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Research Article

Theme: Leveraging BCS Classification and in-silico Modeling for Product Development
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Use of Preclinical Dog Studies and Absorption Modeling to Facilitate Late Stage Formulation Bridging for a BCS II Drug Candidate

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Abstract. Formulation changes are common during drug development either due to clinical or manufacturing considerations. These changes especially at later stages of drug development oftentimes raise questions on the potential impact of a new formulation on bioavailability. In this work, the preclinical assessment of formulation bridging risk for a Biopharmaceutics Classification System II development compound is presented. Early clinical studies were conducted using a liquid-filled capsule (LFC). To assess the feasibility of a conventional solid dosage form, an initial analysis was conducted using absorption modeling which indicated conventional formulation of micronized active pharmaceutical ingredient (API) could be a viable option. Subsequently, test formulations were prepared and tested *in vivo* in dogs. The solid formulations were able to match exposures of the LFC capsule in the dog model; in addition, a sensitivity to API PSD was observed in line with the modeling predictions. When tested in the clinic, the conventional solid formulation resulted in exposures of approximately 25% lower compared to the LFC on an equivalent dose basis; however, bridging with a small dose adjustment would be feasible. The outcome of the clinical study was better predicted by the modeling approach while the dog model appeared to somewhat overestimate absorption. Through the use of preclinical tools and modeling and simulation, a risk assessment around formulation bridging can be conducted and inform formulation decisions or subsequent clinical study designs.

KEY WORDS: absorption modeling; formulation bridging; GastroPlus; preclinical screening; relative bioavailability.

INTRODUCTION

In an effort to quickly advance drug candidates for clinical evaluation, many pharmaceutical companies have adopted fit-for-purpose formulation strategies in early clinical development where compounds are incorporated in "platform" formulations with relatively limited development work prior to first-in-human (FIH) studies. The goal of these formulations is largely to provide adequate bioavailability in the early studies, sufficient to allow for the characterization of the pharmacokinetics and safety profile of the drug candidate. Selection of these early stage formulations is oftentimes facilitated by the use of "decision trees." Different decision trees have been published in the literature; some are based on resource considerations (1), whereas others focus more on linking the formulation selection to specific compound properties. In many of these later cases, the Biopharmaceutics Classification System (BCS) (2) or variations of that are used to guide formulation selection (3–5); however, more complex approaches have also been suggested (6).

As compounds progress through development, additional formulation considerations need to be taken into account. While maintaining bioavailability remains a paramount formulation deliverable, late stage formulation selection considers many additional factors such as long-term physical and chemical stability, processing and commercialization robustness, and costs. It is also possible that clinical factors (*e.g.*, change in estimated therapeutic dose, identification of specific drug interactions) may also trigger need for a new formulation. Thus, it is not uncommon for formulation switches to take place throughout development.

Any formulation switch usually raises questions on the potential impact on bioavailability. While an understanding of the formulation effect on bioavailability is important throughout development, earlier in development, changes in bioavailability may not be a major development concern as the detailed characterization of the compound safety and efficacy may not be fully complete. However, post-generation of more extensive safety or efficacy data, it is highly desirable, and depending on the stage even required from a regulatory perspective, that formulation changes are judged based on bioequivalence criteria.

Formulation changes are routinely evaluated in the clinic in the form of relative bioavailability or bioequivalence studies. However, such studies can be time and resource consuming; thus,

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it would appear very beneficial if project teams can obtain reasonable assurance of formulation equivalence preclinically, prior to commitment to the clinical studies. Preclinical evaluation of formulations historically has entailed the use of dissolution methods and preclinical animal models. In the more recent years, the developments in the area of biorelevant dissolution as well as physiologically based pharmacokinetic (PBPK) modeling have provided formulators and biopharmaceutics scientists additional tools to project clinical risks for developed formulations. Successful application of such models to formulation questions such as effect of active pharmaceutical ingredient (API) properties (7), prediction of food effect (8,9), modified-release formulations (10), or to guide quality by design implementation (11) has been reported in the literature.

In this manuscript, we present a case study on the preclinical evaluation, using absorption modeling and animal studies, of late stage formulation bridging for a BCS II drug development candidate. The compound studied was a high lipophilicity (logP 6.5) molecule with an aqueous solubility of less than 1 μg/mL over the intestinal pH range. Due to the poor solubility of the compound and based on the projected FIH study doses, solubilization technologies were screened early on, and eventually, a liquid-filled capsule (LFC) formulation containing a solution of the API was utilized. However post-phase I studies, efficacious dose estimates were significantly revised suggesting that lower doses could be sufficient. Thus, the need for use of a solubilization technology becomes unclear and simpler formulations may become an option. We detail here the preclinical screening of simple conventional formulations to replace the liquid-filled capsule using *in vitro*, *in vivo*, and *in silico* approaches. The accuracy of the preclinical models is discussed relative to the outcome of a subsequent relative bioavailability study in the clinic.

MATERIALS AND METHODS

Compound Physicochemical Properties

MK-0594 is a weak base with a pK_a of 2.4. It is highly lipophilic, with a logP of 6.5. Thus, the compound exhibits poor solubility across the physiological pH range. Solubility was measured at ~2 μg/mL at pH 1.3 and <0.1 μg/mL at pH 2–8 aqueous buffers. For the purpose of the discussion in this manuscript and as input for simulations, the compound can be considered neutral given the anticipated stomach pH in humans (~1.8) and dogs (>3). Compound solubility significantly increases in the presence of surfactant in line with the compound's logP value. Solubility in biorelevant media was measured at 6 μg/mL in fasted state simulated intestinal fluid (FaSSIF) and 79 μg/mL in fed state simulated intestinal fluid (FeSSIF).

Due to poor mass balance observed in an early Caco-2 cell study, likely due to compound high lipophilicity and nonspecific binding, permeability was estimated *via in situ* rat intestinal perfusion. The rat intestinal perfusion model is widely regarded as one of the most accurate preclinical tools for the prediction of human permeability of passively absorbed compounds and is accepted for formal BCS classification. The perfusion study followed a protocol previously described in the literature (12). The model was previously qualified internally (Merck data in file) against compounds of known human permeability or fraction absorbed as listed in the FDA BCS guidance. All animals were housed in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited facility in accordance with USDA guidelines. The study was conducted under a protocol approved by the West Point Institutional Animal Care and Use Committee (IACUC). The permeability (P_{eff}) of MK-0594 was estimated at $4.3\pm0.8\times10^{-5}$ cm/s. In the same setup, the P_{eff} of the high permeability reference compound metoprolol was measured at $3.7\pm0.3\times10^{-5}$ cm/s. Therefore, the compound was classified preclinically as a BCS Class II compound. The BCS classification of the compound was in line with the moderate–high bioavailability seen in preclinical species (72–85% in rats at 1–5 mg/kg doses and 54–68% in dogs at 1–16 mg/kg doses, dosed in an Imwitor/Tween 80 50:50 vehicle) and with the observations in the clinical ADME study where the majority of radioactivity was recovered in the form of metabolites.

Formulations

For FIH and early clinical studies, a liquid-filled capsule formulation was utilized. The compound was solubilized in Imwitor 742 with 0.1% BHA vehicle and was filled in gelatin capsules. The Imwitor 742 formulation was shown preclinically in dogs at a dose of 4 mg/kg to result in a significant (approximately fourfold) exposure increase compared to crystalline API solid formulations.

The formulations included in the preclinical evaluation discussed in this report are shown in Table I. Formulations were prepared either *via* roller compaction (RC), fluid bed granulation (FBG) or wet granulation (WG). Sodium docecyl sulfate was used at levels between 1% and 5% as the surfactant to facilitate wetting and improve the dissolution rate. Micronized API was used for all formulations; the effect of particle size within the range of ~7 and ~22 μm was evaluated. Formulations in dissolution and dog studies were dosed as 1- or 3-mg tablets or capsules.

While the focus of the evaluation was on conventional formulations, a dried nanosuspension was also evaluated to assess the potential impact of further reduction of API particle

Table I. Formulation Composition for MK-0594 Solid Formulations

Formulation	Process	% drug loading	% SLS	API PSD (μm)
Formulation 1 (RC-1)	RC	5	5	6.7±4.2
Formulation 2[a] (RC-2)	RC	5	5	6.7±4.2
Formulation 3 (RC-3)	RC	5	5	22.2±17.9
Formulation 4 (FBG)	FBG	5	2.5	6.7±4.2
Formulation 5 (WG)	WG	0.5	1	6.7±4.2

RC roller compaction, *FBG* fluid bed granulation, *WG* wet granulation, *API PSD* active pharmaceutical ingredient particle size distribution, *SLS* sodium luaryl sulfate

[a] Difference between RC-1 and RC-2 is in the ratio of inactive ingredients

size in exposures. The nanosuspension manufactured by milling had a mean particle size of 200 nm and was dosed as a capsule.

Animal Study Procedures and Sample Analysis

Fasted male beagle dogs (Marshall Farms) were used for the studies described herein. All animals were housed in an AAALAC-accredited facility in accordance with the USDA guidelines. The Guide and Animal Welfare regulations were followed in the conduct of the animal studies. Veterinary care was given to any animals requiring medical attention. Formulation dosing studies were conducted under a protocol approved by the West Point IACUC. In the morning of the study, formulations were dosed orally to three or six animals depending on the specific study protocol followed by 3.5 mL/kg water administered *via* oral gavage. Animals had been fasted for 16 h prior to dosing and food was returned at 4 h after dosing. Blood was drawn from a 21-g catheter placed into the cephalic vein at predose and 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h after dosing. The plasma was separated by centrifugation (15 min at 2,500×*g*) and kept frozen at −70°C until analysis.

A sensitive analytical method using liquid chromatography/electrospray ionization tandem mass spectrometry for the quantitation of MK-0594 in dog plasma was developed and validated. The method employed a protein precipitation procedure using acetonitrile to isolate MK-0594 from the biological matrix. An analog of MK-0594 was used as the internal standard.

Area under the curve (AUC_{0-24h}), observed maximum plasma concentration (C_{max}), and time of C_{max} (T_{max}) were calculated using the linear trapezoidal, noncompartmental model in WinNonLin v5.2. Plasma concentration values below LOQ were set at zero for pharmacokinetic parameter calculation purposes.

Oral Absorption Modeling

GastroPlus v8.0 software was used for all simulations reported in this manuscript. Initial simulations during product development were conducted with an earlier version of the software; the outcome of the models from different versions of the software was qualitatively the same. Input physicochemical properties are shown in Table II.

The underlying principles of the model have been detailed in the literature (13). The model allows for simulation of drug absorption from the GI tract based on the compound physicochemical properties (such as solubility and permeability) and/or formulation information (*e.g.*, API particle size) in conjunction with relevant physiology parameters of the GI tract, such as instestinal surface area, pH, and water volumes for different intestinal regions. Different physiological absorption models are available in the software for different species. The default physiological Opt-logD SA/V v6.1 model was used for clinical simulations; the default cecum and colonic absorption scale factor (ASF) value (a scaling parameter to convert permeability to absorption rate) was modified to match that of the ileum. In our experience, default colonic ASF values tend to over predict the absorption of poorly soluble compounds; therefore, the assumption was made that permeation rate will be similar in lower small intestine and large intestine. Stomach pH was set at 1.8. For simulations on dog data, the theoretical SA/V model was used. For both human and dog simulations, *in vivo* solubilization as a function of bile salt concentration was implemented using the relevant function in the software based on solubility in biorelevant media. This allows to account for gradual lower solubility in the lower small intestine due to reabsorption of bile salts. The estimated duodenal solubility for the simulations was 5.6 μg/mL for humans and 31 μg/mL for dogs. For projection of the dog solubility, both FaSSIF and FeSSIF data were taken into account since they bracket the expected dog bile salt concentrations. Permeability was projected at 2.03×10^{-4} cm/s based on the rat perfusion data. Same permeability value was used for human and dog simulations; in conjunction with the ASF values, this results in comparable absorption rates between species which reflects our experience with the dog model.

Table II. Physicochemical Parameters Used for GastroPlus Modeling

Parameter	Value
Dosage form	IR solution (for LFC) or IR tablet (for solid)
LogP	6.5
Permeability	2.03×10^{-4} cm/s (estimated from rat intestinal perfusion data)
Solubility at pH 7	0.5 μg/mL
Biorelevant solubility	FaSSIF, 6 μg/mL; FeSSIF, 79 μg/mL
In vivo solubilization ratio	Human 204,000 (calculated in software based on buffer and FaSSIF) Dog 676,000 (calculated in software based on FaSSIF and FeSSIF)
Precipitation time	10,000 s (only relevant to LFC)
Diffusion coefficient	0.552×10^{-6} cm/s (constant)
Density	1.2 g/mL (default value)
Particle size	From Table I

IR immediate release, *LFC* liquid-filled capsule, *FaSSIF* fasted state simulated intestinal fluid, *FeSSIF* fed state simulated intestinal fluid

For the simulations of the solid formulations in the clinic, human PK parameters were obtained by fitting the oral data of the LFC formulation since intravenous data were not available. Thus, it was assumed that the observed exposures for the LFC formulation represented maximal oral bioavailability. The assumption is supported by the dose linear exposures (area under curve (AUC) and C_{max}) observed within the tested dose range (1–200 mg) and the lack of food effect at 100 mg dose. To reflect the high bioavailability, liquid-filled capsule formulations were simulated as a nonprecipitating solution. The simulation of the liquid-filled capsule only serves to assess whether an acceptable permeability estimate is used.

Simulations for solid dosage forms were based on the input of particle size and solubility. The default (Johnson) dissolution model was used and dissolution was modeled based on the API particle size distribution (no distribution assumed for the parameter sensitivity analysis simulation). The nanoparticle effect was turned off; this has no impact on simulations of dosage forms of interest since micronized API was used.

RESULTS

Oral Absorption Modeling

During formulation development, simulations were conducted to assess the potential of a conventional solid

formulation to achieve exposures comparable to that of the phase I LFC formulation. The sensitivity analysis function of the software allows the user to conduct simulations by varying parameters of interest separately or simultaneously and calculate the effect on absorption. A two-parameter sensitivity analysis was utilized. The model was run across the likely dose range of interest. In addition, since for BCS II compounds dissolution rates are often important for *in vivo* bioavailability, the impact of particle size was explored in the model as an early surrogate for the dissolution kinetics. Figure 1 depicts the outcome of this simulation. The results indicate that fast dissolving formulations (small API particles, <10 μm) and at lower doses, the conventional dosage form could provide exposures comparable to the LFC. This modeling provided initial direction for the development of solid dosage forms that would advance to screening in dogs.

Given that the formulation development discussed in this manuscript occurred post-FIH studies, any absorption modeling conducted at that time focused on clinical simulations. If simulations were conducted prior to FIH, one may have chosen to validate the behavior of the absorption model against preclinical (*e.g.*, dog) data. However, to facilitate interpretation of the dog data presented in this manuscript, an additional simulation of the fraction absorbed as a function of particle size in dogs is presented in Fig. 2. Assuming particle size controlled dissolution, a reduction in absorption as a function of particle size is predicted also in the dogs at the dose of 3 mg (~0.3 mg/kg) that was used for the animal studies. The predicted absorption is somewhat higher and the response less steep compared to the clinical simulations at the corresponding dose (when adjusted for body weight), as expected due to projected higher solubility of the compound in the dog lumen.

Although a nanosuspension was included in the dog studies as an additional reference and it is included in the output of the sensitivity analysis for the dog data, that simulation was not pursued during formulation development. It has been reported that performance of nanosuspension formulations is not readily captured by currently available PBPK models only accounting for particle size effect on dissolution (7). While some success has been reported with detailed dissolution data (14) or with changes in the effective intestinal permeability (15), a projection of nanosuspension behavior was beyond the scope of the intended use of the models.

For the purposes of this manuscript, a retrospective assessment of the ability of the absorption model to correctly "predict" the outcome of the clinical relative bioavailability study was also carried out. Figure 3 depicts the simulated *vs.* observed data for LFC (4 mg) and the two developed solid formulations (small API PSD, 5 and 6 mg). The model appears to do a reasonable job in projecting exposure of the formulation based on the assumption of API-based dissolution. Average C_{max} is generally accurately captured; some overprediction of exposures post-T_{max} is observed, suggesting that the colonic absorption projected may be higher than observed; a simulation without colonic absorption (also shown in Fig. 2—inset; only 5 mg simulation shown; same observation holds for 6-mg simulations) does appear to describe the declining phase of the plasma concentration better.

Pharmacokinetic Evaluation of Test Formulations in Beagle Dogs

During FIH formulation screening, data for the liquid-filled capsule in dogs were already generated at a dose of 4 mg/kg. For late stage formulation screening, an initial study tested the exposure of a conventional formulation against the same LFC capsule at a dose of 1 mg (0.1 mg/kg). These two

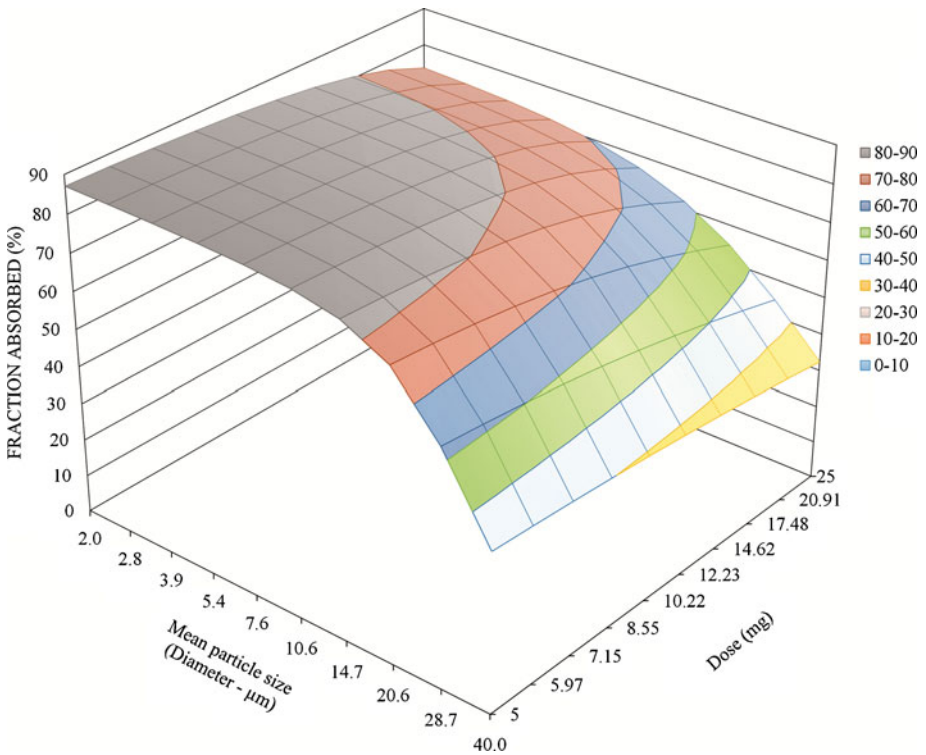

Fig. 1. Parameter sensitivity analysis for fraction absorbed in humans for BCS class II MK-0594 as a function of dose and average API particle size (particle diameter-monodispersed API assumed)

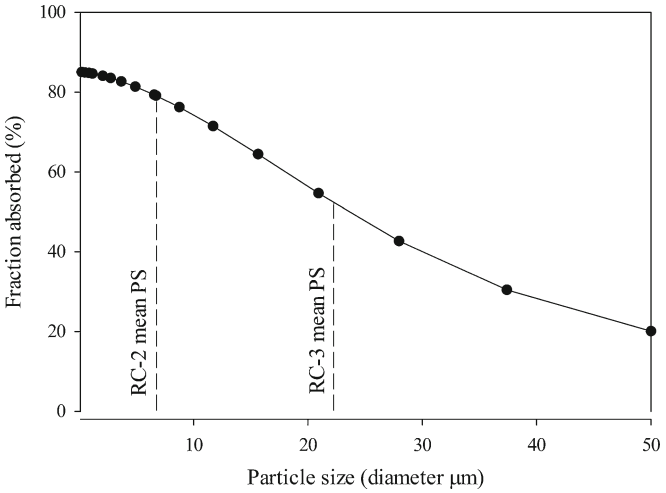

Fig. 2. Parameter sensitivity analysis for fraction absorbed in dogs for a 3-mg dose of BCS class II MK-0594 as a function average API particle size (particle diameter-monodispersed API assumed)

studies suggested that exposures for LFC were linear with dose, and absorption from the formulation was not compromised with increasing doses. Metabolic saturation was also not a concern based on data from earlier bioavailability studies across an even wider range. For the screening of final formulation candidates, we concentrated on the pharmacokinetic assessment of solid-only formulations. Studies were conducted in such a fashion to maximize available information within the short timelines allowed for formulation development. While a direct comparison to the liquid-filled capsule in the same dose which would perhaps, retrospectively, be considered ideal was not attempted, dose normalized exposures for the LFC were used for comparison.

Figure 4 shows the observed plasma concentration *vs.* time profiles for the conventional solid formulations along with the nanosuspension at a dose of 3 mg (0.3 mg/kg) in dogs. The corresponding pharmacokinetic parameters are shown in Table III while

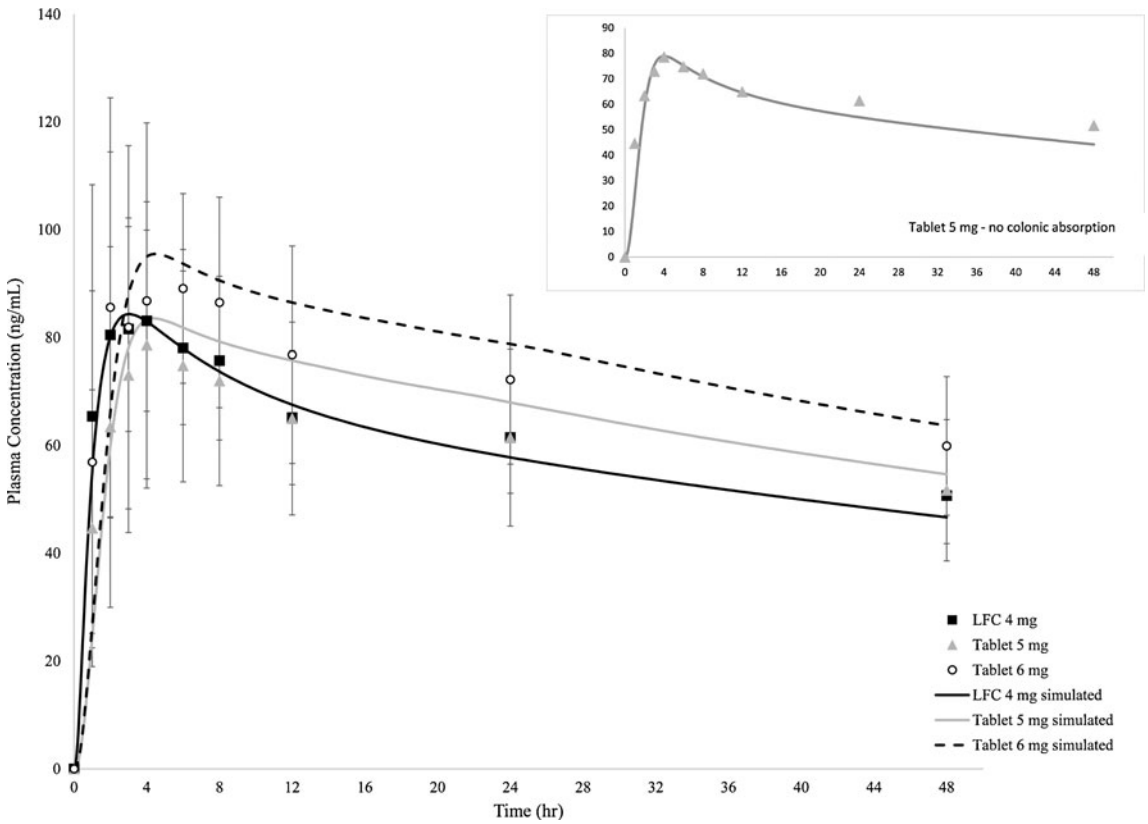

Fig. 3. Observed (mean ± SD) *vs.* absorption model-predicted plasma concentration *vs.* time for MK-0594 in humans following a 4-mg dose LFC or a 5- or 6-mg dose of a solid formulation. In the *inset*, the 5-mg simulation without colonic absorption is shown

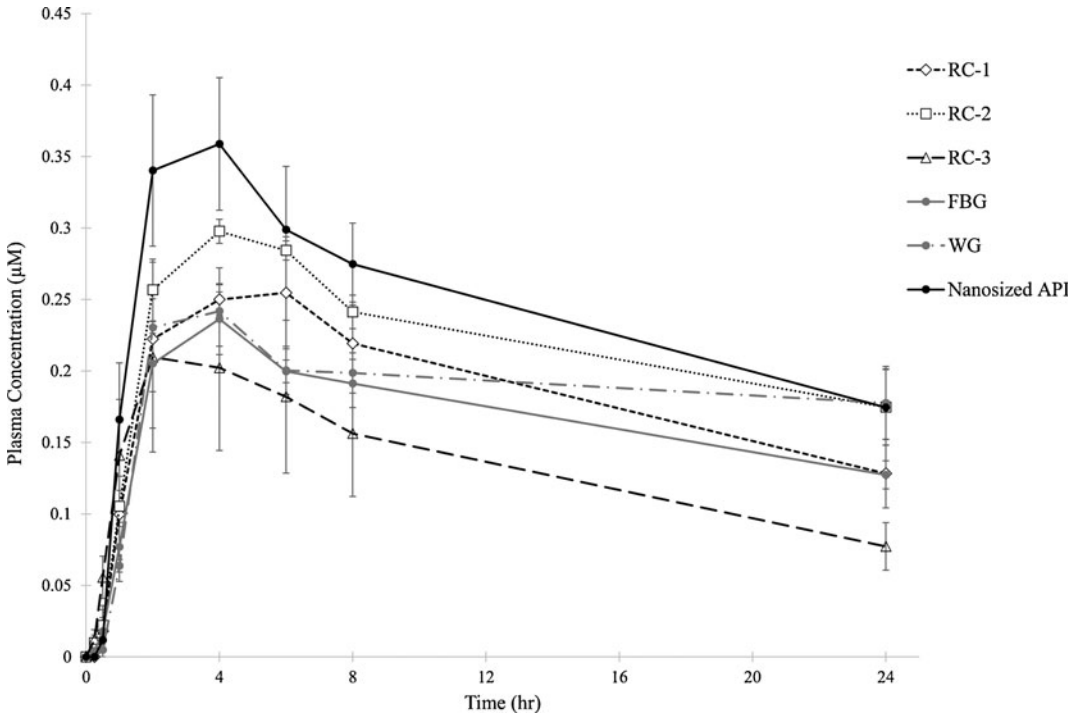

Fig. 4. Plasma concentration *vs.* time profile for MK-0594 following dosing of solid formulations in male beagle dogs at a dose of 3 mg (mean ± SE)

Table IV shows the relative exposure (AUC/milligrams per kilogram) for all formulations relative to the LFC across doses. The comparison focuses on AUC as total exposure was considered the most critical pharmacokinetic parameter for efficacy. All conventional formulations with the ~7-μm API regardless of process used resulted in acceptable exposures. Within the RC formulations, the two formulations with the small API resulted in comparable exposures. Formulation 3 which contained larger particle size API (mean particle size 22 *vs.* ~7 μm) resulted in somewhat reduced exposure based on mean data (mean AUC of 62% compared to formulation 2). While admittedly such preclinical studies are not powered to allow for conclusive formulation comparisons within such small exposure ranges (*e.g.*, the difference between RC2 and RC3 cannot be shown to be statistically significant; $p=0.10$ two-sided *t* test), the larger API formulation also exhibited the higher variability within the animals tested (CV% 45% compared to 10–15% for the rest, approximately threefold AUC differences within the three dogs tested with RC3), which was another sign of potential concern with the larger API. Moving from RC to either WG or FBG did not affect the overall formulation performance.

It is worth noting that the dried nanoparticles in capsule resulted in modestly higher exposure levels (both AUC and C_{max}; $p=0.03$ *vs.* RC20 that was tested in same three dogs; paired two-sided *t* test). While this approach was not pursued further given the acceptable performance of simpler formulation options, the data served as a further indication of the potential sensitivity of exposure to API particle size.

DISCUSSION

MK-0594 is a highly lipophilic, poorly soluble drug candidate, representative of a significant portion of the currently developed drug candidate compounds across the industry. The low solubility represents a significant challenge for biopharmaceutics and formulation scientists. Especially for FIH studies where the goal is to explore a relatively wide dose range in order to characterize both the pharmacokinetics of the compound as well as its safety at higher doses, poor solubility can be a significant limitation.

The Biopharmaceutical Classification System was introduced in 1995 and was subsequently adopted as a regulatory tool to facilitate bioequivalence assessment for marketed products. However, the BCS system or variations thereof are also highly utilized in preclinical development to guide formulation options. To be able to utilize these proposed decision trees, some confidence in the early BCS classification is needed. However, accurate permeability estimates for highly

Table III. Mean Pharmacokinetic Parameters for Fasted Beagle Dogs Administered Orally 3 mg MK-0594 Solid Formulations (Mean ± Std Dev, $n=3$)

	RC-1	RC-2	RC-3	FBG	WG	Nanosuspension in capsule
AUC_{0-24} (μM×h)	4.43±0.477	5.21±0.575	3.24±1.49	3.99±0.604	4.49±0.564	5.82±0.887
C_{max} (μM)	0.285±0.050	0.298±0.015	0.215±0.113	0.244±0.046	0.256±0.039	0.359±0.080
T_{max} (h)	4.0±2.0	4.0±0	2.7±1.2	3.3±1.2	2.7±1.2	4.0±0

RC roller compaction, *FBG* fluid bed granulation, *WG* wet granulation, *AUC* area under curve

Table IV. Summary of Dose-Normalized $AUC_{0-24\,h}$ (μM×h/mpk) for Solid Formulations of MK-0594 (Mean ± S.D.) Tested at 0.3 mpk (3 mg)

	Dose-normalized $AUC_{0-24\,h}$ (μM×h/mpk)						
	RC-1	RC-2	RC-3	FBG	WG	Nanosuspension (dried)	LFC
0.1 mpk							12.3±0.5
0.3 mpk	14.8±0.92	17.4±1.11	10.8±2.86	13.3±1.16	15.0±1.1	19.4±1.7	
4 mpk							14.9±1.93

Data with LFC (Imwitor) tested at a lower and higher dose are included as reference
RC roller compaction, *FBG* fluid bed granulation, *WG* wet granulation

lipophilic/poorly soluble compounds may not be easy to obtain in the commonly used cell line assays. In some cases, available preclinical information (*e.g.*, bioavailability in rats or dogs) may facilitate data interpretation. For MK-0594, in addition to the preclinical information that suggested acceptable permeability, an accurate BCS classification was achieved *via* the use of the rat intestinal perfusion setup, which, based on our experience, represents the most accurate method to obtain a permeability estimate.

Based on the BCS classification of the compound and the very low solubility, a solubilized formulation (liquid-filled capsule in Imwitor 742) was used in the FIH study. The single rising dose study confirmed the good bioavailability from this formulation as a dose proportional increase in exposure across the studied dose range of 1–200 mg was observed. Furthermore, no food effect was observed for the formulation when dosed with a high-fat breakfast at 100 mg, providing additional confirmation of the practically complete absorption of the compound when solubilized with the Imwitor 742 LFC. However, moving towards later development, development of a solid dosage form was highly desirable, especially since projected efficacious doses were significantly lower. A solid dosage form can generally provide a simpler manufacturing path, alleviate any potential concerns with physical or chemical stability in a lipid system, and potentially serve in the future as the basis of fixed dose combinations.

The advances in modeling and simulation in the field of biopharmaceutics provide scientists with tools to understand the *in vivo* absorption behavior of drug compounds and quickly test what if scenarios to understand formulation options. For the case of formulation bridging, these models allow for an early assessment of feasibility of the potential formulation candidates. For MK-0594, a parameter sensitive analysis conducted early on indicated that fast dissolving formulations (simulated as micronized API) would allow for adequate bioavailability and warrant addition exploration.

Prior to committing to costly clinical evaluation, prototype formulations were evaluated in the dog model. Within the limited number of animals employed in the study, it was not expected that statistical significance of small exposure differences will be concluded. The primary goal of the animal studies was to provide proof of concept that a solid dosage form could provide exposures comparable to the liquid formulation. For the solid formulations that are the focus of this report, all the conventional solid formulations (RC, FBG, and WG) resulted in comparable exposures in the dog model while the dried nanoparticles in capsule resulted in somewhat higher exposure levels (both AUC and C_{max}). Despite the potentially higher exposure for the nanoparticles, the approach was not further pursued as exposure from other solid formulations was satisfactory and generally comparable to the LFC. The results from the nanoparticles demonstrated the potential of using reduction in particle size to increase exposure; however, due to the low targeted dose, the advantage is minimized. Between the different processing options for the formulations, all formulations with the small API PSD resulted in relatively comparable exposures. The main impact was observed preclinically in dogs when a larger API PSD formulation was tested that appeared to result in lower exposure (~40% reduction compared to the exposure of identical formulation composition but with smaller API PSD). An increase in particle size also resulted in a significant increase in variability. While full statistical significance could not be concluded based on the study design, it was decided that a small API PSD would be utilized for the clinical studies to maximize the possibility of success of the solid formulation. Despite the viability of all the formulation options, at the end, the RC was preferred accounting also for potential stability and ease of manufacturing considerations.

The results of the clinical study for MK-0594 confirmed our expectations of adequate exposures from a conventional formulation. The 5-mg tablet AUC was comparable, with a geometric mean ratio (GMR) of 1.01 (95% CI, 0.90–1.15), relative to the LFC, although C_{max} trended somewhat lower (GMR 0.83 (0.77–0.90)). Six-milligram tablet AUC and C_{max} trended slightly higher (GMR of 1.22 (1.10–1.36) and 1.09 (1.00–1.19), respectively); the study (n=16) was not intended to assess definitive bioequivalence. The tablet formulations exhibited a delayed T_{max} (median T_{max} of 3.5 and 4 h) relative to the LFC (2 h). The results in the clinic appear more in line with the absorption simulations rather than the dog studies in that in the clinic a 20–25% reduction in exposure is observed (if one accounts for the dose difference) which may not be clear to discern from the dog studies given the variability with a small number of animals. The expected higher solubility of the compound in the dog lumen appears to contribute to this difference. The difference is more pronounced for C_{max} compared to AUC.

In an ideal scenario, preclinical tools are developed sufficiently to potentially even eliminate the conduct of clinical studies for formulation bridging, assuming no specific regulatory agency requirement (such as post-phase III changes or SUPAC level III changes) applies. The BCS system provides a framework for this at least for BCS I and potentially BCS III compounds. However, the vast majority of the pharmaceutical industry pipelines are insoluble compounds, mostly BCS II. In those cases, the use of preclinical tools like dog studies and absorption modeling appears to be useful in terms of at least understanding the risk with clinical bridging. Whether the tools and especially the modeling aspects are accurate enough to completely eliminate a clinical study for relative bioavailability of formulations can be extensively debated and is beyond the scope of this research article. However, in our experience at the end, it largely depends on the comfort level and experience within each individual organization as well as the criticality of the decision that needs to be made and the specific compound characteristics (*e.g.*, the safety/efficacy margins for a compound may dictate the tolerated deviations

from bioequivalence). In an ideal scenario, sufficient clinical data are available to validate the model, following similar internal and external validation concepts as routinely applied to IVIVC approaches based on available guidance from regulatory agencies. However, we acknowledge that this may not always be practically feasible. In the case of MK-0594 discussed here, simulations retrospectively appeared to adequately predict the outcome of the clinical study at the two dose levels tested.

Perfect predictions from either preclinical models or absorption modeling are difficult to obtain or at least fully validate. For animal models, inherent physiological differences may affect the formulation behavior. Oftentimes for absorption models, there are assumptions in the models that are difficult to fully qualify. Even solubility or permeability estimates that are typically stated as inputs should not be really considered as true absolute values as any extrapolation of preclinical to clinical data comes with a potential error (which could be related to the inherent variability of the parameter estimated). It is also not uncommon that some model assumptions result in contradicting behavior to other assays, *e.g.*, the assumption of particle size-controlled dissolution of a tablet formulation may not be always replicated *in vitro* or *in vivo*. Thus, a careful consideration of the model input and output and exploration of multiple what if scenarios is oftentimes required to drive decision. These concepts are in line with what has also been proposed by other researchers in the field (16). In the case of MK-0594, the modeling was utilized initially to obtain an estimate of feasibility, defined as adequate fraction absorbed in the clinic. That assessment does not need to be precise as it is meant to be only used as a trigger for subsequent experimentation. Subsequently, the model was utilized to assess the impact of API particle size, a quite common question for BCS II compounds. The directional signal that the model provided that larger particles may compromise absorption triggered an additional confirmatory preclinical study. The directional agreement of the two models was sufficient at that stage to inform the design of clinical formulations at least for the initial clinical comparison; the goal was to maximize the possibility of success for formulation bridging. It would be fair to state that the dog study potentially overestimated the performance of the solid formulation especially for C_{max} given the higher (on milligram per kilogram) dose tested in these studies; this is likely to higher solubility expected in dogs for a compound like MK-0594 that solubilizes significantly in bile micelles. It is known that bile micelle concentrations are higher in dogs compared to humans. The retrospective simulations on dog fraction absorbed (Fig. 2) point to that direction. However, one could state that even the initial clinical absorption simulations (Fig. 1) that did not account for the distribution around the mean particle size or allowed for colonic absorption also somewhat overestimated exposures. In the clinic, on a per milligram basis, the LFC appears to result in a more robust exposure. At the end though, this 25% reduction did not represent a hurdle for further development of the solid formulation. Thus within the acceptable range of outcomes that would enable decision making, the preclinical models were adequate to inform the selection of appropriate formulations to be tested in the clinic.

One additional point is worth additional discussion. During preclinical formulation development for MK-0594, dog studies were utilized as the primary tool for formulation decisions and are reported in this manuscript. However, with the advancements in the recent years in understanding of the luminal contents and the development of biorelevant media, it is a common practice that biorelevant dissolution is incorporated as part of the formulation development. For MK-0594, extensive dissolution experiments were conducted after selection of the clinical formulations reported here. Both biorelevant media (FaSSIF) as well as more compendial media (buffer with surfactant) were utilized for this screening. As expected, dissolution studies in both biorelevant and compendial media correctly demonstrated the dissolution difference of neat API powder of differing PSDs (*e.g.*, 30 min dissolution of 85% *vs.* 66% for 7 and 20 μm API, respectively, tested in 0.7% Tween 80 media). Interestingly enough, much smaller differences were observed between final tablets of the same APIs with the differing PSD (30 min dissolution of ~80% for tablets based on 7 or 20 μm API). This could be seen as a contrasting result to the trends observed in the dog study. The reasons for the discrepancy cannot be fully clear at this stage. It is possible that the dog model exaggerated smaller dissolution differences, that there are inherent differences in the *in vivo vs. in vitro* behavior of the dosage form, or that early prototype formulations tested in the dog did not fully reflect behavior of the formulation after scale up. Since only a small API PSD lot was utilized in the clinical study to maximize possibility of success, clinical data to validate the observation of the dissolution differences between API and tablets are not available. However, this observation highlights the potential to receive differing information from different preclinical tools; a final decision on formulation may need to rely on the level of comfort with each of the tools and assessment of the risks and benefits of each decision in terms of both impact on clinical exposures and also on the formulation/manufacturing aspects. In the case of MK-0594, at least at the point of decision making for pursuing the clinical study, the general agreement between absorption modeling and dog data was seen as sufficient supporting evidence from the project team.

CONCLUSION

Formulation changes are routine practice during clinical development. For product development teams, such changes often raise concerns on clinical performance especially for BCS II and IV compounds. The advances in understanding of *in vivo* processes including the development of biorelevant *in vitro* measurements, in conjunction with parallel advances in *in silico* oral absorption modeling, provide biopharmaceutics scientists and formulators additional tools to the traditionally utilized preclinical animal models. In this manuscript, a case study of the complementary use of such tools was presented. For MK-0594, a very poorly soluble BCS II compound, the FIH formulation was a solubilizing lipid system. *Via* the use of absorption modeling for early feasibility assessment along with proof of concept studies in dogs, a conventional crystalline API formulation that provided sufficient exposure as confirmed by clinical data was developed. While clinical studies, as also employed here, may still be required to finalize such formulation changes, preclinical biopharmaceutics tools such as absorption modeling provide invaluable input during risk assessment to enable appropriate allocation of resources and inform clinical possibility of success.

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REFERENCES

1. Hariharan M, Ganorkar LD, Amidon GE, *et al*. Reducing the time to develop and manufacture formulations for first oral dose in humans. Pharm Technol. 2003;2003:68–84.
2. Amidon GL, Lennernäs H, Shah VP, Crison JR. A theoretical basis for a biopharmaceutics drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm Res. 1995;12:413–20.
3. Ku MS. An oral formulation decision tree based on biopharmaceutical classification system for first-in-human clinical trials. Bull Tech Gattefosse. 2006;98:89–102.
4. Li P, Zhao L. Developing early formulations: practice and perspective. Int J Pharm. 2007;341:1–19.
5. Butler JM, Dressman JB. The developability classification system: application of biopharmaceutics concepts to formulation development. J Pharm Sci. 2010;99:4940–54.
6. Branchu S, Rogueda PG, Plumb AP, *et al*. A decision-support tool for the formulation of orally active, poorly soluble compounds. Eur J Pharm Sci. 2007;32:128–39.
7. Kesisoglou F, Wu Y. Understanding the effect of API properties on bioavailability through absorption modeling. AAPS J. 2008;10:516–25.
8. Parrott N, Lukacova V, Fraczkiewicz G, Bolger MB. Predicting pharmacokinetics of drugs using physiologically based modeling—application to food effects. AAPS J. 2009;11:45–53.
9. Heimbach T, Xia B, Lin TH, He H. Case studies for practical food effect assessments across BCS/BDDCS class compounds using in silico, in vitro, and preclinical in vivo data. AAPS J. 2013;15:143–58.
10. Lukacova V, Woltosz WS, Bolger MB. Prediction of modified release pharmacokinetics and pharmacodynamics from in vitro, immediate release, and intravenous data. AAPS J. 2009;11:323–34.
11. Zhang X, Lionberger RA, Davit BM, Yu LX. Utility of physiologically based absorption modeling in implementing quality by design in drug development. AAPS J. 2011;13:59–71.
12. Fleisher D, Sheth N, Griffin H, McFadden M, Aspacher G. Nutirent influences on rat intestinal phenytoin uptake. Pharm Res. 1989;6:332–7.
13. Agoram B, Woltosz WS, Bolger MB. Predicting the impact of physiological and biochemical processes on oral drug bioavailability. Adv Drug Deliv Rev. 2001;50 Suppl 1:S41–67.
14. Shono Y, Jantratid E, Kesisoglou F, Reppas C, Dressman JB. Forecasting in vivo oral absorption and food effect of micronized and nanosized aprepitant formulations in humans. Eur J Pharm Biopharm. 2010;76:95–104.
15. Sugano K. Possible reduction of effective thickness of intestinal unstirred water layer by particle drifting effect. Int J Pharm. 2010;387:103–9.
16. Rose JP. Effective absorption modeling in relative bioavailability study risk assessment. AAPS J. 2012;14:895–903.