lines. Oral/jejunal reference administration, colon administration of solutions and non-solutions are colored in blue, red and green, respectively.

Figure 6. Accuracy of the Orginal (blue) and the new GI-Sim colon Model 1 (High Volume) (red) predicted $Fabs_{colon}$, $Frel_{colon}$ and AUC_{0-t} in relation to the effective permeability (Peff) used in the predictions for the model drugs administered to the colon as solutions. The horizontal solid and dotted lines indicate a 2-fold deviation from the ideal prediction performance. The vertical dotted line corresponds to the Peff of the high permeability drug metoprolol as a divider of low and high permeability.

Figures

926 Figure 1.



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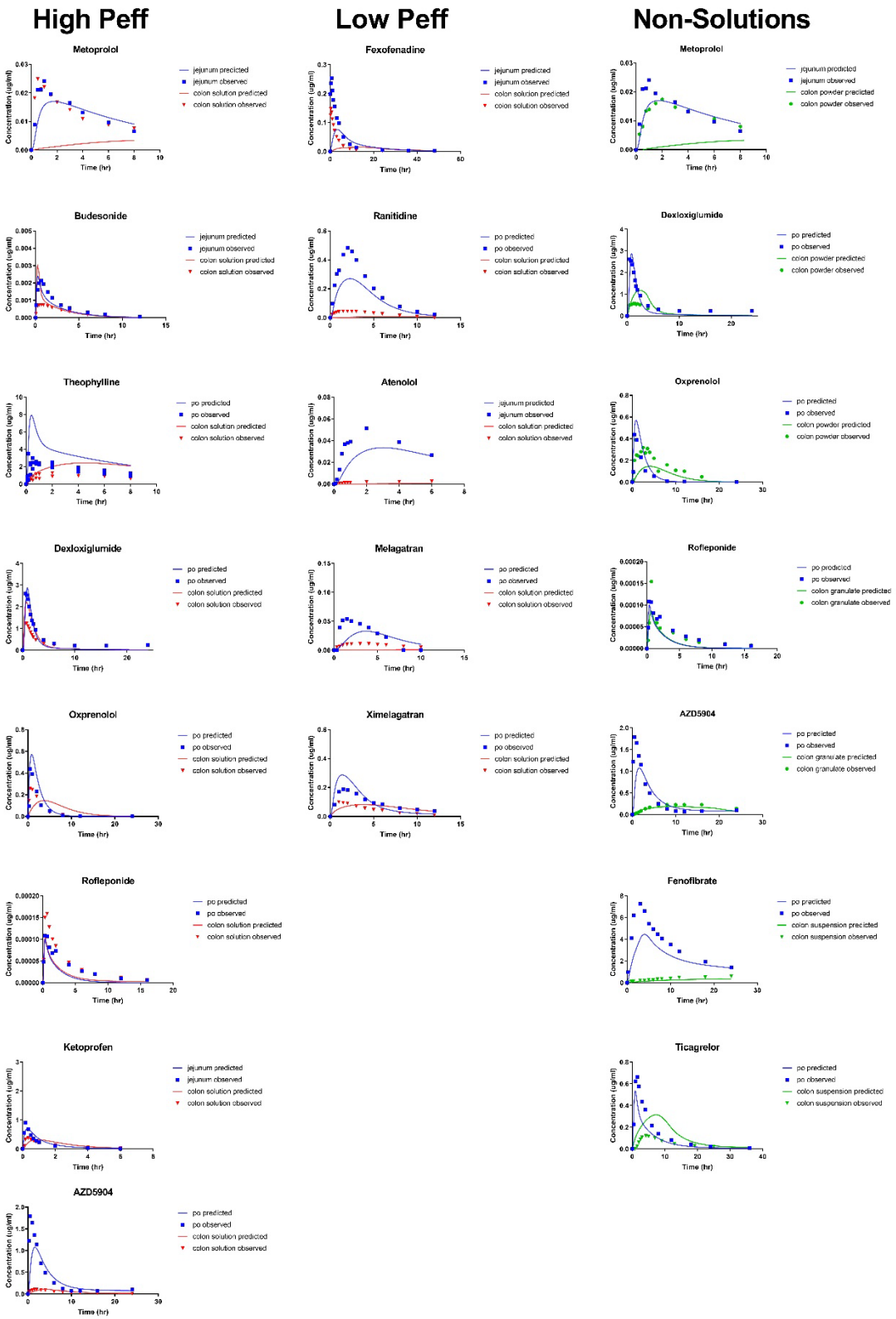
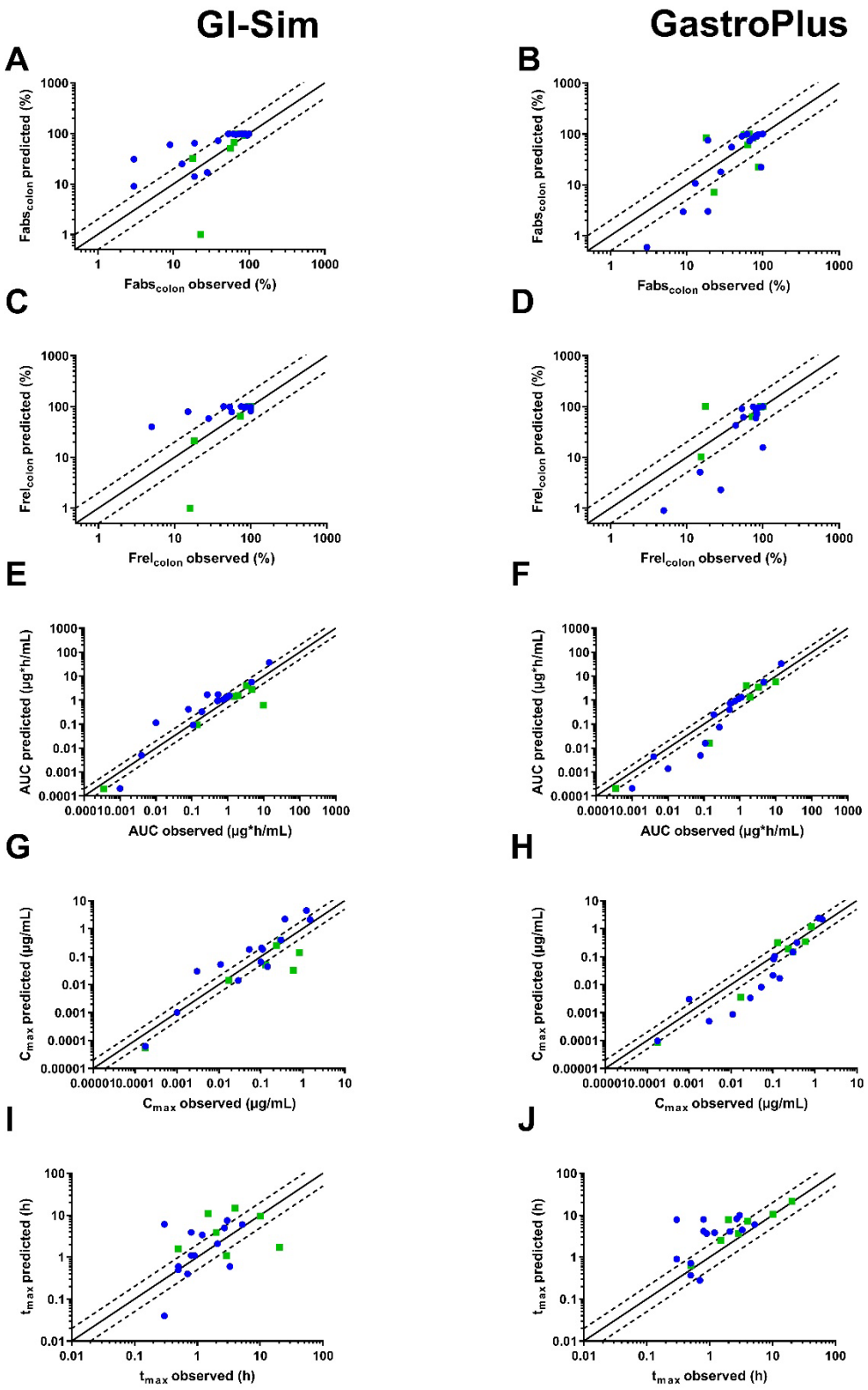
928 Figure 2.



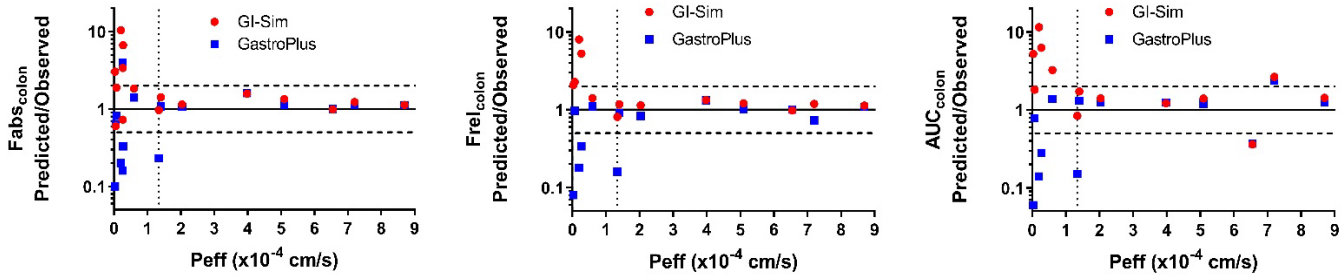
929

930 Figure 3.



931

932 Figure 4.



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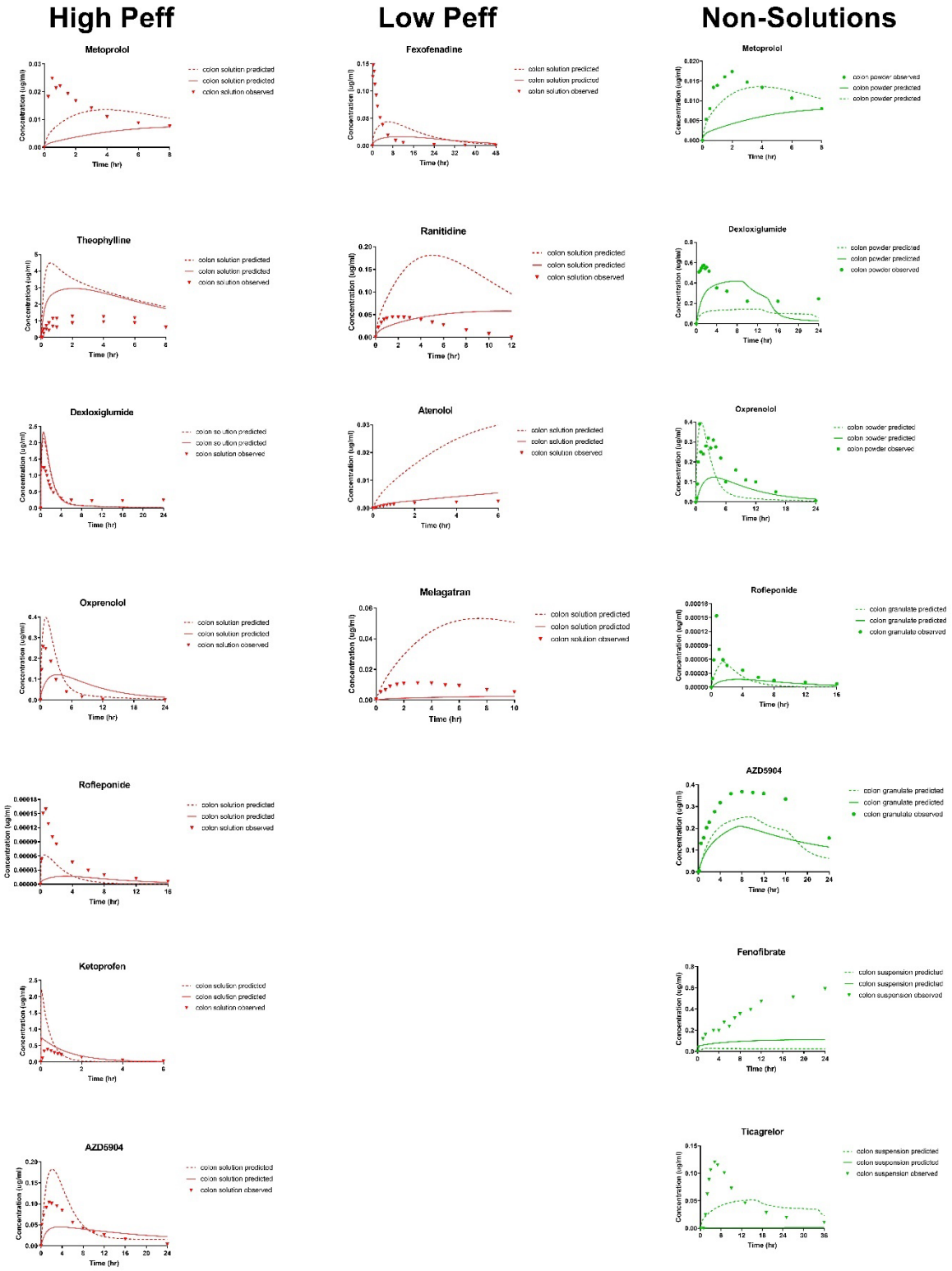
934 Figure 5.



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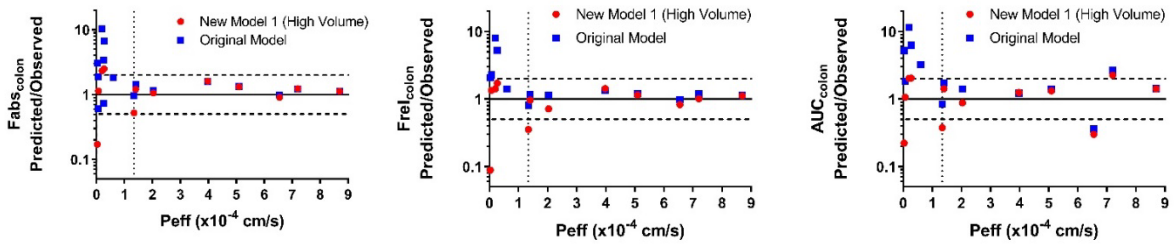
936 Figure 6.



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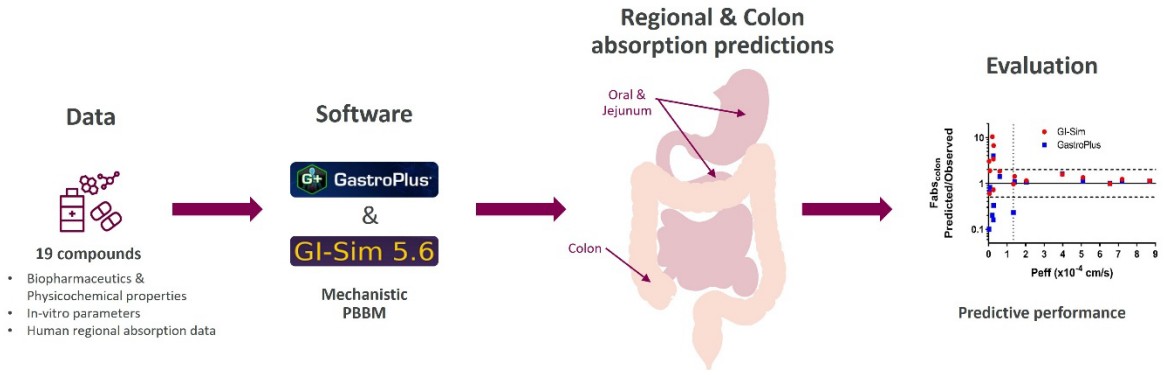
Graphical Abstract



Supporting Information

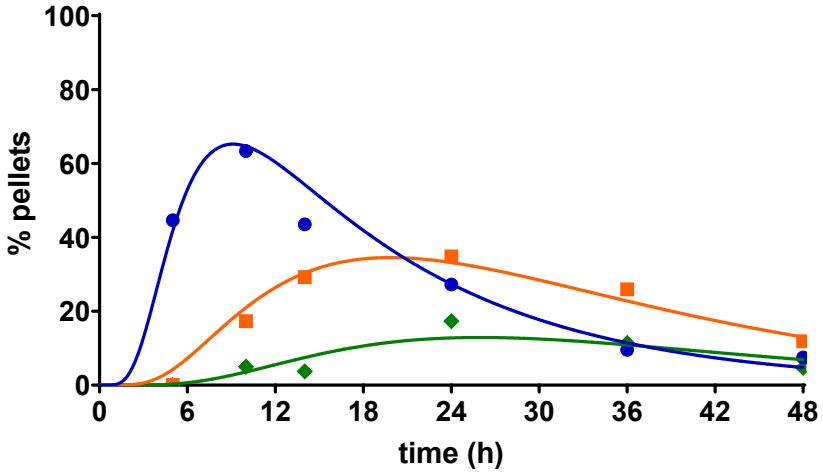


Figure 1S. The predicted and observed mean (n=8) distribution of the pellets in different regions of the colon after oral intake as reported by Abrahamsson et al. 1996[54]. Symbols represent observed values (dots: ascending colon; squares: transverse colon and diamonds: descending colon) and lines the model fitted distribution profiles.

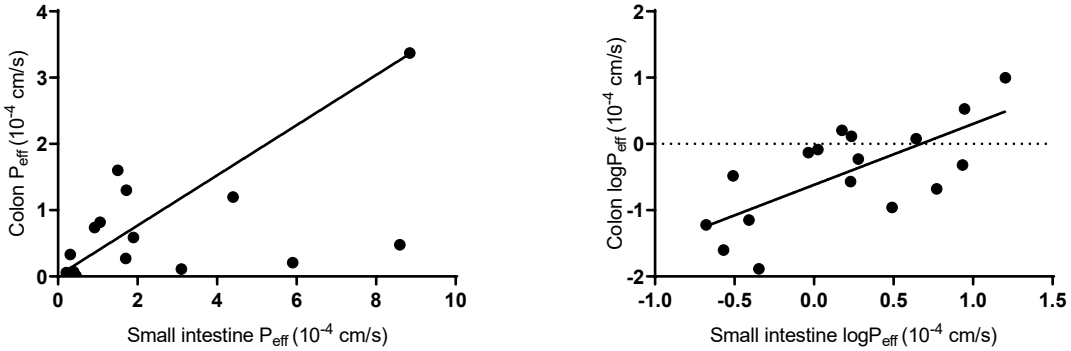


Figure 2S. Linear (a), logarithmic (b) correlation between human small intestinal and colon Peff reported by Sjögren et al 2015[41].

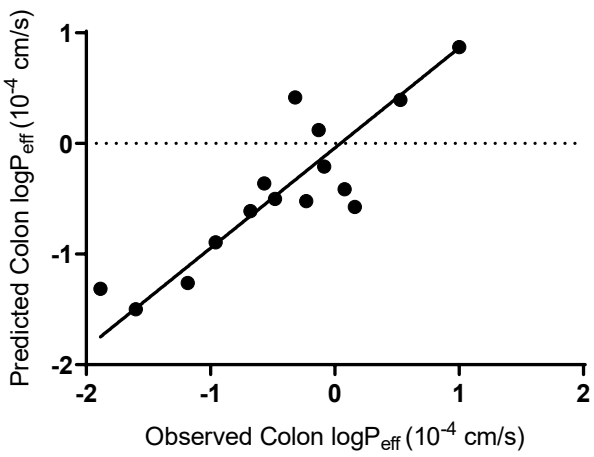


Figure 3S. Correlation between predicted and observed human colon Peff (c). The predicted colon Peff was calculated based on the estimated jejunal Peff and the molecular descriptors polar surface area (PSA), number of hydrogen bound donors (HBD) and number of rotating bonds (Rotbond). Filled circles and solid lines represents observed values and the established linear correlations, respectively.