RESEARCH ARTICLE

# Compartmental Absorption Modeling and Site of Absorption Studies to Determine Feasibility of an Extended-Release Formulation of an HIV-1 Attachment Inhibitor Phosphate Ester Prodrug

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**ABSTRACT:** BMS-663068 is a phosphonooxymethyl ester prodrug under development for the treatment of HIV/AIDS. The prodrug is designed to overcome the solubility-limited bioavailability of the active moiety, BMS-626529. BMS-663068 is not absorbed from the gastrointestinal (GI) tract and requires enzymatic conversion by alkaline phosphatase to BMS-626529 immediately before absorption. In the light of the known short *in vivo* half-life of BMS-626529, compartmental absorption modeling was used to predict the potential feasibility of extended-release (ER) delivery to achieve target $C_{max}$:$C_{min}$ ratios. To further refine the model with respect to colonic absorption, the regional absorption of BMS-626529 following delivery of BMS-663068 to upper and lower GI sites was characterized through a site of absorption study in human subjects. A refined model was subsequently applied to guide the development of ER tablet formulations. Comparisons of results from the refined model to the *in vivo* human pharmacokinetic data for three selected ER formulations demonstrate the utility of the model in predicting feasibility of ER delivery and in directing formulation development. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

**Keywords:** dissolution; colonic drug delivery; site-specific absorption; *in silico* modeling; prodrugs

## INTRODUCTION

There have been significant advances in antiretroviral drugs over the last three decades yielding over 30 drugs in use, and more in development, that are highly effective, convenient, and safe for newly diagnosed and treatment experienced patients.[1,2] The value of treatment is such that a 2006 analysis determined that treatment had yielded a total of at least 3 million years of patient survival benefit in the US alone over 20 years.[3]

However, the desire to improve on existing drugs, especially to deal with the development of resistance to existing agents, has spurred efforts to identify new approaches in interfering with the virus life cycle.[2] The prevention of viral entry into the host cells offers an attractive approach but existing approved treatments based on this principle, CCR5 antagonists (maraviroc) and fusion inhibitors (enfuvirtide), have limitations because of the need for tropism testing and a less-convenient administration route, respectively. Recently the identification of gp120 as a viable target for small molecule entry inhibitors was established[4] leading to the characterization of a series of potentially clinically useful candidates.[5,6] From this series, candidates for human clinical study

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were identified. Poor solubility led to drug delivery challenges, which for one candidate, BMS-488043, were mitigated by the creation of pharmaceutically stable amorphous dispersion of the drug in a hydrophilic polymer or by providing the crystalline drug size reduced to the nanometer range in a suitable formulation.[7,8] Prodrug approaches that might overcome solubility-limited oral absorption[9–11] have been suggested as useful drug delivery strategies in the case of HIV therapy[12] and are successfully utilized for an HIV treatment in the case of fosamprenavir.[13,14] Hence, it was rational to apply a phosphate ester prodrug approach to one of our candidates BMS-626529 and this candidate as its phosphate ester prodrug, BMS-663068 has recently been successfully explored in respect of clinical utility.[15] Structures of BMS-626529 and BMS-663068 are given in Figure 1. *In vivo* the highly soluble prodrug, BMS-663068, is converted to the highly permeable BMS-626529 by alkaline phosphatase in the gut. BMS-663068 has a very low Caco-2 permeability and because of this is very poorly absorbed.

Because of the very short apparent half-life of 1.5 h of BMS-626529[16] extended-release (ER) delivery of the prodrug, BMS-663068, was required to ensure acceptable $C_{max}$ and $C_{min}$ to minimize any plasma peak concentration adverse effects and assure viral inhibitory levels are sustained between doses. A maximum target $C_{max}$:$C_{min}$ ratio of 20 was established and the minimum dosing period was defined as 12 h. Immediate-release delivery of BMS-663068 generated unacceptable $C_{max}$:$C_{min}$ ratios in excess of 150 over the 12 h dosing period.

Extended-release delivery was initially considered a high-risk development strategy because of the very short half-life and uncertainty over the bioavailability of parent compound following prodrug delivery and around bioconversion in lower regions of gastrointestinal (GI) tract. The current paper describes how compartmental absorption modeling and site of absorption (SoA) studies were used to build a risk-based, progressive approach to enable ER formulation development.

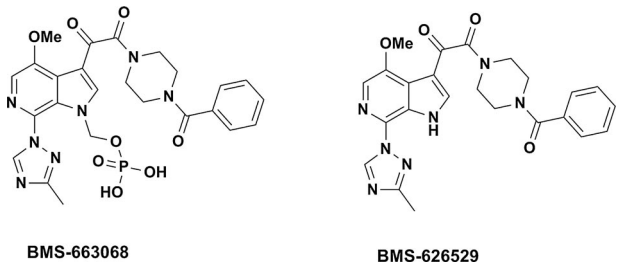


**Figure 1.** Chemical structures of BMS-663068 and BMS-626529.

## EXPERIMENTAL SECTION

### Initial Compartmental Absorption Modeling of Extended-Release Delivery

Commercially available software, GastroPlus, v 6.0 (Simulations Plus, Lancaster, California) was used to model the absorption in humans of the active parent compound BMS-626529 when the prodrug BMS-663068, as the 1:1 tromethamine salt, was administered orally for both immediate-release and theoretical ER delivery. Physicochemical parameters of BMS-663068 and BMS-626529 used in the model, for example, solubility, were experimental values measured at 25 °C. The difference between experimental temperature and physiological temperature is not expected to influence the simulation results because of the highly soluble nature of BMS-663068. The Caco-2 permeability of BMS-626529 was converted to a human jejunal permeability by reference to model compounds within the GastroPlus library. Estimates of volume of distribution and clearance were calculated using WinNonLin v5.0 (Pharsight Corporation, Mountain View, California) using data from the dosing of immediate-release capsules in human subjects.[16] The estimates were used as initial inputs for GastroPlus.

In the absence of knowledge on both the enzyme kinetics of dephosphorylation via alkaline phosphatase and enzyme expression in the lower GI tract, an empirical approach was taken towards the initial compartmental modeling. Simulations assumed that conversion of prodrug to parent was not rate-limiting and that all prodrug dissolved within each compartment of the GI model was available for absorption as the parent compound. In practice, the model employed those properties of the prodrug, BMS-663068, impacting dissolution related processes, for example, aqueous solubility of 250 mg/mL and those properties of the parent, BMS-626529, impacting permeability, distribution, metabolism, and elimination processes, for example, log P, effective permeability, volume of distribution, and clearance. As such, simulations were run using a theoretical molecule.

Within GastroPlus, the human-physiological-fasted condition and optimizable log D method for the calculation and scaling of absorption scale factor (ASF) were chosen to establish the compartmental method. The software's built-in multivariate, nonlinear optimization module was then used to adjust physiological parameters (ASF in the duodenal, jejunal, and ileal compartments) and the initial PK parameters to fit predicted plasma-concentration–time profiles to actual plasma concentration–time profiles measured in human subjects following the administration of BMS-663068 as immediate-release capsule formulation in a single ascending dose study.[16] ASF is a parameter used in GastroPlus software to

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**Table 1.** Model Parameters

| | Parameter | Value (Exploratory Modeling) | Value (Optimized Modeling) |
|---|---|---|---|
| Physical–chemical | Dose | 486.7 mg[a] | 486.7 mg[a] |
| | Log D | 1.7 at pH 6.5 | 1.7 at pH 6.5 |
| | Solubility | 250 mg/mL | 250 mg/mL |
| Physiology | | | |
| | Permeability | $1.34 \times 10^4$ cm/s | $1.34 \times 10^4$ cm/s |
| | Ascending colon compartment transit time | 13.5 h | 2.1–24.0 h[b] |
| Absorption scale factors | | | |
| | Stomach | 0.0 | 0.0 |
| | Duodenum | 25.46 | 5.901–265.6[b] |
| | Jejunum 1 | 26.87 | 6.294–283.3[b] |
| | Jejunum 2 | 30.17 | 7.046–317.2[b] |
| | Ileum 1 | 34.36 | 8.001–360.2[b] |
| | Ileum 2 | 39.73 | 9.348–420.8[b] |
| | Ileum 3 | 47.27 | 11.11–500.0[b] |
| | Cecum | 0.302 | 0.082–0.950[b] |
| | Ascending colon | 0.424 | 0.049–0.450[b] |
| Pharmacokinetic | | | |
| | Oral clearance (CL/*F*) | 0.29 L/(h kg) | 0.22—0.54 L/(h kg)[b] |
| | Apparent volume of distribution (*V*/*F*) | 0.50 L/kg | 0.15—0.57 L/kg |
| | $K_{12}$ | 0.01 L/h | 0.02—0.13 L/h |
| | $K_{21}$ | 0.06 L/h | 0.13—0.70 L/h |

[a] Dose of BMS-626529 equivalent to 600 mg BMS-663068.
[b] Range of values (minimum–maximum) employed in optimized models ($n = 8$) of individual subjects.

calculate the absorption rate coefficient, which in turn determines the predicted rate of absorption. The derived human data is assumed to reflect small intestine absorption due the very rapid *in vitro* dissolution of the drug from the capsule (>80% in 10 min) and the rapid time to maximum plasma concentration ($t_{max}$ = 45 min) observed in human subjects.

Absorption of BMS-626529 (parameterized as ASF) in the cecum and colonic compartments was unoptimized. It was predicted by the software from measured Caco-2 permeability values and the theoretical ASF value, which reflects the surface area available for absorption in these compartments.[17] Modeling parameters employed are listed in Table 1.

To investigate the influence of delivering BMS-663068 in an ER formulation, a series of drug release profiles, typical of hydrophilic matrix tablets, and releasing 100% of the content of drug over time periods in the range of 4–20 h were entered as controlled release profiles into the model. Pharmacokinetics (PK) was simulated using the model described above. Simulations were performed for single doses of 600 mg BMS-663068, the effects of accumulation from multiple doses were not considered at this stage.

## Site of Absorption Study

A human *in vivo* site of absorption (SoA) study was undertaken by Scintipharma Inc., Lexington, Kentucky utilizing InteliSite® capsules to deliver drug to specific areas of the GI tract. InteliSite® capsules were prepared containing 100 mg of BMS-663068-03 as powder mixed with mannitol labeled with 25 μCi Indium-111 chloride. Before the preparation of the capsules, the mannitol and Indium-111 chloride were dissolved in water and then dried to produce a solid material consisting of radiolabel adsorbed onto the mannitol. One hundred milligrams was selected as a suitable dose for delivery taking into account the projected dose for ER and the capacity of the InteliSite® capsule.

The study comprised eight subjects and for each subject, InteliSite® capsules were targeted to the proximal small intestine, the distal small intestine, and the ascending colon in a cross-over fashion.

Administration of the InteliSite® capsule was followed by administration of 240 mL of water, which contained a maximum of 50 μCi Tc-99m diethylene triamine pentaacetic acid (DTPA). The radioactive isotope Tc-99m DTPA provided an outline of the anatomy of the GI tract to identify specific locations when tracking the capsule. Movement of the InteliSite® capsule through the GI tract was assessed by scintigraphic imaging of the Indium-111 chloride capsule contents. When the target location within the GI tract was achieved, the capsule was activated to release its contents via a radio controlled mechanism. Monitoring via gamma scintigraphy was continued post-release to track the movement of dissolved contents. Each subject also received an immediate-release dose consisting of 100 mg BMS-663068 filled into a hard gelatin capsule, which was administered orally with 240 mL of water as a reference

For all doses, blood sampling was undertaken 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, and 24 h post-release to measure plasma concentrations of both

BMS-626529 and BMS-663068. The study protocol was approved by Chesapeake Research Review Inc.

### Analysis of BMS-626529 and BMS-663068 in Plasma

BMS-626529 and BMS-663068 were extracted from plasma samples via a solid phase extraction method. Plasma samples were spiked with internal standard and buffered with 300 μL of 0.05 M ammonium formate. Samples were mixed on a vortexer before being loaded onto an Evolute 96-well SPE plate. After washing with water and 10% methanol, the samples were eluted with two portions of 0.2 mL 0.22 M formic acid in 1:1 (v:v) methanol–acetonitrile. The eluants were evaporated to dryness and reconstituted in 100 μL of 1:9 (v:v) Mobile Phase B–Mobile Phase A, where Mobile Phase B composed of 5 mM ammonium bicarbonate with 0.05% ammonium hydroxide in 45:45:10 (v:v:v) acetonitrile–methanol–water and Mobile Phase A comprised 5 mM ammonium bicarbonate with 0.05% ammonium hydroxide in water.

Samples were analyzed by liquid chromatography–tandem mass spectrometry using a linear gradient method and a Gemini C18 ($2.0 \times 50\,mm^2$, 5 μm) column. Detection was performed via a Sciex API 3000 mass spectrometer and utilized electrospray with positive ionization. A flow rate of 300 μL/min was employed with a linear gradient pattern in which mobile phase B at 10% relative to mobile phase A was ramped to 70% from 0 to 2 min, held for 0.6 min, decreased from 70% to 10% in 0.2 min and held for 0.7 min.

### Calculation of PK Parameters

Plasma concentration versus time data were analyzed by noncompartmental methods using the program Kinetica (Thermo Scientific, Philadelphia, Pennsylvania). Actual sampling times were used for PK calculations.

The peak concentrations in plasma ($C_{max}$), the times to reach peak concentrations ($T_{max}$), and concentrations at 12 and 24 h postdose (C12 and C24, respectively) were recorded directly from experimental observations. The area under the concentration–time curve from time zero to the last quantifiable plasma concentration, $AUC_{(0-T)}$ was calculated by log- and linear-trapezoidal summations.

### Refinement of Compartmental Absorption Model Post Site of Absorption Study

To better predict absorption throughout the colonic as well as the intestinal compartments, ASF parameters in all ACAT compartments and the transit time in the colonic compartment were optimized to fit the simulated regional plasma concentration–time profiles to the actual profiles from the SoA study. In simulating delivery to the upper small intestine, the human-physiological-fasted condition and optimizable log D method for the calculation and scaling of ASF were used. The optimization scheme built-in to the GastroPlus software matched simulated data for 100 mg BMS-663068 delivered as a bolus dose to the duodenum compartment with *in vivo* data from the oral administration of the immediate-release capsule formulation. The same optimization process was used to match simulated data for 100 mg BMS-663068 delivered as a bolus to the cecum compartment to *in vivo* data from the InteliSite® capsule targeted to the ascending colon; however, to achieve satisfactory fitting, it was necessary to use the user-defined method for calculating and scaling the ASF.

The optimization process was performed separately for data from each individual subject ($n = 8$) from the SoA study resulting in the creation of eight individual models optimized for both small and large intestinal absorption. The PK parameters were also calculated for each individual subject from the immediate-release capsule data using WinNonLin (Version 5.0) and the calculated values used in the individual models. Model parameters are listed in Table 1.

To simulate ER using the refined model, selected ER profiles were applied to each individual subject model and the resulting simulations averaged to generate a mean profile.

### Human PK Study

Fifteen healthy subjects were dosed 600 mg of BMS-663068 from three ER formulations based on the same hydrophilic matrix delivery technology and having *in vitro* release rates matching those used in the optimized modeling (USP type 1 apparatus, 100 rpm, pH 6.8 phosphate buffer). A cross-over design study was employed and drug was administered in the fasted condition. Blood levels of the parent drug BMS-626529 and prodrug BMS-663068 were monitored. Sample times were 0, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 20, 24, 30, 36, and 48 h postadministration. The study protocol was approved by the New England Institutional Review Board.

## RESULTS

### Development of an Initial Compartmental Model to Simulate ER Delivery

The initial model provided good predictions for a 600 mg dose delivered as an immediate-release formulation (Fig. 2) with observed versus predicted values for $C_{max}$ and AUC having percent errors of less than 5% and 10%, respectively.

The series of typical drug release profiles for ER formulations shown in Figure 3a was applied to the model. The results presented in Figure 3b show at

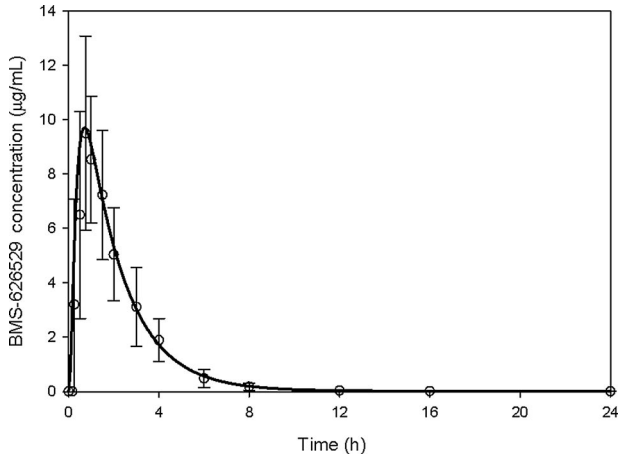


**Figure 2.** Plasma concentration–time profiles of BMS-626529 delivered as 600 mg BMS-663068 as immediate-release formulation: (o) observed mean data ± SD, (−) simulated profile from exploratory compartmental modeling.

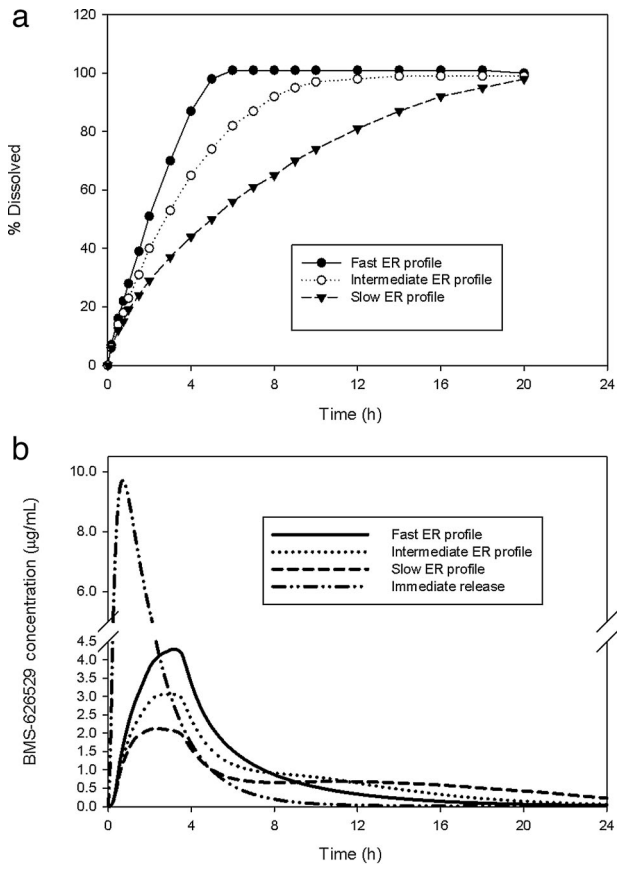


**Figure 3.** (a) Simulated drug release profiles used in initial compartmental modeling of extended release. (b) Simulated plasma concentration–time profiles of BMS-626529 from initial compartmental modeling delivered as 600 mg BMS-663068 in extended-release and immediate-release formulations.

least a two-fold reduction in $C_{max}$, and a four-fold increase in $C_{min}$ at 12 h (C12) achieved for formulations releasing drug over 5 h or longer. The predicted ratios of $C_{max}$:$C_{12}$ for all three release rates were less than 20 and were therefore within a range considered as acceptable based on available knowledge of the pharmacology and toxicology of the parent compound, BMS-626529. This finding supported the initial feasibility of an ER approach for BMS-663068.

## Site of Absorption Study

In the SoA study, 100 mg of BMS-663068 was delivered to the stomach, proximal small intestine, distal small intestine, and ascending colon. The delivery to the stomach was employed as a reference against which the absorption from other regions was compared.

Slightly higher exposure ($C_{max}$ and AUC) of BMS-626529 was achieved following delivery of BMS-663068 to the proximal small intestine compared with the stomach, however, following delivery of BMS-663068 to the distal small intestine comparable exposures compared with the stomach were recorded (Table 2). Lower variability (%CV) in exposure (BMS-626529 AUC) when BMS-663068 was released directly in the proximal small intestine (25%) and distal small intestine (21%) were evident when compared with the reference capsule formulation (30%), suggesting that gastric emptying may contribute to the variability of BMS-626529 bioavailability. Chemical stability of BMS-663068 in acid solution at 37°C has been studied previously and although degradation to BMS-626529 does occur, the slow rate of conversion does not explain the difference in exposure observed between delivery to the stomach and distal small intestine (BMS data in file).

**Table 2.** Summary of BMS-626529 Pharmacokinetic Parameters from Site of Absorption Study

| | Treatment | | | |
|---|---|---|---|---|
| Pharmacokinetic Parameter | IR | PSI | DSI | Col |
| $C_{max}$ (μg/mL), geometric mean (CV%) | 2.04 (35) | 2.32 (25) | 1.95 (15) | 0.16 (56) |
| $AUC_{(0-T)}$ (μg h/mL), geometric mean (CV%) | 3.05 (30) | 3.54 (25) | 3.35 (21) | 1.20 (47) |
| $T_{max}$ (h), median (minimum–maximum) | 0.50 (0.50–1.00) | 0.25 (0.25–0.50) | 0.50 (0.25–0.75) | 1.50 (0.75–3.00) |
| T-HALF (h), mean (SD) | 2.09 (0.41) | 1.84 (0.29) | 2.26 (0.26) | 6.26 (2.88) |

IR, immediate release; PSI, proximal small intestine; DSI, distal small intestine; Col, ascending colon.

**Table 3.** Comparison of Pharmacokinetic Parameters Derived from Site-Specific Delivery of 100 mg BMS-663068 with Parameters Derived from Immediate-Release Capsule Delivery to the Stomach

| Parameters | Treatment ($n = 8$) | Treatment Comparison | Geometric Mean (CV%) | Ratio of Adjusted Geometric Means Point Estimate (90% CI) |
|---|---|---|---|---|
| $C_{max}$ (μg/mL) | IR | – | 2.04 (35) | – |
| | PSI | PSI vs. IR | 2.32 (25) | 1.13 (0.99–1.30) |
| | DSI | DSI vs. IR | 1.95 (15) | 0.96 (0.78–1.18) |
| | Col | Col vs. IR | 0.16 (56) | 0.08 (0.05–0.13) |
| $AUC_{(0-T)}$ (μg h/mL) | IR | – | 3.06 (30) | – |
| | PSI | PSI vs. IR | 3.54 (25) | 1.16 (1.02–1.32) |
| | DSI | DSI vs. IR | 3.36 (21) | 1.10 (0.96–1.26) |
| | Col | Col vs. IR | 1.27 (43) | 0.39 (0.25–0.61) |

IR, immediate release; PSI, proximal small intestine; DSI, distal small intestine; Col, ascending colon.

Delivery to the preferred target of the ascending colon showed a reduction in AUC to approximately 40% relative to the reference and a reduction in $C_{max}$ to less than 10% of the reference (Table 3). Absorption data from the ascending colon exhibited higher variability compared with absorption from the small intestine and additionally, $T_{max}$ was extended.

BMS-663068 was not measurable in any of the treatments, indicating that BMS-626529 was rapidly formed following oral dosing of BMS-663068.

## Refinement of a Compartmental Absorption Model Using Site of Absorption Data

For each subject, two optimization processes were run using SoA data, one to fit a model to the 100 mg immediate-release capsule delivery and one to fit a model to 100 mg InteliSite® colonic delivery. Percent error in predicted versus observed $C_{max}$ and $AUC_{(0-T)}$ values ranged from less than 1% up to 27% for each parameter in the individual simulations. Percent error for mean values was calculated as less than 15% for both $C_{max}$ and $AUC_{(0-T)}$. The refined model optimized from the SoA study data also successfully predicted the *in vivo* PK of the 600 mg immediate-release capsule dosed in the single ascending dose study[16] with mean observed versus predicted values for $C_{max}$ and AUC having percent errors of 16% and 11%, respectively.

## Use of the Refined Model to Predict Extended-Release Performance

The optimized ASF values for all ACAT compartments and the optimized colonic transit values for the colonic compartments were combined into a single model for each subject. This was then used to predict the PK resulting from the three different ER *in vitro* release rates shown in Figure 4a. The resulting individual simulations generated were averaged to provide the mean profiles shown in Figure 4b.

The release rates employed here differed from those used in initial modeling. Although all of the original input rates were predicted to meet the $C_{max}$:$C_{12}$ target it was considered that beneficial changes in PK could be achieved by further slowing the profiles. The originally modeled fastest initial rate (50% release in 2 h) was therefore eliminated and a new slower profile (50% release in 7 h) was introduced.

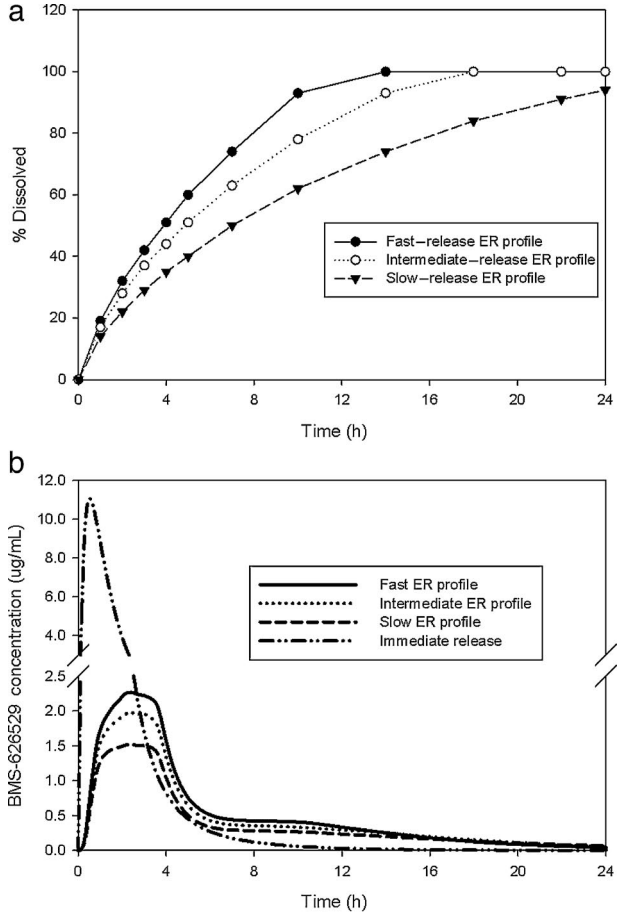


**Figure 4.** (a) Experimental drug release profiles used in refined compartmental modeling of extended release. (b) Simulated plasma concentration–time profiles of BMS-626529 from refined compartmental modeling delivered as 600 mg BMS-663068 in extended-release and immediate-release formulations.

**Table 4.** Model-Predicted (Pred.) Versus Observed (Obs.) Parameters Following Delivery of 600 mg BMS-663068 as Fast-, Intermediate-, and Slow-Releasing Extended-Release Tablets

| | | Extended-Release Tablet Formulation | | |
|---|---|---|---|---|
| Pharmacokinetic Parameter | | Fast | Intermediate | Slow |
| $C_{max}$ (μg/mL)[a] | Pred. (n = 8) | 2.20 (28) | 1.92 (28) | 1.48 (28) |
| | Obs. (n = 15) | 5.21 (29) | 4.41 (31) | 1.81 (56) |
| $AUC_{(0-T)}$ (μg h/mL)[a] | Pred. (n = 8) | 12.68 (24) | 11.33 (23) | 9.12 (22) |
| | Obs. (n = 15) | 21.41 (34) | 18.96 (29) | 9.42 (50) |
| $C_{12}$ (μg/mL)[a] | Pred. (n = 8) | 0.31 (40) | 0.27 (42) | 0.21 (42) |
| | Obs. (n = 15) | 0.13 (54) | 0.16 (84) | 0.09 (69) |

[a]Geometric mean (CV%)

The plasma concentration–time profiles simulated by the refined model predict reduced $C_{max}$:$C_{12}$ ratios for the ER formulations relative to the immediate-release formulation. The faster releasing ER profiles are predicted to produce higher $C_{max}$ and $C_{12}$ values compared with the slower releasing profiles as shown in Figure 4b.

### Tablet Evaluation in Human PK Study

Three prototype 600 mg ER tablet formulations with distinct slow, medium, and fast *in vitro* release profiles matching those used in the optimized modeling were evaluated in human volunteers. The three prototypes used the same hydrophilic matrix drug release rate controlling technology.

The observed versus model-predicted PK parameters for the three ER formulations are provided in Table 4. The rank order of the $C_{max}$ and AUC values for the three formulations was shown to be correctly predicted with the fast-releasing formulation being associated with the highest $C_{max}$ and AUC. The model was therefore shown to successfully predict relative changes in PK associated with changes in *in vitro* dissolution profile.

The refined model was shown to overpredict $C_{min}$ and underpredict $C_{max}$ and AUC for all three formulations. Observed versus predicted PK parameters were closest for the slow releasing formulation. Although the model had predicted that all three of the prototype formulations would achieve the required $C_{max}$:$C_{12}$ ratio of 20, *in vivo* data confirmed that the target ratio was only achieved for the slow formulation. The slow releasing formulation was subsequently selected for further development.

## DISCUSSION

The rationale for prodrug delivery of BMS-663068 focused upon the synthesis of a highly soluble prodrug species by the addition of a hydrophilic methylphosphate ester to the parent molecule. Although it is highly soluble, the hydrophilic prodrug is poorly permeable due to a presumed reliance upon paracellular transport across the intestinal epithelia. Therapeutic levels of the parent drug, BMS-626529, may however be achieved via a proposed mechanism involving conversion of prodrug to highly permeable parent via alkaline phosphatase at the epithelial surface and the transfer of the parent compound into the epithelia.

The stepwise risk-based development process described here is proposed as a suitable process to follow where significant risk has been assigned to the delivery strategy. The initial model employed provided for an early feasibility assessment of ER, however the challenge with the initial model is the absence of consideration of the real change in permeability as the ER formulation liberates drug in the lower regions of the GI tract. This is particularly important as the absorption is dependent upon hydrolysis of the prodrug to the parent drug by alkaline phosphatase in the GI epithelium immediately before absorption. The SoA study reported here was undertaken specifically to characterize absorption of parent compound following delivery of a prodrug to the colonic regions in human subjects.

The 40% colonic bioavailability measured relative to an orally administered immediate-release formulation is considered favorable, as a significant risk to ER delivery is expected if colonic bioavailability is 30% or less.[18]

The increased variability in absorption data and extension to $T_{max}$ observed from delivery to the ascending colon compared to the small intestine are likely to be related to a variable extent of dispersion resulting from sporadic colonic motility, variability in the dissolution of the Intelisite® capsule's contents in the colonic region,[19] and slower permeability or biotransformation of the prodrug in the colon.

The significant absorption of BMS-626529 achieved following the delivery of BMS-663068 to the ascending colon and the subsequent PK modeling confirms that adequate phosphatase activity for phosphate prodrug delivery is available in these regions. This is initially surprising as evidence of low phosphatase activity in colonic tissue taken from the rat led authors previously to suggest that phosphate

prodrugs may not be good candidates for colonic delivery.[20]

It is known that alkaline phosphatase is non-homogeneously expressed along the length of the GI tract in many vertebrate species. In the dog, mouse and adult rat it decreases axially from the upper to lower GI regions.[21] High phosphatase levels in the small intestine are consistent with the proposed physiological role of the enzyme, to assist in the absorption of dietary phosphate in the small intestine.[21] It is not clear what, if any role alkaline phosphatase could perform in the colonic regions where the primary activities are the completion of fermentation of the gut contents and water absorption.[22]

Interestingly, phosphatase activity has been shown to be a property of bacterial colonies of all major species of the family *Enterobacteriaceae*,[23] a component of the gut microflora. It is therefore possible that colonic bacteria could be contributing to the absorption of BMS-626529 from BMS-663068 delivered to the ascending colon. Although the actual source of phosphatase activity requires further investigation, this study has demonstrated the utility of SoA studies for molecules such as produgs whose colonic absorption is difficult to predict based on simple physicochemical properties and *in vitro* biopharmaceutical measurements.

In reviewing multiple prodrugs, Heimbach et al.[24] and Brouwers et al.[14] propose that the maintenance of parent drug in a supersaturated state post bioconversion and pretransfer into epithelial cell membranes is important to the successful oral delivery of the parent compound. The SoA data confirms that even in the colonic regions in which mixing is sporadic, the availability of water is lower and the risk of parent drug precipitation is correspondingly higher, delivery of 100 mg of BMS-663068 enables the absorption of a favorable proportion of BMS-626529. The solubilizing properties of the colonic contents as described by Vertzoni et al.[25] may assist in maintaining the BMS-626529 liberated from the prodrug in solution thereby facilitating absorption of this chemical species.

An under prediction of $C_{max}$ and AUC particularly for the intermediate- and fast-releasing formulation prototypes was apparent when simulated data were compared with *in vivo* data. A greater than dose-proportional absorption of BMS-626529 has been observed for BMS-663068 delivered as immediate-release formulation at doses of 20 mg or greater.[16] This non-linearity is consistent with saturation of gut wall metabolism or P-glycoprotein (P-gp) efflux and could explain the underprediction of $C_{max}$ and AUC if the luminal concentrations of dissolved drug in the intestinal compartments of the Gastroplus model differ between the 100 mg immediate-release capsule used to establish the model and the 600 mg ER tablet used in ER simulations. A nonlinear dose–AUC relationship impacting the early phase of absorption from ER tablets is expected to particularly influence $C_{max}$ and AUC predictions as has been shown by Watson et al.[26] for another drug which is a Cytochrome P450 (CYP3A4) substrate and a P-gp substrate. In the present study, analysis of dissolved drug concentration in the small intestinal (duodenal and jejunal) compartments using the Gastroplus model showed the concentration of dissolved drug to be approximately two-fold higher from a 100 mg immediate-release capsule given orally compared to a 600 mg ER tablet. Analysis in the large intestine compartments showed similar drug concentrations from an InteliSite® capsule released in the cecum compartment compared with an orally administered 600 mg ER tablet. Dissolved drug concentrations calculated subsequently in the ascending colon were two-fold higher from the ER tablet compared with the same InteliSite® capsule. From single ascending dose data, the maximum of a two-fold difference in compartmental concentration from the level associated with 100 mg immediate-release dosing is not consistent with the simulation error in $C_{max}$ and AUC described for fast and intermediate formulations in Table 4.

The underprediction of $C_{max}$ and AUC for the fast and intermediate formulations could however be related to the *in vitro* test methodology used. The affected formulations contain a lower viscosity polymer grade compared to the slow formulation resulting in a system for which erosion, subject to shear force, plays a greater role in drug release.[27] These dosage forms may require higher agitation rates in *in vitro* dissolution tests to reflect *in vivo* release than dosage forms less sensitive to erosive forces.[28] This raises the possibility of slower *in vitro* release compared to *in vivo* release and the potential for underprediction of $C_{max}$ and AUC particularly for the fast and intermediate formulations. Increasing the release rate for each of the formulations in the Gastroplus model leads to much improved correlation between the simulated and observed PK profiles. This finding suggests that poor *in vitro* to *in vivo* correlation of the dissolution methodology used is the major contributor to the less than optimal predictions rather than compartmental concentration differences.

## CONCLUSIONS

In this study, the use of a compartmental absorption model using physicochemical information fitted to immediate-release PK data was used to provide an initial assessment of ER feasibility of a phosphate ester prodrug assuming no limitation of colonic absorption from alkaline phosphatase levels in the region.

A SoA study subsequently determined approximately 40% bioavailability of prodrug delivered to the colon relative to oral administration, confirming the

potential for use of phosphate ester prodrugs in ER delivery.

When SoA data was subsequently incorporated into a refined compartmental model, the model successfully predicted the rank order of $C_{max}$ and AUC of three distinct ER tablet prototypes. The refined model tended to underpredict $C_{max}$ and AUC especially for the faster releasing prototypes. Further optimization of *in vitro* release methodology and the GastroPlus model to incorporate efflux and metabolism effects would likely be required to achieve improved absolute predictions of *in vivo* performance for all three prototypes. Although the modeling approach requires further development to optimize fit for all prototypes and develop an *in vitro–in vivo* correlation, the compartmental modeling approach together with the SoA data has furthered understanding of and developed the potential for ER delivery of BMS-663068/BMS-626529. Furthermore, it has assisted in a risk-based development process to select and progress a formulation of the compound into clinical studies.

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