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The Absolute Bioavailability and Effect of Food on the Pharmacokinetics of Odanacatib: A Stable-Label i.v./Oral Study in Healthy Postmenopausal Women[S]

Stefan Zajic, Stefaan Rossenu, David Hreniuk, Filippos Kesisoglou, Jacqueline McCrea, Fang Liu, Li Sun, Rose Witter, Don Gauthier, Roy Helmy, Darrick Joss, Tong Ni, Randall Stoltz, Julie Stone, and S. Aubrey Stoch

Merck & Co., Inc., Kenilworth, New Jersey (S.Z., S.R., D.H., F.K., J.M., F.L., L.S., R.W., D.G., R.H., D.J., T.N., J.S., S.A.S.); and Covance Clinical Research Unit, Evansville, Indiana (R.S.)

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ABSTRACT

A stable-label i.v./oral study design was conducted to investigate the pharmacokinetics (PK) of odanacatib. Healthy, postmenopausal women received oral doses of unlabeled odanacatib administered simultaneously with a reference of 1 mg i.v. stable ^{13}C-labeled odanacatib. The absolute bioavailability of odanacatib was 30% at 50 mg (the phase 3 dose) and 70% at 10 mg, which is consistent with solubility-limited absorption. Odanacatib exposure (area under the curve from zero to infinity) increased by 15% and 63% when 50 mg was administered with low-fat and high-fat meals, respectively. This magnitude of the food effect is unlikely to be clinically important. The volume of distribution was ∼100 liters. The clearance was ∼0.8 l/h (13 ml/min), supporting that odanacatib is a low–extraction ratio drug. Population PK modeling indicated that 88% of individuals had completed absorption of >80% bioavailable drug within 24 hours, with modest additional absorption after 24 hours and periodic fluctuations in plasma concentrations contributing to late values for time to C_{max} in some subjects.

Introduction

Odanacatib is an oral, selective cathepsin K inhibitor that is in development as a treatment for osteoporosis (Langdahl et al., 2012). The pharmacokinetics (PK) of odanacatib have been investigated in phase 1 single-dose (Stoch et al., 2013) and multiple-dose (Stoch et al., 2009; Anderson et al., 2014) studies. In addition, an absorption, distribution, metabolism, and excretion study was conducted in humans (Kassahun et al., 2014), after detailed characterization of metabolism in rats, dogs, and monkeys (Kassahun et al., 2011). PK in humans are characterized by a relatively long apparent terminal half-life ($t_{1/2}$) of approximately 80 hours, which is consistent with low metabolic intrinsic clearance, and less than dose-proportional increases in concentration and exposure [area under the curve (AUC)] with increasing dose. Metabolism (principally mediated by CYP3A) and biliary and/or intestinal excretion of intact parent compound account for approximately 70% and 30%, respectively, of the clearance of odanacatib in humans (Kassahun et al., 2014). Odanacatib is a biopharmaceutics classification system class II compound with high permeability (consistent with absorption via passive diffusion) and very low solubility across the gastrointestinal tract (<0.001 mg/ml in water).

The primary objectives of this study were to determine the absolute bioavailability (and other fundamental PK parameters, such as volume of distribution and clearance) of odanacatib in fasted and fed conditions to assess the effect of food (low-fat and high-fat meals) on PK, to characterize the absorption profile, and to assess the safety and tolerability of odanacatib and ^{13}C-odanacatib. The secondary objectives were to evaluate whether the systemic disposition is linear, and to determine the influence of ^{13}C isotopic labeling on odanacatib i.v. PK (as a control). As part of characterizing absorption and disposition, this study also aimed to evaluate secondary peaks and late time to C_{max} (T_{max}) values occasionally observed in the concentration–time profile. The anticipated clinical dose of odanacatib is 50 mg, and this dose was studied in fasted and fed meal conditions. A 10-mg dose was also studied in one period to characterize the dependence of bioavailability on dose.

To accomplish these objectives, a relatively unusual study design was used: in each period of the study, stable-labeled (^{13}C) odanacatib was administered i.v., whereas unlabeled odanacatib was administered either orally or i.v. (in one period, as a control to test the effect of labeling). In contrast, traditional assessment of bioavailability typically involves crossover study designs in which the oral and i.v. PK of a drug are assessed in separate periods in the same group of individuals, and then compared (Musib et al., 2013). The i.v. labeled/oral unlabeled approach has been available for some time (Strong et al., 1975), although its use has been relatively limited (Dobson et al., 1994; Bianchetti et al., 1995; Bode et al., 1996; Yeh et al., 1999; Schwab et al., 2013).

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ABBREVIATIONS: AUC, area under the curve; $AUC_{0-\infty}$, area under the curve from zero to infinity; C_{eoi}, $C_{end\text{-}of\text{-}infusion}$; CI, confidence interval; *F*, bioavailability; GCP, Good Clinical Practices; GMR, geometric mean ratio; PK, pharmacokinetics; $t_{1/2}$, half-life; T_{max}, time to C_{max}; V_{ss}, volume of distribution at steady state.

Materials and Methods

Study Design

This was an open-label, randomized, four-period, partial crossover study (protocol number 045). Postmenopausal women (N = 24, 46–65 years of age) received single doses of 1 mg i.v. ^{13}C-odanacatib in each of four treatment periods, plus unlabeled odanacatib 1 mg i.v. (as a control to test the effect of the label on PK), 50 mg oral fasted, or 10 mg oral fasted in the first three periods. In the fourth period, 12 subjects also each received 50 mg of oral odanacatib with either a low- or high-fat meal. There was at least a 28-day washout between doses. For PK assessment, blood samples were collected over 336 hours after odanacatib administration. The allocation schedule for this study is shown in Table 1.

The protocol and other applicable study documentation was reviewed and approved by Independent Investigational Review Board, Inc. In addition, this trial had an investigator meeting at the outset to review all protocol procedures and investigator responsibilities under Good Clinical Practices (GCP). The investigator understood that by signing the Protocol Investigator Signature Page he/she provided commitment to comply with applicable GCP regulations and guidance, and to conduct the study in accordance with the protocol. This study was conducted in conformance with GCP standards, the Declaration of Helsinki, and applicable country and/or local statutes and regulations regarding ethics committee review, informed consent, and the protection of human subjects participating in biomedical research.

Synthesis of Stable-Labeled Odanacatib

The synthesis of [phenyl-U-^{13}C]-odanacatib follows a similar route (Fig. 1) to a previously reported nonlabeled version of odanacatib (Hughes et al., 2007). Commercially available [$^{13}C_6$]-4-bromobenzenemethylsulfone **1** (Sigma-Aldrich) was converted to boronic acid **2**. Suzuki coupling of **2** with arylbromide **3** produced hydrated ketone **4**. Reductive amination of **4** with fluoroleucine bisulfate **5** was mediated by $ZnCl_2$ and $NaBH_4$ to give the desired diastereomer **6** with high selectivity. The final coupling with amino **7** was promoted with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and hydroxybenzotriazole to produce [phenyl-U-^{13}C]-odanacatib with greater than 99% purity.

Formulation of Stable-Labeled Odanacatib

The i.v. formulation was provided as a 0.1 mg/ml solution of ^{13}C-odanacatib (as a neutral form) in 35% w/v sulfobutylether beta-cyclodextrin sodium salt (Captisol solubilizer/stabilizer; CyDex Inc., Lenexa, KS) and 0.01% w/v Polysorbate 80 (surfactant) in water for injection with a final pH ranging from 3 to 7. The final density of the resulting formulation was 1.14 mg/ml. The unlabeled i.v. formulation was prepared in an identical solution.

Drug Concentration Assays

Plasma odanacatib and ^{13}C-odanacatib concentrations were determined simultaneously using an analytical method that involved supported-liquid extraction followed by liquid chromatography–tandem mass spectrometry (Sun et al., 2012). The lower limit of quantitation for this method was 0.500 ng/ml with a linear calibration range from 0.500 to 500 ng/ml for both analytes.

Plasma samples basified with ammonium hydroxide were extracted with methyl tert-butyl ether on a 96-well supported-liquid extraction plate. The eluent was evaporated to dryness, and the residue was reconstituted in a 50%:50% v/v acetonitrile/water solution. A portion of the resulting solution was then injected into the liquid chromatography–tandem mass spectrometry system interfaced with a turbo ion spray source operated in positive ionization mode. For LC separation, the mobile phases consisted of 65/35 acetonitrile/0.1% formic acid in water (solvent A) and acetonitrile (solvent B). The analytes (odanacatib and $^{13}C_6$-odanacatib) and internal standard ($^{13}C_{12}$-odanacatib) were eluted under an isocratic mobile phase composition of 100% solvent A at a flow rate of 0.2 ml/min. A step gradient was then applied to wash the column with 100% solvent B at a flow rate of 0.4 ml/min, then the column was re-equilibrated at 100% solvent A. The retention time for both odanacatib and $^{13}C_6$-odanacatib was approximately 1.5 minutes. MS ion transitions used for odanacatib, $^{13}C_6$-odanacatib, and $^{13}C_{12}$-odanacatib detection were m/z 526 → 313, 532 → 319, and 538 → 325, respectively.

Assay intrarun and inter-run variability was assessed by the analysis of plasma quality control samples prepared at concentrations of 1.5, 37.5, and 375 ng/ml. For odanacatib and $^{13}C_6$-odanacatib, intrarun mean accuracy (N = 5) was between 98.7% and 105% of the nominal concentrations, with precision between 1.12% and 3.38%, and inter-run mean accuracy (N = 100) was between 94.7% and 99.6% of the nominal with precision between 2.80% and 6.34%.

TABLE 1

Allocation of subjects to treatment

Allocation number	Period 1	Period 2	Period 3	Period 4
007, 023	A	B	C	D
001, 016	B	C	A	D
010, 024	C	A	B	D
009, 022	A	C	B	D
003, 019	B	A	C	D
002, 013	C	B	A	D
006, 015	A	B	C	E
012, 014	B	C	A	E
008, 017	C	A	B	E
004, 018	A	C	B	E
011, 020	B	A	C	E
005, 021	C	B	A	E

A, ^{13}C-odanacatib 1-mg i.v. solution coadministered with unlabeled odanacatib 1-mg i.v. solution in a fasted state; B, ^{13}C-odanacatib 1-mg i.v. solution after a 50-mg oral dose of odanacatib in a fasted state; C, ^{13}C-odanacatib 1 mg i.v. solution after a 10-mg oral dose of odanacatib in a fasted state; D, ^{13}C-odanacatib 1 mg i.v. solution after a 50-mg oral dose of odanacatib after a low-fat meal; E, ^{13}C-odanacatib 1 mg i.v. solution after a 50-mg oral dose of odanacatib after a high-fat meal.

PK Analysis

For i.v. treatments, plasma AUC from 0 to ∞ ($AUC_{0\text{-}\infty}$), $C_{end\text{-}of\text{-}infusion}$ (C_{eoi}), terminal $t_{1/2}$, clearance, and steady-state volume of distribution were derived from the concentration–time profiles of ^{13}C-odanacatib and unlabeled odanacatib for each individual. For oral treatments, plasma $AUC_{0\text{-}\infty}$, C_{max}, T_{max}, and apparent terminal $t_{1/2}$ were determined from the concentration–time profiles of unlabeled odanacatib for each individual. Certain PK parameters, including $AUC_{0\text{-}\infty}$ and $t_{1/2}$, could not be determined for all subjects due to insufficient data in the terminal phase.

Absolute bioavailability (F) was calculated as the dose-adjusted ratio of $AUC_{0\text{-}\infty}$ values for the oral and i.v. treatments, as shown below in eq. 1.

$$F(\%) = 100 \times \frac{Dose_{IV}}{Dose_{oral}} \times \frac{AUC_{oral}}{AUC_{IV}}. \quad (1)$$

Plasma concentrations for odanacatib, converted into molar units using the molecular weight of 525.57 g/mol for unlabeled odanacatib or 531.50 g/mol for ^{13}C-odanacatib, and actual sampling times were used to estimate PK parameters for each treatment in each subject. Apparent terminal $t_{1/2}$ values and $AUC_{0\text{-}\infty}$ values (the calculation of which requires terminal elimination rate constant) were determined only for those subjects in which at least three odanacatib plasma concentrations were collected at 96 hours postdose or greater, with at least one time point collected at 240 hours (approximately three times the estimated $t_{1/2}$ of odanacatib) or greater. For i.v. treatments, clearance was calculated as the ratio of dose to AUC, and steady-state volume was calculated as the product of clearance and mean residence time.

In some subjects, nonzero predose concentrations of odanacatib were observed as a result of treatment in a prior period. In all cases, these values were less than 5% of C_{max} and were not used to adjust the PK analysis. The PK analysis was conducted using the Phoenix software package (Certara, Princeton, NJ).

Statistical Analysis

Primary Estimation of Absolute Bioavailability. Individual ratios (F) of [dose-normalized (to 1 mg) 10-mg oral fasted $AUC_{0\text{-}\infty}$ over 1-mg labeled i.v. $AUC_{0\text{-}\infty}$], and [dose-normalized (to 1 mg) 50-mg oral fasted $AUC_{0\text{-}\infty}$ over 1-mg labeled i.v. $AUC_{0\text{-}\infty}$] were calculated within each treatment period and for each individual in the first three periods. Actual doses administered rather than planned doses were used in the analysis. A log transformation was applied to the individual ratio data and analyzed using a mixed-effects model appropriate for a three-period crossover design. The model contained treatment and period as fixed effects, and subject as a random effect with a compound symmetry–heterogeneous variance structure (i.e., the CSH covariance structure in SAS, in which variances can differ across different repeated measures, but covariances, across subjects, are identical)

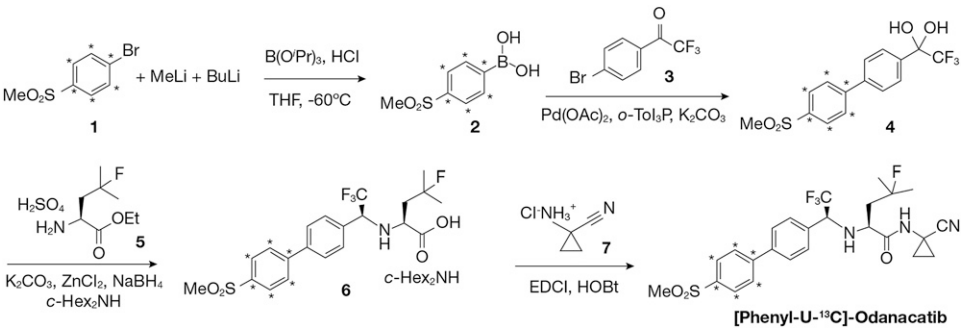

Fig. 1. Synthesis of ^{13}C-odanacatib.

(Kincaid, 2005) to model the errors. The assumption of compound symmetry covariance structure was examined by Levene's test, and the *P* value was 0.0295 (less than $\alpha = 0.05$), which indicates that the null hypothesis of equality of variances was rejected. As there was evidence of unequal variances, a heterogeneous compound symmetry covariance structure was used. The geometric mean and 90% confidence interval (CI) for true ratio *F* after 10- and 50-mg doses was generated from the above mixed-effects model to estimate the absolute bioavailability of 10- and 50-mg oral doses of odanacatib administered in the fasted state. Additional individual absolute bioavailability ratios of (dose-adjusted 50-mg oral with low-fat meal $AUC_{0-\infty}$ over 1-mg labeled i.v. $AUC_{0-\infty}$) and (dose-adjusted 50-mg oral with high-fat meal $AUC_{0-\infty}$ over 1-mg labeled i.v. $AUC_{0-\infty}$) were also estimated. A log transformation was applied to the individual ratio data and analyzed using a *t* test to generate the geometric mean and 90% CI.

Primary Estimation of Food Effect. A linear mixed-effects model with treatment (fasted, low-fat and high-fat) as a fixed effect and subject as a random effect was used to analyze log-transformed $AUC_{0-\infty}$ using data from a 50-mg oral dose of odanacatib in the fasted and fed states. Compound symmetry was used as the covariance structure. Two-sided 90% CIs were constructed for the geometric mean ratios (GMRs) (low-fat/fasted and high-fat/fasted) of $AUC_{0-\infty}$. In addition, 90% CIs were obtained for GMRs of C_{max}.

Absorption Modeling

A population PK model was developed to characterize the rate and duration of absorption, and to explore the mechanism of the secondary peaks in the odanacatib concentration–time profile. The population PK dataset contained 2562 PK samples from 24 postmenopausal women (age range 45 to 65 years). Measurements below the limit of quantification and missing values were excluded from the dataset. Nonlinear mixed-effects modeling was performed using NONMEM, version 7.2 (ICON Development Solutions, Ellicott City, MD) in a UNIX environment with Intel FORTRAN Complier, version 11.1 (Intel Corporation, Santa Clara, CA) using the Navigator interface (Mango Solutions, Chippenham, UK). Xpose (xpose.sourceforge.net), PsN (psn.sourceforge.net), and R (R-project, www.r-product.org, version 2.14.1 or higher) were used for exploratory analyses and postprocessing of NONMEM output.

Population PK model development was staged, with separate structural models for i.v. and oral data used as a starting point to characterize the distribution and absorption profiles, respectively, which were then combined into a base reference model. Because dose and food (light and heavy meal) effects on bioavailability were known (Stoch et al., 2009), these covariates were included a priori in the model. The influence of food on absorption was tested as a covariate in the model, the residual error model structure was evaluated, and outlier evaluation was conducted to arrive at a final model. Model selection was based on the log-likelihood criterion, goodness-of-fit plots, and scientific plausibility. Reliability of the final model was checked with diagnostic plots and visual predictive check. The final model included a Hill function to describe input from absorption to provide sufficient flexibility to capture a range of absorption behaviors:

$$Hill\ function = \frac{D_{max}.T^n}{\left(T_{50}^n + T^n\right)}, \quad (2)$$

where D_{max} is the maximum bioavailable dose, *T* is time, T_{50} is the time at which 50% of the drug is bioavailable, and *n* is the Hill factor. This was used to simulate the input profile after oral administration. Deconvolution of individual concentration–time profiles, using the corresponding i.v. profiles as reference, was also used to determine absorption profiles in a model-independent manner.

Safety

Subjects were queried daily regarding the occurrence of adverse experiences; none were suggested, and all reported adverse experiences were documented. Subjects were monitored throughout for adverse experiences. All adverse experiences reported by the subject or observed by the investigator were graded with respect to maximum intensity, seriousness, action taken, and relationship to the drug. Adverse experiences rated as possibly, probably, or definitely were considered to be drug related. Safety was also assessed by physical examination, collection of vital signs, electrocardiogram, hematology, serum chemistry, and urinalysis.

Linear Systemic Disposition of Odanacatib

To assess the linearity of the systemic disposition of odanacatib, the labeled i.v. PK parameter data from all five treatments were used in a mixed-effects model, with treatment as a fixed effect and subject as a random effect (and a compound symmetric covariance structure). An overall *F* test was used to test whether the labeled i.v. $AUC_{0-\infty}$ differed among the five treatments. Note that the study was not sufficiently powered for this test. If the test was not statistically significant at the significance level of 0.05, then there was not enough evidence from the study to claim that the i.v. PK was different among the five treatments; that is, the systemic disposition of odanacatib was linear, otherwise, it would be concluded that the systemic disposition of odanacatib is not linear. C_{eoi} was analyzed in a similar manner.

The Effect of ^{13}C Isotopic Labeling

The effect of the ^{13}C isotopic labeling of odanacatib was assessed by estimating the true ratio *F* after the unlabeled i.v. dose along with a 90% CI. If the 90% CI was contained within the interval (0.80–1.25), then it was concluded that odanacatib was not affected by ^{13}C isotopic labeling. Additional individual ratios testing the effect of the ^{13}C isotopic labeling, including 1-mg unlabeled i.v. C_{eoi} over 1-mg labeled i.v. C_{eoi} and 1-mg unlabeled i.v. terminal $t_{1/2}$ over 1-mg labeled i.v. terminal $t_{1/2}$ were also estimated. A log transformation was applied to the individual ratio data and analyzed using a *t* test to generate the geometric mean and 90% CI.

Results

Absolute Bioavailability at 10 and 50 mg

The absolute bioavailability of 10- and 50-mg oral doses of odanacatib was 70% and 30%, respectively, as presented in Table 2. The absolute bioavailability of 50-mg oral doses of odanacatib administered with low-fat and high-fat meals was 36% and 49%, respectively.

Determination of Volume of Distribution and Clearance

The volume of distribution at steady state (V_{ss}) of odanacatib was estimated to be approximately 100 liters. Because this exceeds the

TABLE 2

The absolute bioavailability of 10- and 50-mg oral doses of odanacatib

Dose and food status	N^a	Geometric mean (90% CI)
10 mg fasted	17	0.70 (0.64–0.77)
50 mg fasted	18	0.30 (0.27–0.34)
50 mg with low-fat meal	9	0.36 (0.31–0.42)
50 mg with high-fat meal	9	0.49 (0.43–0.57)

All doses listed above reflect oral administration, and were administered with a reference i.v. dose of 1 mg ^{13}C-labeled odanacatib to determine absolute bioavailability.

[a]N is less than 24 for fasted treatments and less than 12 for fed treatments because, for some subjects, $AUC_{0-\infty}$ could not be determined due to insufficient data in the terminal phase.

volumes of extracellular fluid (approximately 10 liters) and total body water (approximately 60 liters), this supports the idea that odanacatib distributes into tissues. Total clearance from plasma was estimated to be 0.8 l/h (approximately 13 ml/min or approximately 9 ml/min after taking into account an in vitro blood/plasma ratio of approximately 0.7), which is small relative to hepatic blood flow (approximately 1 to 1.5 l/min), supporting that odanacatib is a low extraction ratio drug. Detailed summary statistics for V_{ss} and total clearance can be found in Tables 3 and 4.

Effect of Low- and High-Fat Meals on Exposure/Bioavailability

In this study, administration with food moderately increased odanacatib concentrations and exposures, with higher fat content associated with greater increases, as shown in Fig. 2. Specifically, the administration with a low-fat meal resulted in increases of 15% for $AUC_{0-\infty}$ and 16% for C_{max} relative to fasted, whereas administration with a high-fat meal resulted in increases of 63% for $AUC_{0-\infty}$ and 91% for C_{max} relative to fasted (Table 5). Observed PK values for all treatment groups for both labeled and unlabeled odanacatib can be found in Tables 3 and 4.

Absorption Modeling

The PK of oral and i.v. administered unlabeled odanacatib were best described by a three-compartment model with first-order absorption and first-order elimination. The initial combined model used a first-order absorption rate, but the final model replaced this with a Hill function to describe absorption input as this allowed for greater flexibility in describing the range of absorption profile shapes. Model-based estimates of bioavailability were closely consistent with observed values, and, as shown in Fig. 3, periodic fluctuations in concentration after the first absorption peak (clearly visible in the mean profiles presented in Fig. 2; individual profiles for two individuals after oral doses are also included in Fig. 6; and i.v./oral profiles of 12 individuals are shown in Supplemental Fig. 1) were adequately described by a circadian rhythm function on the volume of the central compartment with the following equation:

$$\text{circ} = \cos((clock - acro).\pi/12).amp \quad (3)$$

where clock is the actual clock time of PK sampling, acro is the acrophase, and amp is the amplitude. This approach is compared graphically with model-independent deconvolution in Supplemental Fig. 2. Although the two approaches were qualitatively similar, a circadian rhythm–based model was better able to capture the periodic fluctuations in the profiles, which deconvolution treated as additional drug input. The population PK parameter estimates for the final i.v. and oral combined model of data from this study are included in Supplemental Table 1, and visual predictive check plots for this model are presented in Supplemental Fig. 3.

This model of odanacatib absorption allowed for simulation of the cumulative absorption profile. On average, the majority of the compound is absorbed between 6 and 10 hours postdose (i.e., in 97% of individuals, >50% of the amount of drug that will be absorbed has been absorbed by 10 hours postdose) with >80% absorbed within 24 hours (i.e., in 88% of individuals, >80% of the amount of drug that will be absorbed has been absorbed by 24 hours postdose), as illustrated in Fig. 4. In addition, Fig. 4 also illustrates that a high-fat meal increases the extent and slows the rate of absorption (i.e., the absorption rate constant) of odanacatib, whereas a low-fat meal more modestly increases the extent of absorption with no impact on rate.

Periodic Fluctuations, Evidence for Systemic Effect

Both oral and i.v. administration of odanacatib resulted in periodic fluctuations in the postdose concentration–time profiles, as illustrated in Fig. 5, which compares profiles after administration of 50 mg orally after fasting and 1 mg i.v., suggesting that these fluctuations are the result of systemic effects, rather than due to continued absorption in the second and third days postdose, after oral doses. In addition, the examination of individual concentration–time

TABLE 3

Summary statistics for PK parameters of unlabeled odanacatib after single doses of ^{13}C-odanacatib 1 mg i.v. with oral or i.v. doses of unlabeled odanacatib

PK parameter	Dose of unlabeled odanacatib				
	1 mg i.v. (N = 21–24)	10 mg fasted (N = 23–24)	50 mg fasted (N = 24)	50 mg with low-fat meal (N = 12)	50 mg with high-fat meal (N = 12)
	Geometric mean (%CV)				
$AUC_{0-\infty}$[a] (μM-h)	2.7 (41.0)	15.8 (40.3)	33.6 (44.3)	40.5 (55.9)	52.5 (40.2)
$AUC_{0\text{-last}}$[a] (μM-h)	2.1 (44.2)	14.9 (37.1)	33.1 (40.2)	37.0 (50.8)	49.7 (36.9)
C_{max}[a] (nM)	56.2 (43.9)	120.8 (19.9)	240.3 (20.6)	283.3 (20.4)	450.4 (19.7)
T_{max}[b] (h)		7.5 (1.7, 48.0)	6.0 (1.7, 96.0)	5.0 (2.8, 84.0)	10.5 (2.8, 24.1)
V_{ss}[a] (liters)	103.1 (20.4)				
Cl[a] (l/h)	0.7 (52.9)				
Terminal $t_{1/2}$[c] (h)	104.4 (32.7)	85.8 (21.7)	84.5 (19.7)	87.9 (20.9)	78.7 (13.2)
$AUC_{\%extrap}$[a]	11.0 (38.6)	6.6 (103.8)	6.0 (76.7)	6.4 (102.7)	4.5 (67.7)

N is less than 24 for certain PK parameters because for some subjects $AUC_{0-\infty}$ and $t_{1/2}$ values could not be determined due to insufficient data in the terminal phase. $AUC_{0\text{-last}}$, AUC from 0 to last time point; $AUC_{\%extrap}$, percentage of AUC extrapolated beyond last time point; Cl, clearance.

[a]Geometric means for indicated parameters are directly calculated, not model based.

[b]Values are reported as the median (minimum, maximum).

[c]Harmonic mean (pseudo-S.D.). Values are observed for terminal $t_{1/2}$ for i.v. treatments and apparent terminal $t_{1/2}$ for oral treatments.

TABLE 4

Summary statistics for PK parameters of ^{13}C-odanacatib after single doses of ^{13}C-odanacatib 1 mg i.v. with oral or i.v. doses of unlabeled odanacatib

PK parameter	Dose of unlabeled odanacatib				
	1 mg i.v. (N = 20–24)	10 mg fasted (N = 18–24)	50 mg fasted (N = 18–24)	50 mg low-fat meal (N = 9–12)	50 mg high-fat meal (N = 9–12)
	Geometric mean (%CV)				
$AUC_{0-\infty}$[a] (μM-h)	2.3 (30.7)	2.4 (32.4)	2.3 (23.1)	2.7 (15.8)	2.2 (26.7)
$AUC_{0\text{-last}}$[a] (μM-h)	1.8 (34.3)	1.8 (33.7)	1.8 (32.8)	2.0 (39.5)	1.8 (36.0)
C_{eoi}[a] (nM)	52.6 (43.2)	52.5 (34.1)	55.9 (39.5)	58.2 (27.5)	54.9 (29.2)
V_{ss}[a] (liters)	114.8 (17.4)	103.4 (19.7)	93.8 (14.8)	96.8 (17.3)	88.6 (16.8)
Cl[a] (l/h)	0.8 (37.8)	0.8 (40.5)	0.8 (35.0)	0.8 (41.6)	0.9 (35.2)
Terminal $t_{1/2}$[b] (h)	108 (27.3)	95.6 (29.9)	81.7 (21.0)	100.3 (19.9)	73.9 (21.0)
$AUC_{\%extrap}$[a]	10.7 (38.7)	8.9 (57.8)	7.6 (35.8)	8.0 (46.3)	6.5 (39.9)

N is less than 24 for certain PK parameters because for some subjects, $AUC_{0-\infty}$ and $t_{1/2}$ could not be determined due to insufficient data in the terminal phase. $AUC_{0\text{-last}}$, AUC from 0 to last time point; $AUC_{\%extrap}$, percentage of AUC extrapolated beyond last time point; Cl, clearance.

[a]Geometric means for indicated parameters are directly calculated, not model-based.

[b]Harmonic mean (pseudo-S.D.).

profiles (a representative set is shown in Supplemental Fig. 1) indicates that secondary fluctuations generally occur at the same time in oral and i.v. profiles.

As the nature of these fluctuations is not fully understood, two different possible mechanisms that could explain the secondary peaks were explored as part of the population PK modeling. First, the increase of odanacatib plasma concentrations every 24 hours was modeled as an extra input from a peripheral compartment into the central compartment; this structure was intended to mimic an enterohepatic recycling mechanism. Alternatively, a circadian rhythm function on the volume of the central compartment was introduced to mimic a mechanism of diurnal variation in plasma protein concentrations, and thus the distribution of odanacatib (which is highly protein bound) between plasma and tissue. Both model structures could describe the fluctuations satisfactorily, and thus the results support that either mechanism is feasible. The second, circadian rhythm, model was selected as it was more parsimonious and resulted in shorter runtimes.

In several phase 1 studies (Stoch et al., 2013), including this one, multiple peaks were observed in individual concentration–time profiles, typically an initial peak at around 4–10 hours and a peak at 24 hours or later, which was occasionally higher than the first peak. Thus, T_{max} values of 24 hours or as late as 72 hours have been observed. Results from the population PK modeling indicate that, although absorption is largely finished within 24 hours, in some individuals late T_{max} values can arise from a combination of a sustained minor absorption component combined with the periodic fluctuations in concentration (described in the model as a circadian function on the volume of the central compartment), as illustrated in Fig. 6, which shows observed concentration–time profiles for two individuals. Note that even when late T_{max} values occur, a majority of the bioavailable drug is still absorbed during the first day (i.e., delayed absorption is not the cause of the late T_{max} values).

Safety

Odanacatib was generally well tolerated in postmenopausal women in this study. No serious clinical adverse experiences were reported, and no subject discontinued participation in the study because of an

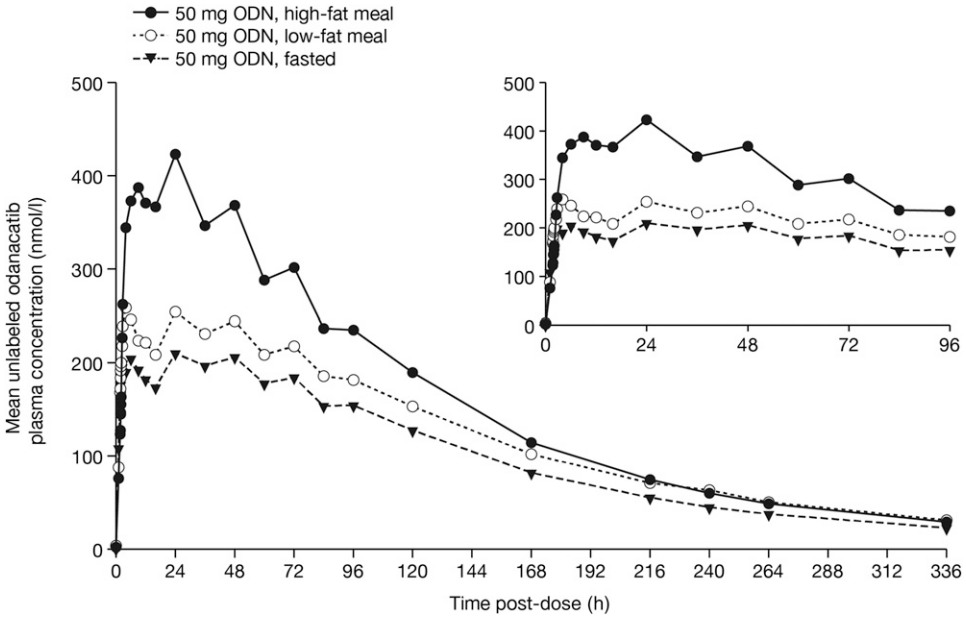

Fig. 2. Odanacatib exposures were increased when administered with food (N = 24 for fasted, N = 12 for high-fat and low-fat meals). ODN, odanacatib.

TABLE 5

Administration with food moderately increases odanacatib plasma concentrations and exposures

PK parameter	Low-fat meal/fasted	High-fat meal/fasted
$AUC_{0-\infty}$	1.15 (1.04–1.28)	1.63 (1.47–1.81)
C_{max}	1.16 (1.07–1.25)	1.91 (1.77–2.07)
T_{max}[a]	5.0 (2.8, 84.0)	10.5 (2.8, 24.1)
$t_{1/2}$[b]	87.9 (20.9)	78.7 (13.2)

Data are reported as the GMR (90% CI), unless otherwise indicated.
[a]Median values (minimum, maximum), not ratio, are reported for T_{max}.
[b]Harmonic mean values (jack-knife S.D.), not ratio, are reported for $t_{1/2}$.

adverse experience. All adverse experiences were rated mild in intensity, with the exception of one instance of headache that was rated moderate. The most common adverse experience was headache, which was reported by eight subjects. No laboratory adverse experiences were reported. There were no consistent treatment-related changes in laboratory values, vital signs, or electrocardiogram safety parameters.

Systemic Linearity

The PK of 1 mg i.v. ^{13}C-labeled odanacatib, as assessed by the $AUC_{0-\infty}$ and C_{eoi}, were statistically similar (P values of 0.515 and 0.823, respectively; see Supplemental Table 2).

In addition, population PK modeling also supported the linearity disposition for all treatments over the dose range studied of 1–50 mg, with less-than-dose-proportional PK being due to dose-dependent bioavailability, rather than to nonlinear disposition. These two complementary assessments together support that the systemic disposition of odanacatib appears to be linear.

Effect of Isotopic Labeling

The geometric mean $AUC_{0-\infty}$ ratio for unlabeled/^{13}C-labeled was 1.18 (90% CI 1.12–1.24), falling within the prespecified bounds of 0.8–1.25 (Supplemental Table 3). In addition, C_{eoi} and terminal $t_{1/2}$ values were similar. Taken together, these data confirm that ^{13}C-isotopic labeling of odanacatib does not substantially alter the PK of i.v.-administered odanacatib.

Discussion

This study used concurrent administrations of ^{13}C-labeled and unlabeled odanacatib to allow for simultaneous assessments of i.v. and oral PK. In the past, this approach has been used to study the PK of drugs with known or suspected clearance nonlinearities (Yeh et al., 1999), but in this case, the aim was primarily to use the reference stable-labeled i.v. treatment to gain a clearer understanding of the absorption profile for a low–intrinsic clearance drug, including to better understand which aspects of the oral concentration–time profile derived from absorption versus systemic phenomena (i.e., distribution, metabolism, or elimination).

Compared with a traditional (i.e., crossover) bioavailability study design, the use of a stable-label approach was particularly useful in

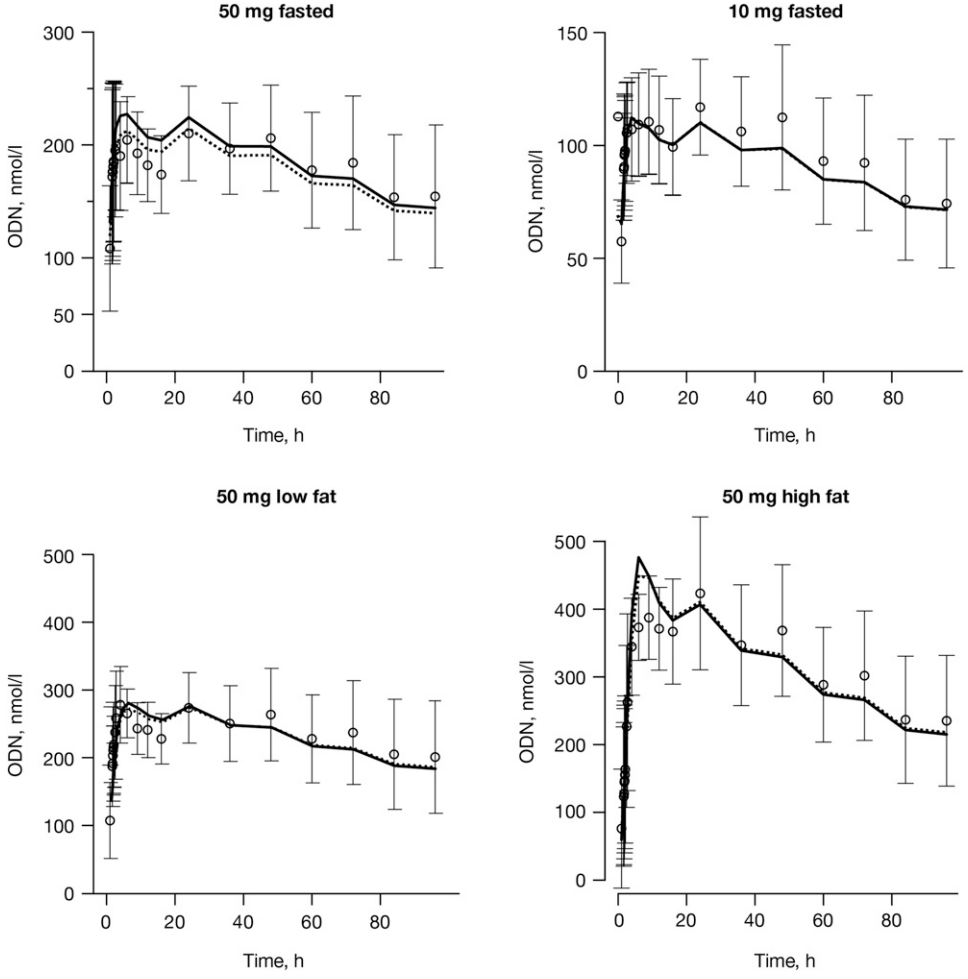

Fig. 3. Population PK model predictions are consistent with observed data (only the first 96 hours postdose are shown for clarity). Dotted line, mean individual i.v. PK profile (including covariate effects); solid line, mean population i.v. PK profile; symbol: mean (S.D.) observed data. ODN, odanacatib.

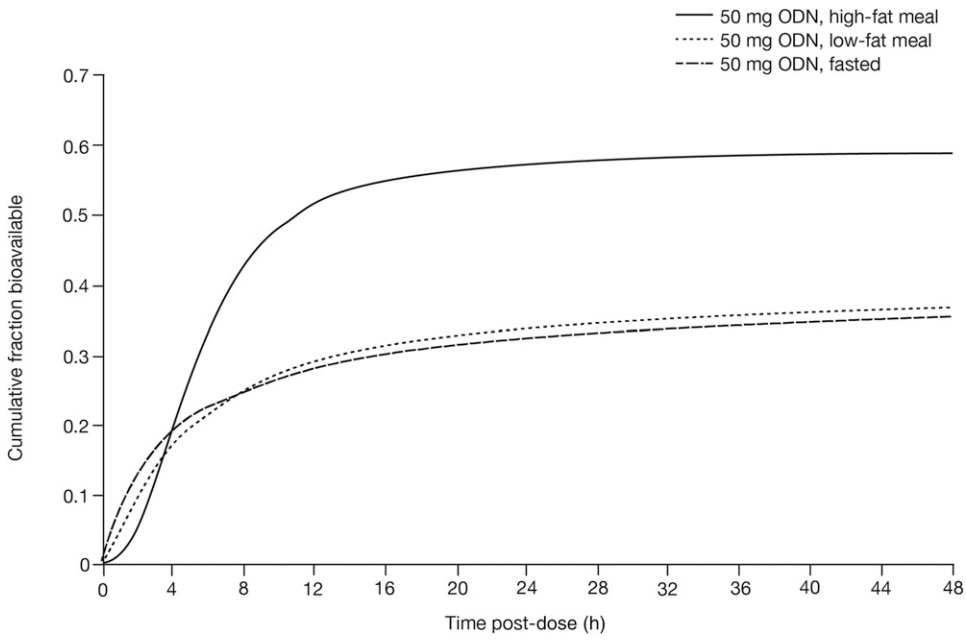

Fig. 4. Simulated mean cumulative absorption profiles (using model with Hill function) illustrate that odanacatib absorption is well behaved in both the fed and fasted states. ODN, odanacatib.

this study because it allowed the evaluation of oral and i.v. profiles simultaneously in the same individuals to provide information about whether certain features of the profiles (e.g., secondary concentration peaks) were due to absorption behavior or factors related to systemic disposition. This approach also corrects for any potential systemic nonlinearities, should they exist, and still provides accurate estimates of bioavailability, unlike crossover designs. Further, this design also produced a dataset that was well suited for model-based assessment of the absorption rate and profile.

The mean oral bioavailability of odanacatib at 50 mg in fasted conditions was moderate at 30%, with reasonable precision (90% CI 27–34%). Odanacatib $AUC_{0\text{-}\infty}$ increased by 15% and 63% when 50 mg was administered with low-fat and high-fat meals, corresponding to bioavailabilities of 36% and 49%, respectively. This is consistent with the expectation that dietary lipids may improve the solubility, and thus bioavailability, of a lipophilic biopharmaceutics classification system class II molecule such as odanacatib (log P = 3.11). This magnitude of food effect is unlikely to be clinically meaningful, which is consistent with the decision to conduct the phase 3 trial for odanacatib with dosing without regard to food (Bone et al., 2015).

The rate and duration of odanacatib absorption is fairly consistent, despite widely varying T_{max} values. Most absorption occurs within 24 hours of dosing, with consistent absorption in the first 10 hours postdose in all subjects with a median of 70% of the bioavailable drug absorbed. Continued absorption results in >80% of the bioavailable drug being absorbed within 24 hours for the majority of subjects (88%).

Both oral and i.v. administration of odanacatib resulted in periodic fluctuations in postdose concentration–time profiles (both mean and individual), suggesting that these fluctuations are due to

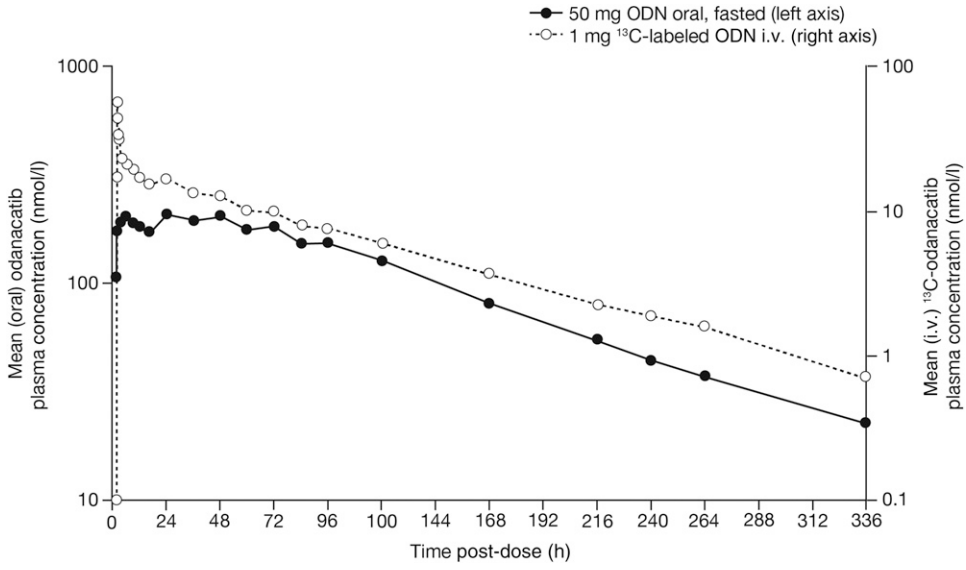

Fig. 5. Periodic fluctuations occur in concentration–time profiles after both oral and i.v. administration (note: left *y*-axis is abbreviated for clarity). ODN, odanacatib.

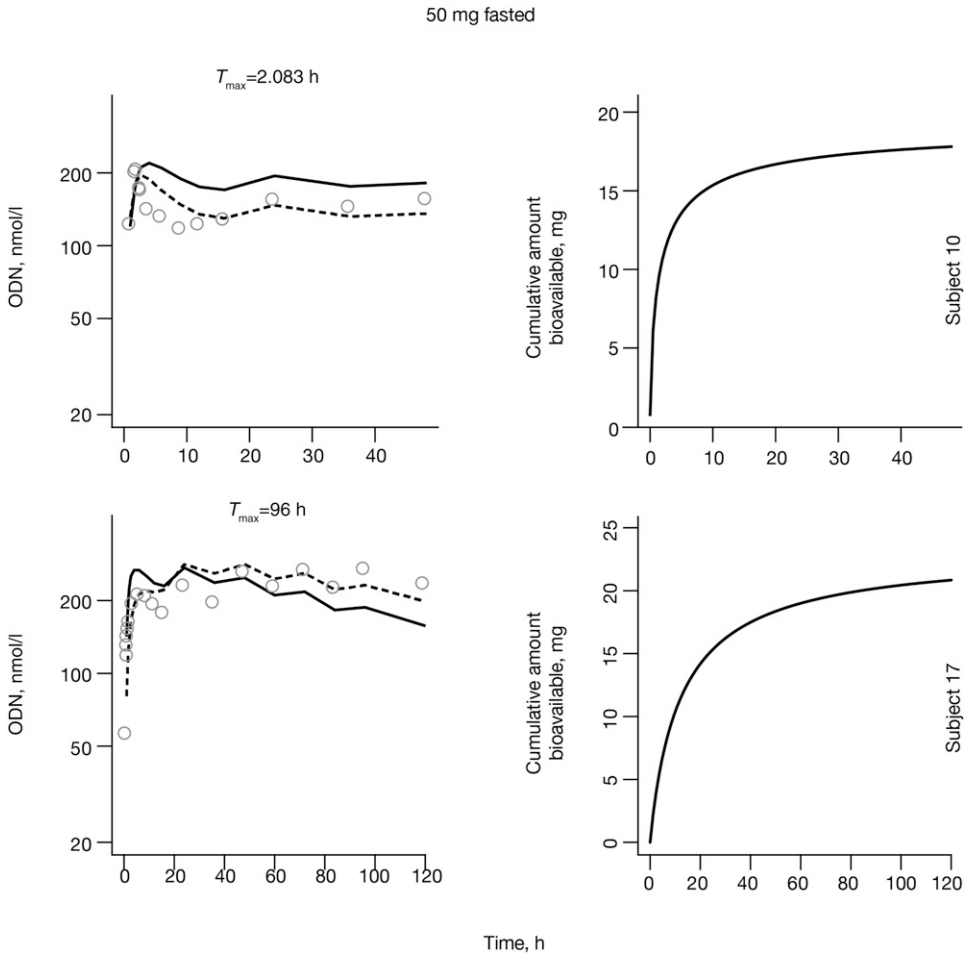

Fig. 6. Late T_{max} values are likely due to a combination of moderate sustained absorption and periodic fluctuation in plasma concentrations: representative examples of model predictions. Dashed black line, individual fit (including covariate effects); gray symbols, observed data; solid black line, population fit (typical profile). ODN, odanacatib.

systemic effects, rather than delayed absorption. Although they have been observed in most phase 1 studies of odanacatib after either single or multiple doses, the cause of these fluctuations is unknown. Potential mechanisms for this behavior include enterohepatic recirculation of intact drug eliminated by hepatic efflux or circadian variation in endogenous levels of plasma proteins or lipoproteins to which odanacatib binds. Lipoprotein binding can alter the PK of lipophilic drugs (Wasan et al., 2008), and the magnitude of the variation observed in this study with odanacatib (approximately 10% of total distribution volume) is consistent with the diurnal variation observed in lipoprotein levels (Bremner et al., 2000). Overall, these fluctuations are relatively small and unlikely to be clinically relevant for a drug that will be administered chronically.

The linearity of disposition was confirmed across a dose range of 1–50 mg by both statistical comparison and model-based assessment. Thus, it is reasonable to continue to attribute the less than dose-proportional oral PK to dose-dependent bioavailability associated with low solubility and saturable absorption.

The estimates of volume (~100 liters) and clearance (~0.8 l/h) observed in this study support the idea that odanacatib distributes into tissues and is a low–extraction ratio drug, respectively. Based on the amounts of odanacatib recovered in feces and urine in the human absorption, distribution, metabolism, and excretion study, it can be estimated that metabolism (principally mediated by CYP3A) and excretion of intact parent compound account for approximately 70% and 30%, respectively, of the clearance of odanacatib in humans (calculations supporting this estimate are described in detail by Kassahun et al., 2014; absorbed drug in feces accounts for approximately 20% of the dose).

In summary, the stable-label i.v./oral design used for this study was valuable in providing insights beyond its previous use to characterize bioavailability in settings of saturable or nonlinear clearance. In addition, results from this study provide a clear understanding of the absorption properties of odanacatib in the intended patient population of postmenopausal women, further supporting its use for the treatment of osteoporosis.

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Authorship Contributions

Participated in research design: Zajic, Hreniuk, Witter, Helmy, Joss, Stone, and Stoch

Conducted experiments: Zajic, Hreniuk, Sun, Witter, Gauthier, Helmy, Ni, Stoltz, and Stoch

Contributed new reagents or analytic tools: Sun, Gauthier, Joss, and Stoch

Performed data analysis: Zajic, Rossenu, Kesisoglou, McCrea, Liu, Sun, Helmy, and Stoch

Wrote or contributed to the writing of the manuscript: Zajic, Rossenu, Kesisoglou, McCrea, Liu, Sun, Witter, Gauthier, Helmy, Stone, and Stoch

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Address correspondence to: Stefan Zajic, Merck & Co., Inc., 2000 Galloping Hill Road, Kenilworth, NJ 07033. E-mail: szajic@gmail.com.