The AAPS Journal (2020) 22: 134
DOI: 10.1208/s12248-020-00511-7

Research Article

Theme: Use of PBPK Modeling to Inform Clinical Decisions: Current Status of Prediction of Drug-Food Interactions
Guest Editor: Filippos Kesisoglou

Physiologically Based Pharmacokinetic Modeling of Oral Absorption, pH, and Food Effect in Healthy Volunteers to Drive Alpelisib Formulation Selection

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Received 10 June 2020; accepted 24 September 2020; published online 18 October 2020

Abstract. A physiologically based pharmacokinetic (PBPK) human model for alpelisib, an oral α-specific class I phosphatidylinositol-3-kinase (PI3K) inhibitor, was established to simulate oral absorption and plasma pharmacokinetics of healthy subjects to allow model-informed drug development. The GastroPlus™ model consisted of an advanced absorption gut model, which was linked to a 2-compartmental model. Systemic clearance and volume of distribution were estimated using population pharmacokinetics (popPK). Various food effect and pH-mediated absorption drug–drug interaction (DDI) scenarios were modeled. In fasted healthy subjects, simulated absorption was lower (ca. 70% for a 300-mg dose) due to pH and bile acid concentration-dependent solubility. Ranitidine showed a significant pH-mediated DDI effect only in the fasted but not fed state. The PBPK model identified that more drug is absorbed in the fed state, and alpelisib intestinal permeability is rate limiting to systemic exposure. Simulations for healthy subject showed a positive food effect with ca. 2-fold increase in plasma Cmax and 1.5-fold increase in AUC0-inf with a meal compared with fasted conditions. The PBPK model was verified using clinical food effect data with pivotal clinical formulation (PCF) and then applied to predict the performance of a commercial formulation (CF) in healthy volunteers. The model successfully predicted the outcome of a clinical bioequivalence study for PCF and CF with included *in vitro* dissolution data, both fasted and fed state. Estimated predictive errors (based on plasma Cmax, AUC0-t) were equal or below 30%. The alpelisib model for healthy subjects enables future bioequivalence formulation assessments, in fasted, fed, or altered pH conditions.

KEY WORDS: alpelisib; bioequivalence; biopharmaceutics; GastroPlus™; physiologically based pharmacokinetic(s) modeling; proton pump inhibitor DDI.

Guest Editor: Filippos Kesisoglou

Electronic supplementary material The online version of this article (https://doi.org/10.1208/s12248-020-00511-7) contains supplementary material, which is available to authorized users.

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Abbreviations: *AUC*, Area under the plasma concentration-time curve; *Cmax*, Maximum plasma concentration; *PBPK*, Physiologically based pharmacokinetics; *PK*, Pharmacokinetics.

INTRODUCTION

Alpelisib is an oral α-specific class I phosphatidylinositol-3-kinase (PI3K) inhibitor belonging to the 2-aminothiazole class of compounds. It showed clinical activity on solid tumors (as a single agent and in combination with fulvestrant) in hormone receptor–positive metastatic breast cancer (1). Alpelisib is a weak base with pH-dependent and bile acid concentration-dependent solubility. Increased pH in the gut leads to decreased solubility, while solubility is increased by bile acids in the fed state. Due to the pH-dependent solubility profile, acid-reducing agents (ARAs) such as H2 receptor antagonists (ranitidine), proton pump inhibitors, and antacids could potentially affect the PK of alpelisib by altering its gastrointestinal absorption. In a fasted human absorption, distribution, metabolism, and excretion (ADME) study of a single 14C-radiolabelled dose in four healthy male subjects, more than 50% of the oral alpelisib dose was rapidly absorbed. Alpelisib is primarily metabolized by

chemical and enzymatic hydrolysis to form its metabolite BZG791 (occurring extrahepatically) and to a lesser extent by CYP3A4, *in vitro*. Following a single oral dose of 400-mg radiolabeled alpelisib under fasted condition, 81% of the administered dose was recovered in feces (36% unchanged, 32% BZG791) and 14% (2% unchanged, 7.1% BZG791) in urine. CYP3A4-mediated metabolites (12%) and glucuronides amounted to approximately 15% of the dose (2). Since alpelisib is a substrate of BCRP, it may be eliminated by hepatobiliary excretion and intestinal secretion. Based on data in bile duct cannulated rats, where both pathways contributed equally, the same was assumed in human. Alpelisib has limited drug–drug interactions as a victim drug. Therefore, it may be used in combination with other drugs for the treatment of cancer.

A first-in-human study in subjects with cancer demonstrated that alpelisib has a favorable safety profile with predictable PK characteristics. Alpelisib was rapidly absorbed under fed (light meal) conditions, and PK plasma profiles were consistent after single and repeated dosing suggesting minimal drug accumulation. Systemic exposure to alpelisib was overall dose-proportional within the tested dose range: 30–450 mg (3). Alpelisib pharmacokinetics were also evaluated under fasting conditions after a 300-mg and 400-mg dose in healthy subjects in a hepatic impairment study control arm (4) and in a human ADME study (2), respectively. Maximal plasma concentration was reached at 2 h and declined in a biphasic manner, with a mean half-life of 11.7–13.7 h, the mean apparent oral clearance (CL/F) was 27.1–39.0 L/h, and the apparent terminal volume of distribution (Vz/F) was 465–838 L (2). No intravenous studies have been conducted to date.

Formulation assessment and food effect studies were conducted in healthy subjects. Two dedicated clinical studies were carried out to evaluate the effect of food with or without an acid-reducing agent (ranitidine) on alpelisib absorption and plasma exposure after a single dose of 300 mg (CBYL719A2103). Additionally, the bioequivalence of two formulations after a single dose of 200 mg was predicted and clinically verified in the fasted and fed state (CBYL719A2109). The results of these studies are the main subject of this work.

GastroPlus™ (version 9.6, Simulations Plus, Lancaster, CA) is a mechanism-based simulation software platform applied in assessment of oral (or alternative administration routes) absorption, biopharmaceutics, pharmacokinetics, and pharmacodynamics in humans and animals. The software is commonly used in industry to predict human pharmacokinetics and dose based on preclinical data prior to first-in-human, evaluate parameters influencing absorption, help in evaluating/selecting the best formulation, and predict organ exposure in preclinical and clinical projects. GastroPlus physiologically based pharmacokinetic (s) models (PBPK) (Simulations Plus, Lancaster, CA) are often applied for orally absorbed drugs, and such models have been used extensively in the industry and by health authorities for PBBM (physiologically based biopharmaceutics modeling) (5–9). GastroPlus™ offers inbuilt Advanced Compartmental And Transit (ACAT™) model, which consists of a set of eighteen gastrointestinal (GI) compartments (stomach, seven compartments for the small intestine, colon, and nine enterocyte compartments). The mathematical model couples linear and nonlinear rate equations used to simulate the effect of physiological conditions on drug absorption as it transits through successive compartments. The model accounts for a drug substance being released from a matrix, dissolved, degraded, metabolized, and absorbed into the systemic circulation. Healthy human physiology files of the gastrointestinal tract are available within the ACAT™ model for fasted and fed state. In-built physiological parameters including pH, transit times, and volumes across the intestinal tract are used to simulate fasted and high-fat–high-calorie (HFHC) meal conditions. GastroPlus models, in the biopharmaceutics space, commonly serve to establish a link between physiologically relevant parameters such as experimental dissolution, solubility, and permeability data to clinical outcomes. A publication by Heimbach *et al.* 2019 (9) summarized case examples of population PK and PBPK approaches using GastroPlus models to inform clinical trials, develop *in vitro*-to-*in vivo* correlations, and setting of clinically relevant dissolution specifications using the bioequivalence safe space approach.

The goal of this work was to establish GastroPlus PBPK/PBBM model for alpelisib to simulate and assess its oral absorption and bioavailability in healthy subjects to allow model-informed formulation selections in various prandial states. Objectives included identification of factors that limit or alter gut absorption to guide formulation selection and bioequivalence predictions. The simulations were performed for different clinical treatment conditions (dose, formulation, prandial states, pH effect via co-administration of ranitidine) and two different formulations: pivotal clinical formulation (PCF) and commercial formulation (CF).

MATERIALS AND METHODS

Physicochemical Properties and Absorption Parameters of Alpelisib

Table I summarizes the measured physicochemical parameters of alpelisib and input parameters to the GastroPlus PBPK model. This table provides the pKa-function fitted pH-solubility data of alpelisib (under fasted and fed conditions). As alpelisib showed an increased solubility in intestinal fluids in the fed state *in vivo*, its absorption was better simulated by using the biorelevant solubility in FeSSIF (fed state simulated intestinal fluid) for the pH-solubility profile obtained via pKa-function fitting (within the GastroPlus™ software) compared with measured solubility in aqueous buffers ($pH > 2$) in connection with the solubilization ratio for fed state. Experimental solubility data included in the fitting were 0.42 mg/mL at $pH = 2$ (in buffers); equilibrium solubility data in biorelevant media 0.04 mg/mL and 0.32 mg/mL in level II fasted and fed state simulated intestinal media, respectively (fasted and fed state simulated intestinal fluid: FaSSIF and FeSSIF (10)). With the pKa-fitted pH-solubility profile, the observed difference in plasma concentration-time profiles between fasted and fed conditions was captured. Two different dissolution models, the Johnson and Takano models (both being an integral part of the GastroPlus™ software), were used in simulations. The Johnson model accounts for an impact of particle size on dissolution, whereas Takano model (Z-factor model) (11) makes use of *in vitro* dissolution data, which is practically

useful when comparing performance of different formulations. The rationale of applying two different dissolution models was to correlate and assess the impact of available *in vitro* dissolution as well as particle size data on *in vivo* absorption of alpelisib (in simulating dissolution, both types of data cannot be used at the same time). Particle size distribution data are provided in Table I for the pivotal clinical formulation (PCF) and commercial formulation (CF). Dissolution tests for 200 mg alpelisib in PCF and CF were carried out in 900-ml biorelevant media at 37°C with a paddle speed of 75 rpm. Results of this study recorded as a percentage of alpelisib dissolved in time (10–75 min) are shown in Fig. 1. Based on the data, slight differences in the dissolution rate between the two formulations in FaSSIF were observed. Similarly, for the fed condition, dissolution of alpelisib was slightly faster with the CF formulation in FeSSIF. A constant Z-factor was fitted (within the GastroPlus™ software) to the experimental *in vitro* dissolution data per case: Z-factor = 7.48E−3 mL/mg/s (FaSSIF, PCF), Z-factor = 9.72E−3 mL/mg/s (FaSSIF, CF), Z-factor = 6.0E−3 mL/mg/s (FeSSIF, PCF), and Z-factor = 6.93E−3 mL/mg/s (FeSSIF, CF). *In vivo*, the alpelisib dissolution rate is a function of pH-dependent solubility which is calculated by multiplying the constant Z-factor with the actual solubility of alpelisib in a given pH (depending on a part of the GI tract). The solubility of alpelisib is the highest at low pH (1); therefore, under fasted conditions, alpelisib is fast dissolved in the stomach.

Table I. Input Parameters to the GastroPlus Human Model for Alpelisib

Parameter	Used value (Novartis data on file)
Molecular Weight (g/mol)	441.47
logP	2.96
pK_a	3.26 (base), SolFactor 205.8 9.40 (acid), SolFactor 50
Biorelevant solubilities (mg/mL)	SGF 1.43 (pH 1.1) FaSSIF 0.04 (pH 6.5) FeSSIF 0.32 (pH 5)
pH-solubility curve for alpelisib when fasted (mg/mL)	pH 1 3.64 pH 2 0.42 pH 3 0.08 pH 5 0.02 pH 6.5 0.04
pH-solubility curve for alpelisib when fed (mg/mL)	pH 1 3.64 pH 2 0.42 pH 4.5 0.32 pH 5 0.32 pH 6.5 0.32
Particle size distribution: in terms of radius (μm)	PCF: 3.14 (cumulative 10%) 18.42 (cumulative 50%) 40.8 (cumulative 90%) CF: 1.0 (cumulative 10%) 14 (cumulative 50%) 38.5 (cumulative 90%)
Caco-2 (A-B)	Papp 3.84×10^{-6} cm/s
Passive intestinal permeability (cm/s)	Peff 1.084×10^{-4} cm/s
B/P ratio, fu%	fu = 10.8%, B/P ratio = 1.03
Bile salt, solubilization ratio	Fed 0, Fasted 5.56E+4
Dosage form	IR tablet
Dissolution model	Z-Factor (Takano) Johnson

SGF gastrointestinal fluid solubility. Simulated gastric fluid (pH ~1.1); prepared according to EP 5.17.1, *FaSSIF* fasted-state simulated intestinal fluid, *FeSSIF* fed-state simulated intestinal fluid, *Peff* effective *in vivo* permeability estimated from Caco-2 P(A-B) using the internal Novartis extrapolation function

Alpelisib Pharmacokinetics in Animals

Preclinical studies showed no species disconnect in disposition parameters. Four female rats (Sprague Dawley) received an *i.v.* bolus of 1 mg/kg of alpelisib. The compound showed a low total plasma clearance of 1.09 L/h/kg (30% liver blood flow), a moderate volume of distribution of 3.63 L/kg, and an elimination half-life of approximately 3.1 h. Three Beagle dogs received an *i.v.* bolus of 0.1 mg/kg of alpelisib resulting in low plasma clearance equal to 0.46 L/h/kg (28% liver blood flow), moderate volume of distribution equal to 1.8 L/kg, and elimination half-life equal to approximately 4 h (Novartis data on file). Preclinical intravenous concentration-time plasma data rat and dog showed a biphasic profile which was best described a two-compartmental PK model. The animal PK profiles were used to estimate alpelisib human *i.v.* PK profile and parameters. Oral bioavailability was moderate to high in rodents and dogs (~ 60–100%).

Alpelisib Pharmacokinetics in Healthy Subjects Under Different Treatment Conditions

Two independent clinical studies were carried out to evaluate the absorption and pharmacokinetics of alpelisib in healthy subjects under different treatment conditions and compare the performance of two formulations: (a) an early pivotal clinical formulation (PCF) and (b) a commercial formulation (CF) (Fig. 2). The first clinical study (CBYL719A2103 (12)) was a single-center, open-label, randomized, five-period, ten-sequence crossover study to investigate the impact of both gastrointestinal pH and different prandial conditions on alpelisib PK. A total of 21 subjects were enrolled, and 20 subjects completed the study per protocol. The study started with a screening period, followed

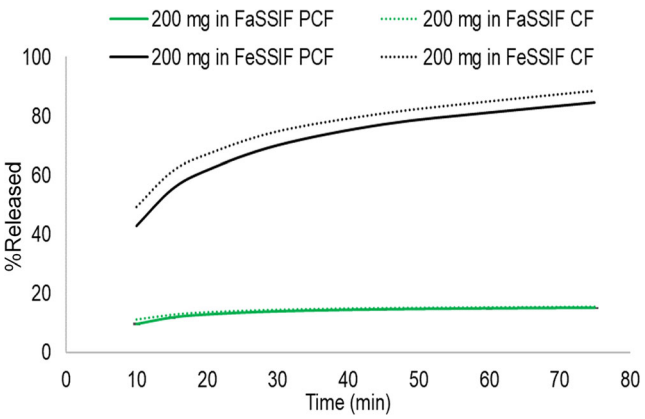

Fig. 1. *In vitro* dissolution results for 200 mg alpelisib in PCF and CF

by five treatment periods separated by complete washout of 8 days (+ 14 days window) between the dose administration days and a safety follow-up call. The impact of ranitidine, which raises stomach pH, as well as low-fat–low-calorie and high-fat–high-calorie breakfast, and their combined effect on the rate and extent of absorption were investigated. The subjects received a single dose of 300 mg of alpelisib administered in PCF formulation with approximately 250 mL of noncarbonated water under the five following conditions: alpelisib in fasted condition (after an overnight fast of at least 10 h), alpelisib with a high-fat–high-calorie (HFHC) meal, alpelisib with low fat, low calorie (LFLC) meal, alpelisib co-administered with ranitidine in fasted condition, and alpelisib co-administered with ranitidine with a LFLC meal. For treatment periods with HFHC or LFLC breakfast, alpelisib was administered 30 min after the start of the meal, and the subjects were asked to consume the entire meal within 30 min. The HFHC breakfast contained 985 cal (kcal) in total—510 kcal came from fat, 336 kcal came from carbohydrates, and 139 kcal came from proteins. The actual amount of fat was ~ 58.1 g, of carbohydrates ~ 83.4 g, and of proteins ~ 34.2 g. The LFLC breakfast contained 334 cal (kcal) in total—78 kcal came from fat, 206 kcal came from carbohydrates, and 50 kcal came from proteins. The actual amount of fat was ~ 8.7 g, of carbohydrates ~ 50.8 g, and of proteins ~ 12.2 g. Ranitidine was dosed 150 mg bid (twice a day), starting 2 days before co-administration with alpelisib to maintain a stable elevated gastric pH. On the day of alpelisib administration, the ranitidine morning dose was given 2 h before alpelisib as peak levels of ranitidine were to occur 2–3 h after a dose of 150 mg according to prescribing information. Twice-daily regimen of ranitidine (with upfront treatment for 2 days) can be considered as a similar scenario to a treatment with proton pump inhibitors (PPIs) with regard to the magnitude of the effect on gastric pH for the purpose of absorption DDI. For example, there were data generated from a study comparing the effect of concomitant administration of omeprazole or ranitidine with the anti-cancer drug erlotinib. Erlotinib pharmacokinetic exposure was found to be comparably reduced (↓AUC0-inf 54% *vs.* 46%; ↓Cmax 61% *vs.* 54%) (13). Similar results were obtained with darunavir and prasugel (14), supporting the interchangeability of PPIs and H2RA as acid-reducing agent for pH-mediated absorption DDI purposes.

The second clinical study (CBYL719A2109) was a single-center, randomized, open-label, two-cohort, two-period crossover study to investigate the bioequivalence of CF and PCF tablet formulation in healthy volunteers in the fasted and fed state. The study consisted of cohort 1 (200 mg after a HFHC meal; fed state) and cohort 2 (200 mg; fasted state). A total of 108 subjects (34 subjects in cohort 1 + 74 subjects in cohort 2) were enrolled, and 95 (24 + 71) were included in the pharmacokinetic analysis set. The study started with a screening period, followed by two treatment periods

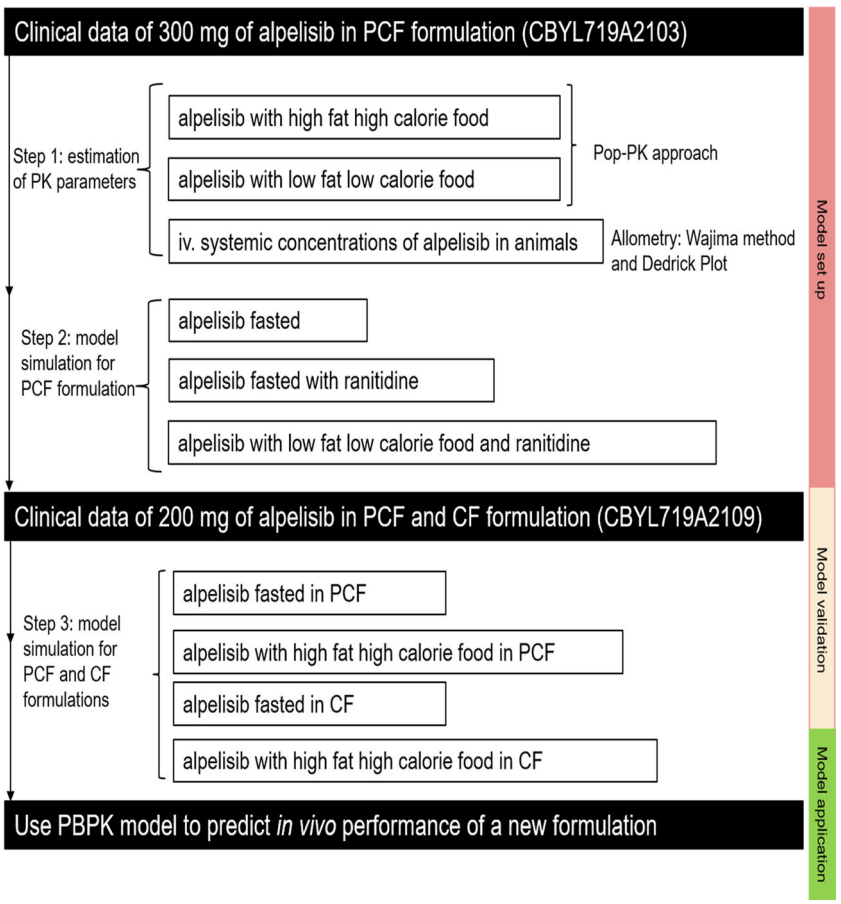

Fig. 2. Modeling strategy for PBPK model construction, validation, and application based on two clinical alpelisib studies in healthy subjects

separated by complete washout of 8 days (+ 14 days window) between the dose administration days and a safety follow-up call. The primary objective for the second study was to assess the bioequivalence between formulations, and the secondary endpoints included assessment of food effects of both formulations at the 200-mg dose across fasted and fed cohorts.

Estimation of Pharmacokinetic Parameters of Alpelisib in Healthy Subjects

Two approaches were taken to estimate clearance and volume of alpelisib in healthy subjects as an input into the GastroPlus model.

Approach 1: Monolix software (version 2018R1) was used to simultaneously fit the pooled fed (low- and high-fat meal) single-dose (number of subject = 41) data from the study CBYL719A2103 at 300 mg using population pharmacokinetic (popPK) modeling. The dataset consisted only of individual fed data due to the observed higher plasma exposure of alpelisib compared with fasted conditions with expected almost complete absorption and bioavailability (due to low systemic clearance of alpelisib). In the popPK approach, bioavailability was therefore fixed to 1, decreasing the number of parameters to estimate. No covariate selection was carried out as the primary aim of the exploratory popPK work was to describe drug kinetics.

Approach 2: experimental intravenous plasma concentrations in rat (1 mg/kg) and dog (0.1 mg/kg) were used to anticipate plasma exposure in human after a single intravenous dose of 1 mg/kg of alpelisib by using the Dedrick Plot (15) along with the PhRMA-recommended Wajima method (16). Human PK parameters were estimated by a compartmental model fitting to the predicted concentration-time profile using PKPlus fitting tool in the GastroPlus™. The fitting was done for the standard body weight of 70 kg.

GastroPlus Human PBPK Model for Alpelisib

The ACAT model for alpelisib for healthy subject was coupled with a compartmental PK model (representing plasma and rest of the body). GastroPlus ACAT in-built human physiologies (Opt-logD SA/V v6.1 model) for fasted and HFHC conditions were used for the simulations. To simulate the food effect for an LFLC meal, adjustments in the ACAT model were made according to Sutton *et al.* 2017 (17) for stomach pH and volume. The transit time in stomach was reduced to 0.25 h as in the case of fasted conditions (default setting). For co-administration with ranitidine, the pH in stomach was set to 6.50 (when fasted and fed) (18). Fluid percentage in small and large intestine was kept equal to 40% and 10%, respectively, as by default. In the fasted state only, transit times in jejunum 1 and 2 compartments were reduced from default GastroPlus settings of 0.93 h in jejunum 1 and 0.74 h in jejunum 2 to 0.26 h for both, as it showed to improve simulations, but the total transit time via small intestine is still within the range of reported transit times for healthy subjects (19,20). Physiological gastrointestinal input parameters to GastroPlus absorption model for different meal and treatment conditions are listed in Table II. Johnson dissolution model was used to simulate the first clinical study for alpelisib administered in PCF under different conditions. The Takano dissolution model was used to simulate the second clinical study—comparing absorption and plasma exposure of alpelisib administered in CF *vs.* PCF. The modeling strategy diagram is presented in Fig. 2. The strategy shows steps in training the model, based on clinical data of alpelisib in healthy subjects, towards its validation and application. The model derived from study CBYL719A2103 was then used to anticipate bioequivalence of two formulations, which was clinically confirmed (CBYL719A2109).

RESULTS

Pharmacokinetic Parameters of Alpelisib in Healthy Subjects

In approach 1, one-, two-, and three-compartmental PK models, all with a first-order rate of absorption, were fitted to the pooled fed (low- and high-fat meal) single-dose data. The two-compartmental model improved the residual error, −2xlog-likelihood, Akaike information criteria, and Bayesian information criteria compared with one- and three-compartmental models. The model assumes complete absorption and bioavailability of alpelisib. Resulting alpelisib PK parameters were low clearance (CL/F) = 20.4 L/h, moderate volume of distribution (Vd/F) = 90.9 L and a calculated elimination half-life of 4.65 h. In approach 2, both Dedrick and Wajima methods showed a good alignment between rat and dog intravenous PK profiles in plasma. Like in approach 1, animal and predicted human concentration-time profiles after single intravenous doses were better described by a two-compartmental PK model. Dedrick Plot predicted clearance (CL) = 21.82 L/h, total volume of distribution (Vd) = 195.10 L, and elimination half-life of alpelisib of 7.34 h. By comparison, the Wajima method predicted similar disposition parameters with clearance (CL) = 22.76 L/h, the total volume of distribution (Vd) = 103.22 L, and elimination half-life of 4.19 h.

The above methods fitted or predicted comparable plasma clearance of 20 L/h. Total volume of distribution of around 100 L (for a body weight of 70 kg) was estimated by the first approach and Wajima method. Dedrick plot derived a higher volume of distribution and consequently a longer elimination half-life of alpelisib in human. Results of the fitting using a two-compartmental model for both approaches are presented in Table A1 in the supplementary data. The table contains estimated clearance (CL), volume of distribution into the central compartment (Vc), and intercompartmental clearance rates (k12, k21). PK parameters estimated in approach 1 were used as an input to the GastroPlus model. The first-pass effect in gut was assumed to be equal to 0 for all simulations, and the maximal liver first-pass effect was estimated to be around 20% (with CL/F = 20.4 L/h, or ~ 0.3 L/h/kg with $F = 1$, or ~ 20% of human liver blood flow). The CL estimate was consistent with 0.27 L/h/kg calculated using dog single species scaling or 0.21 L/h/kg using the rule of exponent method (21). The human bioavailability was estimated using Fa estimates from GastroPlus ACAT module based on biopharmaceutic properties. For simulations, the liver first-pass effect was assumed to be dose-proportional and was set to 10% for 300 mg and 20% for 200 mg.

Table II. Gastrointestinal Parameters in GastroPlus for Different Treatment Conditions

Compartment	Condition	pH	Transit time (h)	Volume (mL)
Stomach	Fasted	1.3	0.25	46.6
	Fed (HFHC)	4.9	1.00	931.2
	Fed (LFLC)	1.3*a*	0.70[a]	600
	Fasted + ranitidine	6.5*b*	0.25	46.6
	LFLC + ranitidine	6.5*b*	0.70	600
Duodenum	Fasted	6	0.26	41.6
	Fed (HFHC)	5.4		
	Fed (LFLC)	6		
	Fasted + ranitidine	6		
	LFLC + ranitidine	6		
Jejunum 1	Fasted	6.2	0.26	154.2
	Fed (HFHC)	5.4	0.93	
	Fed (LFLC)	6.2		
	Fasted + ranitidine	6.2	0.26	
	LFLC + ranitidine	6.2	0.93	
Jejunum 2	Fasted	6.4	0.26	122.3
	Fed (HFHC)	6	0.74	
	Fed (LFLC)	6.4		
	Fasted + ranitidine	6.4	0.26	
	LFLC + ranitidine	6.4	0.74	
Ileum 1, 2, 3	All	6.6, 6.9, 7.4	0.58, 0.42, 0.29	94.3, 70.5, 49.8
Caecum	All	6.4	4.19	47.5
Asc colon	All	6.8	12.57	50.3

[a] (17)
[b] (18)

Clinical Study Results

Clinical PK data with geometric means across different treatment study arms from the two studies CBYL719A2103 and CBYL719A2109 can be found in the supplementary material (Table A2, Table A3). In the first clinical study (CBYL719A2103), a single dose of 300 mg alpelisib tablet was tested in healthy subjects under 5 different treatment conditions: fasted, fasted and co-administered with ranitidine, fed with high-fat–high-calorie food (HFHC), fed with low-fat–low-calorie food (LFLC), and fed with low-fat–low-calorie food co-administered with ranitidine (12,22). The concentration-time profiles of alpelisib were overall higher (in terms of Cmax and AUC) in fed condition (both with LFLC and HFHC meal) as compared with fasted condition. Under all fed conditions, plasma concentrations followed a monophasic decline suggesting limited distribution towards the peripheral tissues. Under fasted condition, plasma concentrations followed a biphasic decline. The terminal elimination phase was relatively long across all treatments, starting around 24 h after administration and extending beyond 72 h. Peak concentration was reached earlier in fasted state compared with fed. When alpelisib was administered with HFHC meal, the absorption was delayed with a time to maximal concentration (Tmax) increase of approximately 1 h. Cmax increased by 84% compared to alpelisib in fasted condition. Similarly, the AUC0-inf increased by ca. 73% with HFHC meal. The administration of alpelisib with LFLC meal led to a delay of 0.45 h in Tmax relative to alpelisib in fasted state. Compared with alpelisib in fasted condition, plasma Cmax increased by approximately 145% and the AUC0-inf, increased by 77%, indicating that both high- and low-calorie meals increase alpelisib bioavailability significantly. The clinically observed somewhat higher Cmax for the LFLC compared with HFHC was not anticipated (1690–3930 ng/mL LFLC *vs.* 1160–2830 ng/mL HFHC—see Table III). Simulations showed a positive food effect of ca. 2-fold with a small (ca 30%) underprediction of Cmax and AUC0-inf after the LFLC meal. The reason for this may be partly due to a reduced gastric emptying time for LFLC leading to higher Cmax, and a reduced Tmax was observed with LFLC (2.45 *vs.* 3 h, Table III). Another reason may be the reduced meal viscosity for LFLC relative to HFHC (23). Administration of different types of meal may result in the physiological differences such as, for example, increased presence of calories, and fat may significantly prolong the drug residence time in stomach and/or increase the bile salt concentration across the GI lumen. In our PBPK model, we used the standard fed-state physiology available in GastroPlus (version 9.6) modified for LFLC condition only in terms of reduced transit time and volume in stomach and equal as in fasted-state bile salt concentrations in the GI tract (17). However, considering that the LFLC breakfast consists of approximately 20% fat of the total 330 caloric content of the meal, a slight increase in bile salt concentrations (compared to the fasted state) could occur in the lumen, and bile salts can impact on the dissolution and drug absorption. Alpelisib rate of absorption decreased rapidly when co-administered with ranitidine under fasted condition but increased when alpelisib was administered with ranitidine combined with LFLC meal, compared with administration of alpelisib alone in fasted condition. The co-administration of alpelisib with ranitidine in fasted condition led to a reduction of the plasma Cmax by 51% and AUC0-inf by 30%. In contrast, when ranitidine was co-

Table III. GastroPlus Simulations *vs.* Measurements: 300 mg of Alpelisib in PCF

Treatment group		Fa (%)	Tmax (h) (range)	Cmax (ng/mL) (range)	Cmax PE[b] (%)	AUC0-inf (ng h/mL) (range)	AUC PE[b] (%)
Fasted	Observed mean[a]	–	1.98 (1.00–2.50)	1230 (341–2390)	–	10,400 (4190–25,200)	–
	Range						
	Simulated	70.6	1.92	1087	11.6	9345	10
High fat high calorie	Observed mean[a]	–	3.00 (1.00–4.07)	2040 (1160–2830)	–	17,500 (7710–28,200)	–
	Range						
	Simulated	99.8	3.6	1752	14	13,210	24.5
Low fat low calorie	Observed mean[a]	–	2.45 (0.967–4.00)	2680 (1690–3930)	–	17,600 (12,300–30,200)	–
	Range						
	Simulated	99.8	2.80	1871	30	13,210	25
Fasted with ranitidine	Observed mean[a]	–	2.03 (1.45–4.12)	579 (230–1240)	–	7110 (3370–11,700)	–
	Range						
	Simulated	51	2.7	550	5	6788	4.5
Low fat low calorie with ranitidine	Observed mean[b]	–	2.52 (1.48–4.05)	1770 (821–2830)	–	14,500 (8110–25,200)	–
	range						
	Simulated	99.6	3.3	1795	1.3	13,190	9

[a] Observed: cycle 1, day 1, geometric mean values calculated for data from individual concentrations ($n = 21$ (low fat low calorie), 20 (other treatments))
[b] % PE = |[(Observed value − Predicted value) / Observed value]| × 100

administered with alpelisib under fed (LFLC) condition, the Cmax of alpelisib showed 56% increase and AUC0-inf-40% increase when compared with fasted conditions without ranitidine. Consequently, administration of alpelisib with ranitidine and LFLC meal led to a clinically observed reduction of both Cmax (36%) and AUC0-inf (21%) with geometric mean ratios of 0.64 and 0.79, respectively, compared with a LFLC with alpelisib alone. This was confirmed by modeling. In all GastroPlus simulations, the calculated arithmetic mean concentration-time profiles were used as reference observed data. Therefore, arithmetic mean PK parameters were derived from the clinical data to compare against simulations as presented in Table III. Calculated *vs.* observed food effect ratios in terms of Cmax and AUC0-inf under different treatment conditions are provided in Table IV.

In the second clinical study (CBYL719A2109), a dose of 200 mg of alpelisib in CF and PCF tablet formulation was tested in healthy volunteers in the fasted and fed state. The PK parameters of alpelisib in plasma were comparable between CF and PCF. The estimated geometric mean ratios (CF/PCF) with respect to AUC0-inf, AUC0-last, and Cmax were 0.961, 0.957, and 0.932 respectively. The two-sided 90% CI for all three primary PK parameters (AUC0-inf, AUClast, and Cmax) were completely within the predefined bioequivalence boundary of 0.80 and 1.25 demonstrating bioequivalence between CF and PCF 200 mg under the fasted state.

Table IV. Food Effect for 300 mg of Alpelisib in PCF: Simulations *vs.* Measurements

Treatment group		Cmax ratio (with respect to fasted results)	AUC0-inf ratio (with respect to fasted results)
High fat high calorie	Observed mean[a]	1.7	1.7
	Simulated	1.6	1.4
Low fat low calorie	Observed mean[a]	2.2	1.7
	Simulated	1.7	1.4
Fasted with ranitidine	Observed mean[a]	0.5	0.7
	Simulated	0.5	0.7
Low fat low calorie with ranitidine	Observed mean[a]	1.4	1.4
	Simulated	1.6	1.4

[a] Observed: single dose, geometric mean values calculated for data from individual concentrations ($n = 21$ (low fat low calorie), 20 (other treatments))

Table V. GastroPlus Simulations *vs.* Measurements: 200 mg Alpelisib in PCF and CF

Treatment group		Fa (%)	Tmax (h) (range)	Cmax (ng/mL)	Cmax PE[b] (%)	AUC0-inf (ng h/mL)	AUC PE[b] (%)
High fat high calorie, PCF	Observed[a] ($n = 73$)	–	4	1050.8		9554	
	range		1.50–8.02	864–2140		6110–14,100	
	Simulated	99.9	3.2	1122	7	8176	14
High fat high calorie, CF	Observed[a] ($n = 72$)		4.0	1080		9501.3	
	range		1.00–8.00	787–1610		5410–13,400	
	Simulated	99.9	3.2	1121	4	8176	14
Fasted, PCF	Observed[a] (n = 73)	–	2	666.5		6519.8	
	range		1.00–6.00	248–1840		3340–13,600	
	Simulated	78.4	2.1	641	4	5999	8
Fasted, CF	Observed[a] (n = 72)	–	2	644.2		6360.8	
	range		1.00–8.00	176–2080		2840–13,900	
	Simulated	79.4	2.1	653	1	6070	5

[a] Observed: day 1, geometric mean values calculated for data from individual concentrations
[b] % PE = |[(Observed value − Predicted value) / Observed value]| × 100

The plasma Tmax was 1.50 h for CF and 2.00 h for PCF (when fasted). A positive food effect was observed with both the CF and PCF variants after a 200mg dose given in the fed state on the rate and extent of absorption. Alpelisib in PCF (200 mg dose) given in the fed state increased the extent of absorption (AUC0-inf) by 47% and Cmax by 85%. Alpelisib in CF (200 mg dose) given in the fed state increased the AUC0-inf by 50% and Cmax by 86%. The same magnitude of food effect was observed for both formulations. Tmax in the fed state was longer for both variants (2.50 h -PCF and 3.00 h - CF) compared to Tmax in the fasted state (2.00 h—PCF and 1.50 h—CF). In the similar manner to the first clinical study, arithmetic mean concentration-time profiles and PK parameters per treatment were derived and

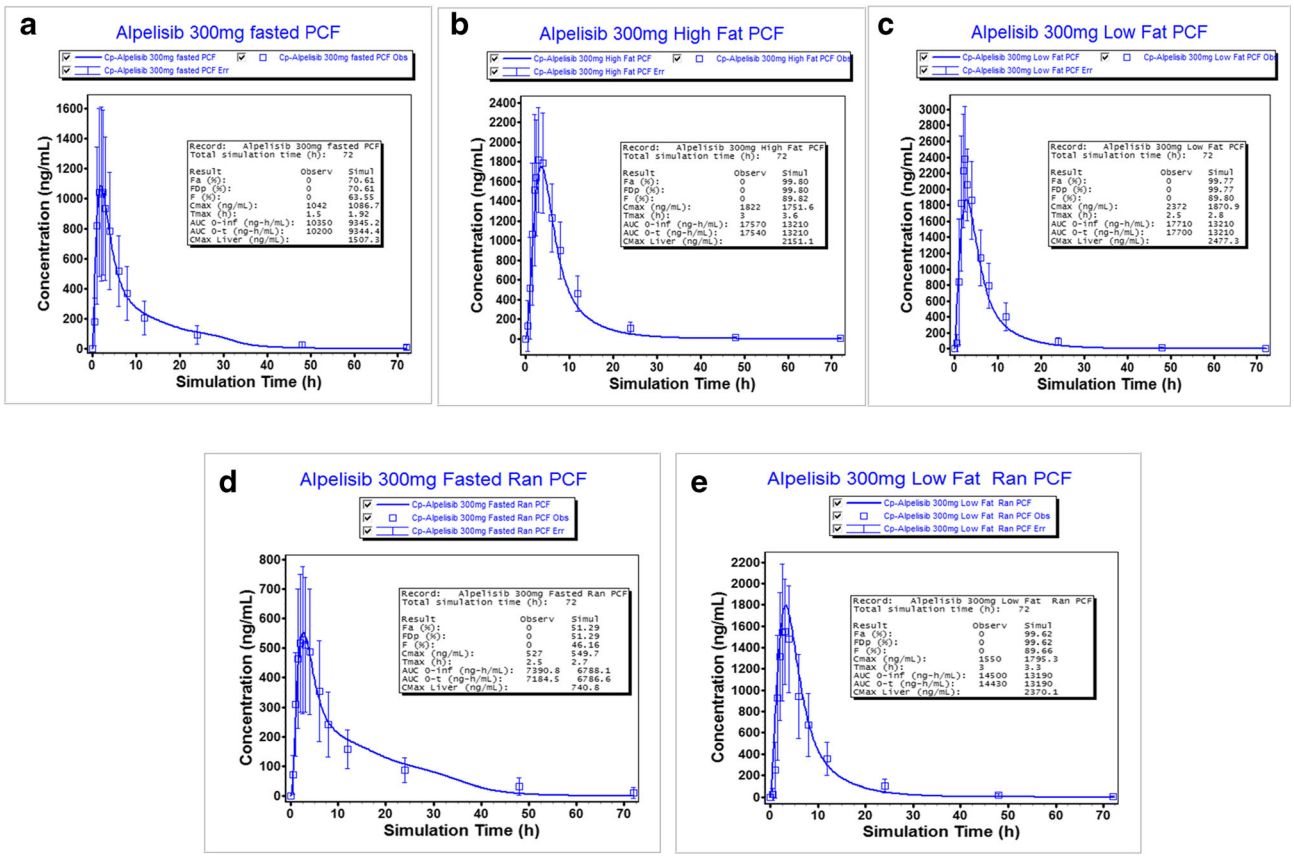

Fig. 3. Simulations *vs.* experimental plasma concentrations of alpelisib administered as a single dose of 300 mg under different treatment conditions. **a** Fasted, **b** fed (high fat, HFHC), **c** fed (low fat, LFLC), **d** fasted (with ranitidine), **e** fed (low fat, LFLC with ranitidine). The observed values displayed in the text box are the arithmetic mean values calculated from alpelisib plasma concentrations of all subjects

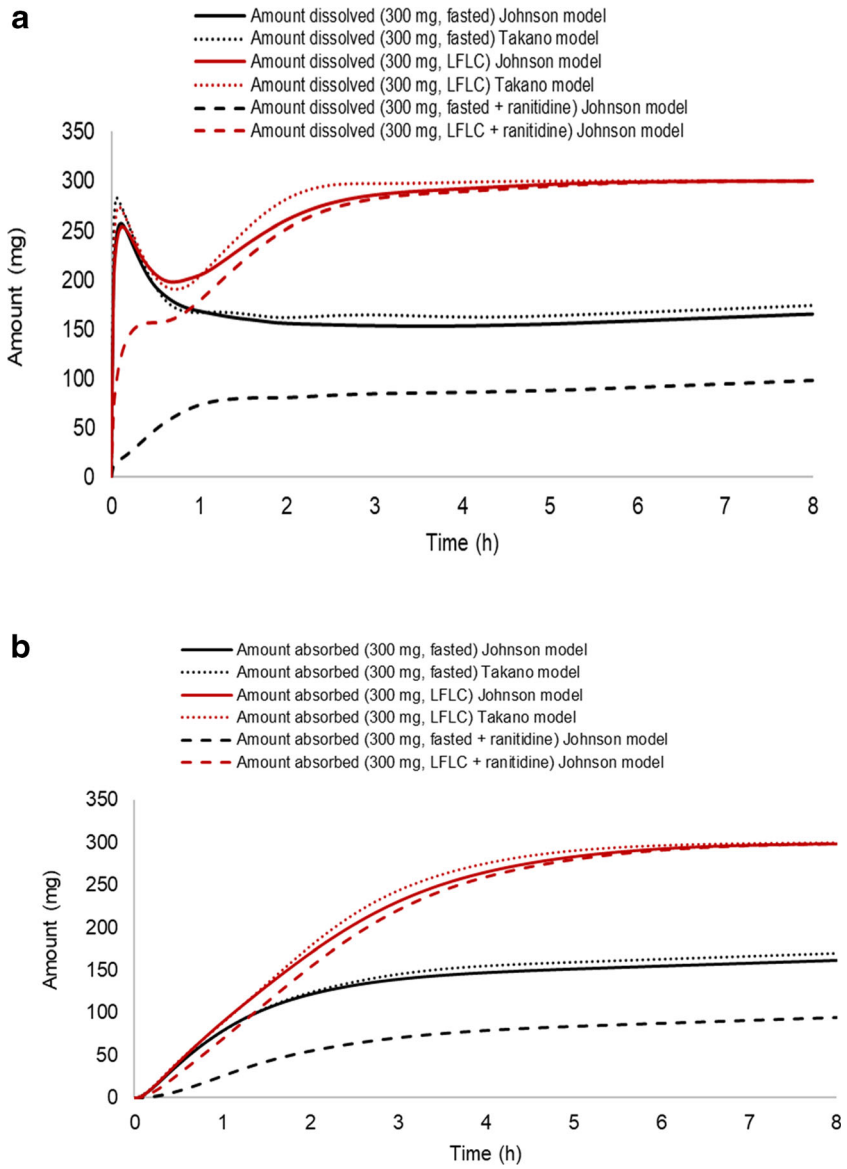

Fig. 4. Absorption kinetics of alpelisib—diagnostic plots. Simulated amount of alpelisib (dose = 300 mg in PCF) dissolved **a** and absorbed and **b** under fasted (black, solid line), fasted with ranitidine (black, dashed line), LFLC meal (red, solid line), LFLC meal with ranitidine (red, dashed line)

compared with simulations (Table V). The modeling results were in agreement with clinical observations.

In simulating clinical CBYL719A2103 and CBYL719A2109, two different dissolution models were used. Both, however, showed comparable results. For the clinical study CBYL719A2103, with alpelisib in PCF formulation, only particle size data of alpelisib were available; therefore, the Johnson dissolution model was applied. For the clinical study CBYL719A2109, *in vitro* dissolution data for formulations CF and PCF were generated, and Takano dissolution model was selected to be able to compare absorption of alpelisib in both formulations in silico assuming a constant particle size of alpelisib. Figure 4 shows a comparison between the two different dissolution models in terms of simulated amount of alpelisib dissolved and absorbed for 300 mg in PCF formulation, with LFLC meal and fasted. On average, simulations are similar with ca 0.13% (fed)–3% (fasted) higher simulated fraction absorbed using the Takano model compared with Johnson model.

Simulation of Oral Absorption of Alpelisib in Healthy Subjects

GastroPlus simulations of plasma concentration-time profiles for all prandial conditions following a single dose of alpelisib 300 mg dose, overlaid against mean food effect study data, are presented in Fig. 3. Overall, simulations matched the mean measurements well with the predictive error equal or below 30% in terms of Cmax and AUC0-inf (Table III).

Diagnostic plots of absorption kinetics for a dose of 300 mg PCF formulation (low-fat–low-calorie and fasted—with and without ranitidine) are shown in Fig. 4. Alpelisib shows permeability-controlled absorption. A fast

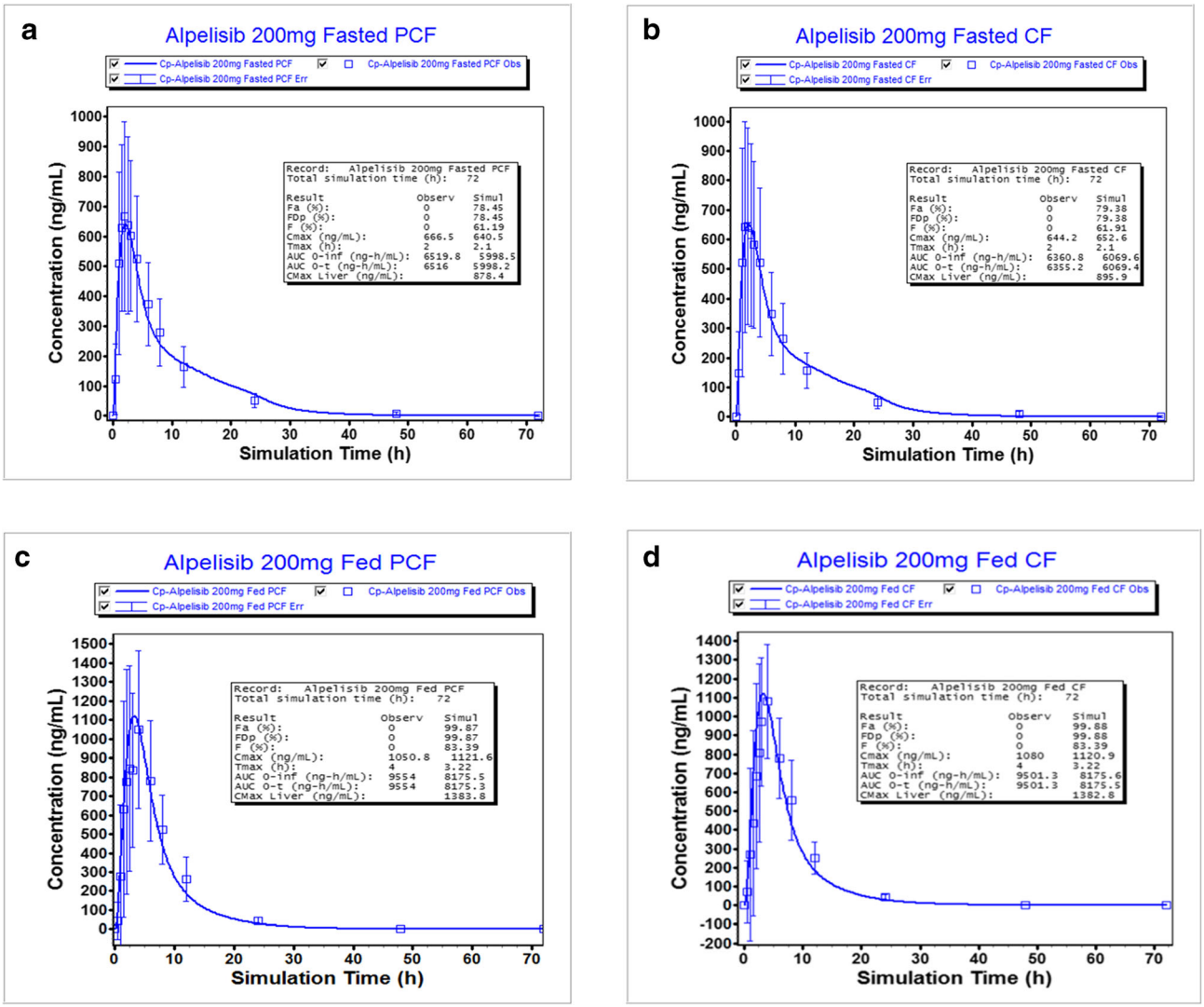

Fig. 5. Simulations *vs.* experimental plasma concentrations of alpelisib administered as a single dose of 200 mg in PCF and CF. The observed values displayed in the text box are the arithmetic mean values calculated from alpelisib plasma concentrations of all subjects

alpelisib dissolution occurs in the fasted state followed by extensive precipitation (the default GastroPlus precipitation kinetics adequately reflected the *in vivo* drug behavior). Dissolution kinetics of the compound was slower in the fed state. However, due to the high drug solubility in FeSSIF a higher amount of the dose was dissolved and absorbed. GastroPlus simulations of plasma concentration-time profiles per each formulation (PCF and CF, alpelisib single dose of 200 mg) overlaid against mean bioequivalence study data are presented in Fig. 5. Mean concentration-time points were calculated from 72 to 73 individual concentration-time profiles what confirms that the model is able to simulate well absorption and PK of alpelisib for a population representative. Table V, in a similar manner to Table III, presents measurements, simulations of fraction absorbed, Cmax, Tmax, and AUC0-inf and resulting predictive error with respect to plasma Cmax and AUC0-inf that was below 15% for both PK parameters. Simulated impact of PCF and CF on absorption of alpelisib is estimated based on dissolution kinetics described by a constant dissolution rate fitted to experimental *in vitro* dissolution data of PCF and CF. The *in vitro* dissolution data show a minimal difference between PCF and CF in dissolution in the fed state, what is consequently described by the GastroPlus model. Simulated fraction absorbed of alpelisib in both PCF and CF in the fed state equals to ca. 99.9%. There is simulated only around 1% lower fraction absorbed of alpelisib in PCF when compared with CF, what reflects in slightly lower PK parameters in plasma. The clinical case study with 200 mg of alpelisib administered in fasted state (CF formulation), due to simulated lower fraction absorbed in healthy subjects, was selected for a parameter sensitivity analysis, considering the following absorption-related parameters: particle size, reference solubility, and intestinal permeability. Parameters were modified by 1/10–10*x* of their input value. The Johnson dissolution model was used to be able to account for a particle size effect on absorption (the Takano model ignores a particle size in the simulated absorption process). The outcomes are shown in Fig. 6a, which indicated that reference solubility and intestinal permeability impact alpelisib fraction absorbed. The mean particle size (ranged between 0.7 and 70 μm) did not show large effect on absorption. Other

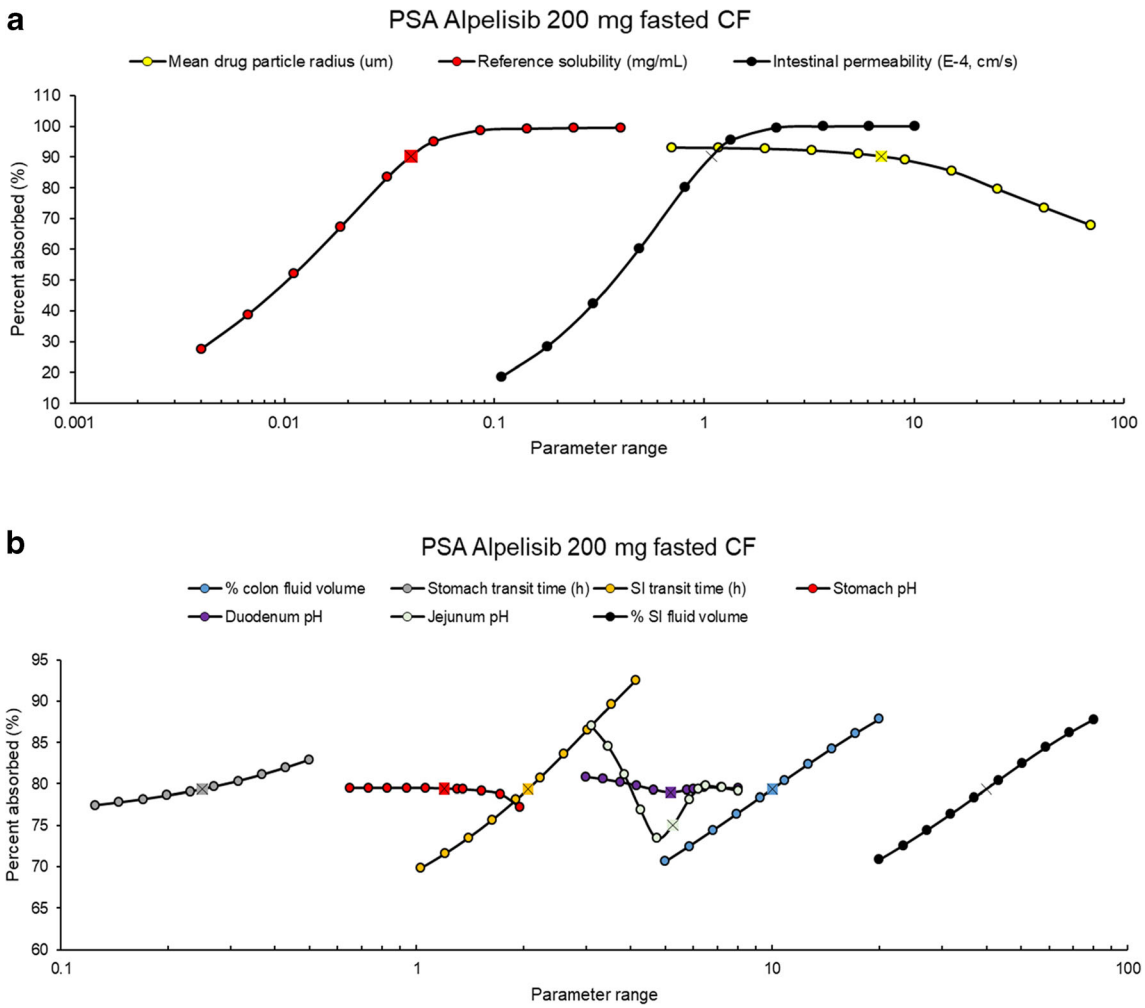

Fig. 6. Parameter sensitivity analysis. PSA for alpelisib administered as a single dose of 200 mg in CF (baseline point indicated by *x*). PSA was conducted using the Johnson dissolution model and the following parameters: **a** radius, reference solubility and intestinal permeability and **b** gastrointestinal transit times, pH, and fluid volumes

investigated parameters, such as percentage of fluid in small intestine and colon, transit times, and pH in stomach and small intestine (modified by ± 50% of their default input values), identified the biggest impact from fluid percentage in colon and small intestine and transit time in small intestine on absorption (Fig. 6b).

DISCUSSION

Alpelisib can be classified as a BCS (*Biopharmaceutics Classification* System) class II/IIb weak base drug, characterized by low and pH-dependent solubility. As a part of successful drug development for a BCSII/IIb oncology drug, the understanding of food effects and the possibility of pH-mediated drug–drug interactions by ARAs (8) are important for final market formulation and dose selection. Alpelisib PBBM was part of model-informed drug development to describe (a) observed food effects with LFLC and HFHC; (b) pH-mediated DDI effect, which was prandial state dependent and occurred only in the fasted state; and (c) *a priori* bioequivalence modeling for a new dose strength, 200 mg, for two formulations, PCF and CF.

Food effect predictions were carried out with confidence using decision tree criteria for two doses and two different formulations, as described by Tistaert *et al.* (7). In this paper, food effect predictions were verified on five case studies (BCS class I and II) with available clinical data. The authors highlighted the need for incorporation of appropriate solubility and dissolution data and for a stepwise validation of food effect projections against clinical data. Alpelisib met key decision tree criteria supporting the reliability of PBPK/PBBM simulations which included BCS class II/IIb drug with linear pharmacokinetics and major mechanism for food effect related to bile solubilization/supersaturation or delay in gastric emptying. This confirmed that the alpelisib GastroPlus model, with included population PK parameters and physiologies for healthy subject population representatives, was adequately verified to predict the PK performance of other formulations and to address absorption-related questions.

Alpelisib oral absorption prediction results (indicating moderate- to- high absorption) were consistent with *in vivo* data (Table III, CBYL719A2103). Alpelisib oral bioavailability can be expected to be close to fraction absorbed due to low systemic clearance and low liver first pass effect. The simulated fraction absorbed in human under fasted conditions

was around 70%, and nearly complete (99%) under fed conditions (both high and low calorie meals). This is in line with a clinically concluded positive food effect in healthy subjects of *ca.* 30% at a dose of 300 mg identified with GastroPlus simulations. A moderate - to- high (or nearly complete) Fa in human is consistent with preclinical data. The oral bioavailability in preclinical species was moderate (~ 57%) in rats after suspension dosing and complete (≥ 100%) in mice and dogs after a single dose of alpelisib as a solution, indicating complete absorption and high permeability. In a rat ADME study, alpelisib absorption had been reported to be 62.5% (range 53–75%) (24).

Potential pH-mediated DDI assessments and study conduct have been of interest to the FDA (25). Successful GastroPlus model applications to simulate pH-mediated DDI of acid-reducing agent on PK of basic drugs with pH-dependent solubility were described in Mitra *et al.* 2019 (8). The authors presented several industrial case studies with simulated effect of ARA on absorption of drugs (via interaction driven mainly via altered gastric pH) and proposed a workflow towards application of validated PBPK/PBBM models to inform clinical and regulatory decisions. For example, Danirixin, when co-dosed with a PPI, showed pH-mediated DDI exposure reductions that were more pronounced (> 50% higher) in the fasted state compared with fed conditions.

For alpelisib, pH-mediated DDI with ranitidine occurred only in the fasted but not the fed state (Table III). While the gastrointestinal pH may be elevated by ranitidine, possibly reducing alpelisib solubility, this is apparently counteracted by an increase in solubility due to high concentrations of bile acids present in the fed state, but not in the fasted state. The pH effect of ranitidine is best observed and simulated under fasted conditions. The fraction absorption (Fa %) of alpelisib decreased (Table III) with ranitidine in the fasted state compared with alpelisib alone. Similarly, plasma Cmax decreased by 49% (1087 to 550 ng/mL) and AUC0-inf decreased by 27% (9345 to 6788 ng h/mL) in the presence of ranitidine (Table III). Increasing pH in the stomach from 1.3 to 6.5 reduced solubility of alpelisib in the stomach from 3.64 mg/mL at pH = 1 to 0.04 mg/mL at pH = 6.5 which explained the observed effect of ranitidine. In contrast, in LFLC meal conditions, simulations showed no significant pH-mediated DDI effect of ranitidine on alpelisib absorption despite an overprediction (the predictive error was 30% with respect to plasma Cmax and 25% with respect to AUC0-inf when alpelisib was administered alone). In a similar manner, the effect of food and ranitidine on plasma pharmacokinetics of saquinavir in healthy volunteers was evaluated (18). It was concluded that plasma concentrations of saquinavir were significantly higher when the drug was co-administered with ranitidine and food when compared with the fasted state with ranitidine or saquinavir administered alone with food (18). Ranitidine has been previously proposed as a selective histamine type 2 receptor antagonist/blocker (26). Moreover, a recent study (27) showed that the administration of famotidine, another H_2-RA, can effectively result in the complete inhibition of gastric acid secretion. In particular, the median value of gastric pH during the first 35-min post water administration to fasted healthy adults was as high as 7.1. Based on this evidence, a higher stomach pH than 4.5 (namely 6.5) was used in the model to reflect the physiological conditions after ranitidine co-administration.

PCF and CF were concluded to be bioequivalent in the clinic in both fasted and fed states. For the 200-mg study, food effect and bioequivalence were correctly predicted *a priori.* The alpelisib PBBM approach exemplifies how food effects and bioequivalence can be predicted with confidence for BCS II/IIb drugs with linear PK as described by Tistaert (7).

CONCLUSIONS

A GastroPlus PBPK absorption model for alpelisib was established and verified with two independent clinical studies in healthy subjects under different treatment conditions (fed, fasted, co-administration with ranitidine, formulations: PCF and CF). The PBPK model allowed the *a priori* identification of PK, food effect, and BE for another dose strength with confidence. A pH-mediated drug interaction between alpelisib and ranitidine was observed in the fasted state, but not with a meal (LFLC), a finding which could be described by the PBPK model. Simulations confirmed the observed positive food effect of alpelisib of *ca.* 2-fold. Alpelisib absorption kinetics was shown to be permeability-limited based on GastroPlus assessment. The Takano (Z-Factor) dissolution model (trained on *in vitro* dissolution data in FaSSIF and FeSSIF) with constant dissolution rate was used to simulate absorption of alpelisib in formulations PCF and CF. In the investigated cases, the model simulations were within observations with predictive error (with respect to clinical mean plasma Cmax and AUC0-inf) and were less or equal to 30%. Two formulations were anticipated to be bioequivalent in the clinics at two dose strengths, which was confirmed by clinical data. The verified alpelisib PBPK model can serve as a foundation for formulation evaluations or pH-mediated DDI assessments and may address alpelisib absorption questions in various prandial conditions.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest The Novartis authors as indicated by their affiliation are Novartis employees and own Novartis stocks. A Sinn and M Velinova have nothing to disclose.

REFERENCES

1. André F, Ciruelos E, Rubovszky G, Campone M, Loibl S, Rugo HS, *et al.* Alpelisib for PIK3CA-mutated, hormone receptor-positive advanced breast cancer. N Engl J Med. 2019;380:1929–40.
2. James A, Blumenstein L, Glaenzel U, Jin Y, Demailly A, Jakab A, *et al.* Absorption, distribution, metabolism, and excretion of [14C]BYL719 (alpelisib) in healthy male volunteers. Cancer Chemother Pharmacol. 2015;76:751–60.
3. Juric D, Rodon J, Tabernero J, Janku F, Burris HA, Schellens JHM, *et al.* Phosphatidylinositol 3-kinase a–selective inhibition with alpelisib (BYL719) in PIK3CA-altered solid tumors: results from the first-in-human study. J Clin Oncol. 2018.
4. Marbury T, El-Hashimy M, Blumenstein L, Letellier F, Sengupta T, Lorenzo S, *et al.* Pharmacokinetics and safety of alpelisib in subjects with hepatic impairment: a phase 1, open-

label, multicenter, parallel-group study. J Cancer. 2019;(In press).
5. Butler J, Hens B, Vertzoni M, Brouwers J, Berben P, Dressman J, *et al*. In vitro models for the prediction of in vivo performance of oral dosage forms: recent progress from partnership through the IMI OrBiTo collaboration. Eur J Pharm Biopharm. 2019;136:70–83.
6. Parrott NJ, Yu LJ, Takano R, Nakamura M, Morcos PN. Physiologically based absorption modeling to explore the impact of food and gastric pH changes on the pharmacokinetics of alectinib. AAPS J. 2016;18:1464–74.
7. Tistaert C, Heimbach T, Xia B, Parrott N, Samant TS, Kesisoglou F. Food effect projections via physiologically based pharmacokinetic modeling: predictive case studies. J Pharm Sci. 2019;108:592–602.
8. Mitra A, Parrott N, Miller N, Lloyd R, Tistaert C, Heimbach T, *et al*. Prediction of pH-dependent drug-drug interactions for basic drugs using physiologically based biopharmaceutics modeling: industry case studies. J Pharm Sci. 2019.
9. Heimbach T, Suarez-Sharp S, Kakhi M, Holmstock N, Olivares-Morales A, Pepin X, *et al*. Dissolution and translational modeling strategies toward establishing an in vitro-in vivo link—a workshop summary report. AAPS J. 2019;21:29.
10. Markopoulos C, Andreas CJ, Vertzoni M, Dressman J, Reppas C. In-vitro simulation of luminal conditions for evaluation of performance of oral drug products: choosing the appropriate test media. Eur J Pharm Biopharm. 2015;93:173–82.
11. Takano R, Sugano K, Higashida A, Hayashi Y, Machida M, Aso Y, *et al*. Oral absorption of poorly water-soluble drugs: computer simulation of fraction absorbed in humans from a miniscale dissolution test. Pharm Res. 2006;23:1144–56.
12. Oncology Clinical Protocol CBYL719C2301 / NCT02437318 [Internet]. SOLAR-1: a phase III randomized double-blind, placebo controlled study of alpelisib in combination with fulvestrant for men and postmenopausal women with hormone receptor positive, HER2-negative advanced breast cancer which progressed on or after aromatas. 2017 [cited 2020 Apr 3]. p. 1–210. Available from: https://clinicaltrials.gov/ProvidedDocs/18/NCT02437318/Prot_000.pdf.
13. Kletzl H, Giraudon M, Ducray PS, Abt M, Hamilton M, Lum BL. Effect of gastric pH on erlotinib pharmacokinetics in healthy individuals: omeprazole and ranitidine. Anti-Cancer Drugs. 2014.
14. Zhang L, Wu F, Lee SC, Zhao H, Zhang L. pH-dependent drug-drug interactions for weak base drugs: potential implications for new drug development. Clin Pharmacol Ther. 2014;96:266–77.
15. Boxenbaum H, Ronfeld R. Interspecies pharmacokinetic scaling and the Dedrick plots. Am J Physiol Regul Integr Comp Physiol. 1983;245:R768–75.
16. Vuppugalla R, Marathe P, He H, Jones RDO, Yates JWT, Jones HM, *et al*. PhRMA CPCDC initiative on predictive models of human pharmacokinetics, part 4: prediction of plasma concentration-time profiles in human from in vivo preclinical data by using the Wajima approach. J Pharm Sci. 2011;100:4111–26.
17. Sutton SC, Nause R, Gandelman K. The impact of gastric pH, volume, and emptying on the food effect of ziprasidone oral absorption. AAPS J. 2017;19:1084–90.
18. Kakuda TN, Falcon RW. Effect of food and ranitidine on saquinavir pharmacokinetics and gastric pH in healthy volunteers. Pharmacotherapy. 2006.
19. Fischer M, Fadda HM. The effect of sex and age on small intestinal transit times in humans. J Pharm Sci. 2016;105:682–6.
20. Urgesi R, Cianci R, Bizzotto A, Costamagna G, Riccioni ME. Evaluation of gastric and small bowel transit times in coeliac disease with the small bowel PillCam®: a single centre study in a non gluten-free diet adult italian population with coeliac disease. Eur Rev Med Pharmacol Sci. 2013.
21. Ring BJ, Chien JY, Adkison KK, Jones HM, Rowland M, Jones R Do, *et al*. PhRMA CPCDC initiative on predictive models of human pharmacokinetics, part 3: comparative assessement of prediction methods of human clearance. J Pharm Sci. 2011.
22. FDA- Approved Drugs [Internet]. New Drug Application (NDA): 212526. [cited 2020 Apr 27]. Available from: https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=overview.process&varApplNo=212526.
23. Tougas G, Eaker EY, Abell TL, Abrahamsson H, Boivin M, Chen J, *et al*. Assessment of gastric emptying using a low fat meal: establishment of international control values. Am J Gastroenterol. 2000;95:1456–62.
24. NDA/BLA multi-disciplinary review and evaluation [Internet]. Application number 212526Orig1s000. 2016 [cited 2020 Jun 4]. p. 1–225. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2019/212526Orig1s000MultidisciplineR.pdf.
25. Framework for assessing pH-dependent drug-drug interactions. Dly J United States Gov [Internet]. 2018;No. 2018–1:23688–9. Available from: https://www.federalregister.gov/documents/2018/05/22/2018-10927/framework-for-assessing-ph-dependent-drug-drug-interactions-establishment-of-a-public-docket-request.
26. Bethesda M. No title. Natl Inst Diabetes Dig Kidney Dis. 2012.
27. Litou C, Vertzoni M, Goumas C, Vasdekis V, Xu W, Kesisoglou F, *et al*. Characteristics of the human upper gastrointestinal contents in the fasted state under hypo- and A-chlorhydric gastric conditions under conditions of typical drug – drug interaction studies. Pharm Res. 2016.