

Altering Metabolic Profiles of Drugs by Precision Deuteration 2: Discovery of a Deuterated Analog of Ivacaftor with Differentiated Pharmacokinetics for Clinical Development^{SI}

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ABSTRACT

Ivacaftor is currently used for the treatment of cystic fibrosis as both monotherapy (Kalydeco; Vertex Pharmaceuticals, Boston, MA) and combination therapy with lumacaftor (Orkambi; Vertex Pharmaceuticals). Each therapy targets specific patient populations: Kalydeco treats patients carrying one of nine gating mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) protein, whereas Orkambi treats patients homozygous for the F508del CFTR mutation. In this study, we explored the pharmacological and metabolic effects of precision deuteration chemistry on ivacaftor by synthesizing two novel deuterated ivacaftor analogs, CTP-656 (*d*₉-ivacaftor) and *d*₁₈-ivacaftor. Ivacaftor is administered twice daily and is extensively converted in humans to major metabolites M1 and M6; therefore, the corresponding deuterated metabolites were also prepared. Both CTP-656 and *d*₁₈-ivacaftor showed in vitro pharmacologic potency similar to

that in ivacaftor, and the deuterated M1 and M6 metabolites showed pharmacology equivalent to that in the corresponding metabolites of ivacaftor, which is consistent with the findings of previous studies of deuterated compounds. However, CTP-656 exhibited markedly enhanced stability when tested in vitro. The deuterium isotope effects for CTP-656 metabolism ($k^V = 3.8$, $k^V/K = 2.2$) were notably large for a cytochrome P450-mediated oxidation. The pharmacokinetic (PK) profile of CTP-656 and *d*₁₈-ivacaftor were assessed in six healthy volunteers in a single-dose crossover study, which provided the basis for advancing CTP-656 in development. The overall PK profile, including the 15.9-hour half-life for CTP-656, suggests that CTP-656 may be dosed once daily, thereby enhancing patient adherence. Together, these data continue to validate deuterium substitution as a viable approach for creating novel therapeutic agents with properties potentially differentiated from existing drugs.

Introduction

Deuterium labeling of compounds has a long history of in vitro and in vivo use, primarily as a method to probe enzymatic mechanisms and metabolic pathways or to allow nonradioactive isotopic tracing (Rittenberg and Schoenheimer, 1937; Baillie, 1981). Deuterated agents as pharmaceuticals are an area of growing interest, as shown by the number of deuterated compounds entering clinical development (Halford, 2016). Additionally, the first deuterated compound, deutetrabenazine (Austedo, 2017), has recently been approved. A key feature of this technology is that deuterium substitution generally has minimal impact on the structure,

physical properties, or pharmacology compared with the all-hydrogen version of a drug. However, deuterium substitution can have a marked effect upon the pharmacokinetics (PK) profile of a compound due to the deuterium isotope effect (DIE), which can lower the rate of carbon–deuterium versus carbon–hydrogen bond cleavage (Kushner et al., 1999; Nelson and Trager, 2003; Harbeson and Tung, 2011). Although the theoretical limit for the DIE is 6-fold to 9-fold, effects this large are rarely observed for enzyme-catalyzed reactions (Northrop, 1975; Jenks, 1987). The magnitude of the DIE for a cytochrome P450-mediated oxidation is unpredictable (Fisher et al., 2006; Harbeson and Tung, 2014). Due to this unpredictability, deuterium-substituted analogs must be synthesized and tested in preclinical studies to assess the magnitude and direction of the DIE. Even then, preclinical observations may not quantitatively, or potentially even qualitatively, predict human clinical results.

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ABBREVIATIONS: AUC, area under the curve; AUC_{0–24hr}, area under the curve from time 0 to 24 hours postdose; AUC_{0–inf}, area under the curve from time 0 extrapolated to infinity; C_{24hr}, concentration at 24 hours postdose; C_{max}, maximum plasma concentration; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; CTP-656, N-(2-(tert-butyl)-4-(tert-butyl-*d*₉)-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide; DIE, deuterium isotope effect; DMSO, dimethylsulfoxide; GLP, good laboratory practice; HBE, human bronchial epithelial; HLM, human liver microsomes; HPLC, high-performance liquid chromatography; I_{eq}, equivalent current; IS, internal standard; K_m, concentration of substrate at one-half V_{max}; LC, liquid chromatography; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MRM, multiple reaction monitoring; PK, pharmacokinetics; SAD, single ascending dose; t_{1/2}, half-life.

The first article in this series presented the data for CTP-347, a deuterated analog of paroxetine (Paxil; GlaxoSmithKline, Research Triangle Park, NC), which is a selective serotonin reuptake inhibitor for the treatment of a number of psychiatric disorders (Paxil, 2012; Uttamsingh et al., 2015). Herein, we report the effects of selective deuterium substitution on another therapeutic agent, ivacaftor (Fig. 1). Ivacaftor (Kalydeco; Vertex Pharmaceuticals, Boston, MA) was approved in 2012 as the first drug to target specific mutational defects in the cystic fibrosis (CF) transmembrane conductance regulator (CFTR) protein (Kalydeco, 2015). It is currently labeled for the treatment of CF patients 2 years and older who have at least one mutant allele from a group of nine gating mutations in the CFTR gene, with G551D being the most common (4.4% of CF patients in the United States) (Aditya, 2012; Kalydeco, 2015). In 2016, ivacaftor was approved as part of the fixed-dose combination drug Orkambi (Vertex Pharmaceuticals), which combines ivacaftor and lumacaftor for the treatment of CF patients 12 years and older homozygous for the F508del CFTR mutation (approximately 45% of CF patients in the United States) (Wainwright et al., 2015; Orkambi, 2016). Lumacaftor is a CFTR “corrector” that improves the formation of active CFTR at the epithelial surface of cells (Van Goor et al., 2011). The CFTR protein is an anion channel that regulates epithelial chloride and bicarbonate concentrations to maintain proper hydration and pH of secretory epithelia (Riordan, 2008). CFTR mutations reduce anion secretion, and the resulting dysregulation of anion concentrations promotes the accumulation of thick, viscous mucus. Ivacaftor is a CFTR “potentiator” and increases the channel open probability, thereby increasing anion conductance, improving mucus hydration and decreasing mucus viscosity (Van Goor et al., 2009). Viscous mucus is particularly problematic for lung function since it results in an inability to efficiently clear airways, which leads to chronic inflammation and infections that contribute to a decline in lung function over time (Ong and Ramsey, 2015).

In this study, deuterated analogs of ivacaftor (Fig. 1) were prepared and characterized with respect to pharmacology, in vitro metabolism, and in vivo PK. Since ivacaftor is extensively metabolized in humans to two major circulating metabolites, M1 and M6, these metabolites and the corresponding deuterated metabolites d_8 -M1 and d_6 -M6 were also prepared and studied (Fig. 2) (Zha et al., 2011). Two compounds— d_9 -ivacaftor (CTP-656) and d_{18} -ivacaftor—showed increased metabolic stability versus ivacaftor in vitro. Deuterium modification did not impact pharmacology, as the two deuterated compounds demonstrated equivalent potency to ivacaftor in multiple in vitro studies. In vitro metabolic stabilization did translate to increases in in vivo exposure parameters when the compounds were orally dosed in rats and dogs, although species differences were observed. Subsequently, both compounds were dosed in healthy volunteers in a single-dose

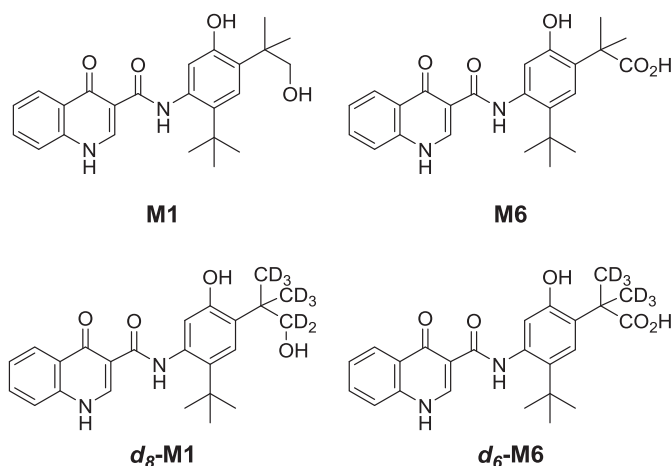
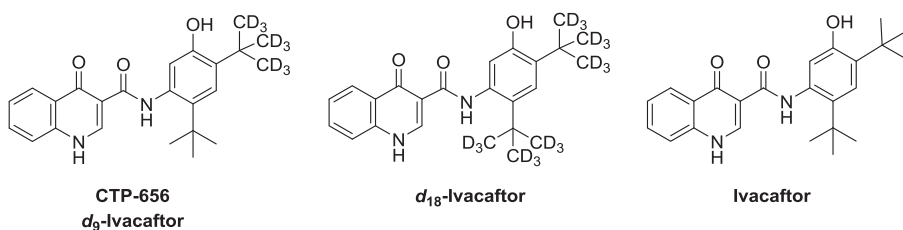


Fig. 2. CTP-656 and ivacaftor are metabolized in humans by CYP3A to two major metabolites.

crossover study. The new chemical entity CTP-656 was identified as the agent for further clinical advancement because of its general trend of increased exposure versus d_{18} -ivacaftor. The observed increase in exposure and half-life ($t_{1/2}$) compared with that reported for ivacaftor supported the potential for CTP-656 to be dosed once daily. These data continue to validate deuterium substitution as a viable approach to creating novel therapeutic agents with properties potentially differentiated from existing drugs.

Materials and Methods

Reagents

Indiplon, CTP-656, d_{18} -ivacaftor, ivacaftor, M1, M6, d_8 -M1, and d_6 -M6 were provided by Concert Pharmaceuticals, Inc. (Lexington, MA). Human liver microsomes (HLM; mixed gender, pool of 200, 20 mg/ml) were from Xenotech, LLC (Lenexa, KS). Cryopreserved human hepatocytes were purchased from In Vitro ADMET Laboratories (Malden, MA). Human cDNA expressed CYP3A4 Supersomes (Corning, Corning, NY) were obtained from BD Biosciences (Woburn, MA). Dimethylsulfoxide (DMSO), acetonitrile, $MgCl_2$, and NADPH were from Sigma-Aldrich (St. Louis, MO) and PEG400 was from J.T. Baker (Center Valley, PA). CF-HBE (human bronchial epithelial) cells were obtained from the Cystic Fibrosis Foundation Therapeutics organization. Forskolin was obtained from LC Laboratories (Woburn, MA). Benzamil and bumetanide were obtained from Sigma-Aldrich. HBE studies were conducted in the laboratory of Dr. Robert Bridges at Rosalind Franklin University of Medicine and Science, Chicago Medical School (North Chicago, IL).

Synthetic Procedures for the Synthesis of CTP-656, d_{18} -Ivacaftor, d_8 -M1, d_6 -M6, M1, and M6

The synthetic methods for the preparation of CTP-656 and d_{18} -ivacaftor have been previously described (Morgan, 2015). Analytical

Fig. 1. Structures of CTP-656 (d_9 -ivacaftor), d_{18} -ivacaftor, and ivacaftor. D, deuterium atom.

characterization of CTP-656 can be found in the Supplemental Material. These include liquid chromatography (LC) purity assessment (Supplemental Fig. 1), mass spectrum (Supplemental Fig. 2), and the $^1\text{H-NMR}$ spectrum (Supplemental Fig. 3). Analytical data for d_{18} -ivacaftor in the Supplemental Material include LC purity assessment (Supplemental Fig. 4), mass spectrum (Supplemental Fig. 5), and the $^1\text{H-NMR}$ spectrum (Supplemental Fig. 6). Synthetic routes and methods for the preparation of CTP-656 metabolites d_8 -M1 and d_6 -M6 are also in the Supplemental Material (Supplemental Figs. 7 and 8, respectively). Analytical data for d_8 -M1 and d_6 -M6 in the Supplemental Material include LC purity assessments (Supplemental Fig. 9), mass spectra (Supplemental Fig. 10), and $^1\text{H-NMR}$ spectra (Supplemental Fig. 11). The M1 metabolite of ivacaftor was prepared by a modification of a synthetic method previously described (Yang et al., 2010). The modified synthetic route (Supplemental Fig. 12) and the methods for the preparation of M1 can be found in the Supplemental Material. Ivacaftor metabolite M6 was prepared as previously described (Yang et al., 2010). Analytical data for M1 and M6 in the Supplemental Material include LC purity assessments (Supplemental Fig. 13), mass spectra (Supplemental Fig. 14), and $^1\text{H-NMR}$ spectra (Supplemental Fig. 15).

Bioanalytical Methods

Preclinical Non-GLP Methods. All plasma samples, in vitro samples, quality control, and standards were treated with acetonitrile during protein precipitation. Acetonitrile containing internal standard (IS; indiplon) was then added, and samples were vortexed at 1450 rpm for 3 minutes and then centrifuged at 3500 rpm for 10 minutes. Ten microliters of prepared sample was injected onto a high-performance LC (HPLC)-tandem quadrupole mass spectrometer (LC-MS/MS), consisting of an Agilent (Santa Clara, CA) 1200 HPLC spectrometer with a Leap HTS PAL auto-sampler (Leap Technologies, Morrisville, NC) equipped with a C6 Gemini phenyl 2.1 \times 50 mm column maintained at 30°C. Analytes were eluted from the column using a gradient, where mobile phase B was linearly increased from 0% to 100% over 1 minute and then returned to 0% over another minute. The composition of mobile phase B was 0.1% formic acid in acetonitrile, whereas the composition of mobile phase A was 0.1% formic acid in reverse osmosis-deionized water.

LC-MS/MS analysis was performed using atmospheric pressure chemical ionization, followed by multiple reaction monitoring (MRM) of each analyte, including the IS. Transitions monitored were 393.2/172.2 m/z for ivacaftor, 402.2/172.2 m/z for CTP-656, 411.2/172.2 m/z for d_{18} -ivacaftor, 417.2/172.2 m/z for d_8 -M1, and 409.2/172.2 m/z for M1, whereas the transition for indiplon (the IS) was 377.2/293.2 m/z. For all analytes, a dwell time of 100 milliseconds was used.

Clinical GLP Method. The determination of CTP-656 and d_{18} -ivacaftor in plasma was performed using a validated method under by CPR Pharma Services (Thebarton, SA, Australia) conducted in compliance with CPR Pharma Standard Operation Procedures. Plasma analysis was performed after protein precipitation with 99% acetonitrile and 1% formic acid using ivacaftor as the IS. The analytes were separated by HPLC using a C18 column (50 \times 2 mm, 3 μm ; Luna C18; Phenomenex, Torrance, CA), where mobile phase A consisted of 10% acetonitrile and 90% water with 0.1% formic acid and mobile phase B consisted of 50% acetonitrile, 50% methanol with 0.1% formic acid. Elution of the analytes from the column was performed at a flow rate of 0.3 ml/min using a gradient where the percentage of mobile phase B was increased linearly from 55% to 100% over 2.5 minutes and held at 100% for 1 minute, after which the mobile phase composition was set to 10% mobile phase B for 0.4 minutes and then returned to initial conditions (55% mobile phase B) for re-equilibration. The total run time for the method was 6 minutes.

The analysis of the eluates was performed using a mass spectrometry system (API 4000 System; AB Sciex, Framingham, MA) in positive MRM mode. The MRM transitions monitored were 402.2/172.2 m/z for CTP-656 and 411.2/172.2 m/z for d_{18} -ivacaftor. Ivacaftor was the IS for both CTP-656 and d_{18} -ivacaftor. For all analytes, the method was linear, and in all

cases the precision and accuracy met the criteria of the Food and Drug Administration and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use.

In Vitro Pharmacology Assessments

Chloride conductance was evaluated with an equivalent current (I_{eq}) assay system using fully differentiated primary cultures of CF-HBE cells grown at an air/liquid interface for 4–6 weeks. Transepithelial voltage and transepithelial conductance were measured under current-clamp conditions using a custom-designed 24-channel current clamp (TECC-24; ChanTest Corporation, Cleveland, OH) and a 24-well electrode manifold. For all experiments, cells were tested in symmetric chloride solution (HEPES-buffered Coon's F-12 modified liquid media without serum or bicarbonate) at $36 \pm 0.5^\circ\text{C}$, and all compounds and test articles were dissolved in DMSO. For chloride conductance measurements, epithelial sodium channels were blocked with 3 μM benzamil; potentiator responses were measured after 10 μM forskolin addition. CFTR specificity was determined by blocking chloride secretion with 20 μM bumetanide. Potentiator responses were quantified by calculating the total peak area under the curve (AUC) of I_{eq} after addition of test article. For studies using a corrector, lumacaftor was added at a concentration of 3 μM 24 hours prior to chloride conductance measurements. All compounds were assayed in triplicate at a single concentration per well, and AUC values were fit to a Hill equation to yield EC_{50} values.

Metabolic Stability in CYP3A4 Supersomes

Stock solutions (10 mM) of CTP-656, d_{18} -ivacaftor, and ivacaftor were prepared in DMSO. The stock solutions were diluted to 50 μM in acetonitrile. Human CYP3A4 Supersomes (1000 pmol/ml) were diluted to 62.5 pmol/ml in 0.1 M potassium phosphate buffer, pH 7.4, containing 3 mM MgCl_2 . Metabolic stability in the diluted Supersomes was assessed as previously described (Morgan, 2015).

Semiquantitative Metabolite Profile in Human Hepatocytes

Stock solutions (5 mM) of CTP-656, d_{18} -ivacaftor, and ivacaftor were prepared in DMSO. Cryopreserved human hepatocytes were thawed, diluted in cold thawing media, and centrifuged. The supernatants were removed, and the cells were resuspended in Krebs-Henseleit buffer. The test articles were added to the hepatocytes in Krebs-Henseleit buffer to achieve a final concentration of 25 μM in 0.5 ml, containing 4×10^6 cells/ml hepatocytes. The reaction mixtures were incubated at 37°C in a cell culture incubator (5% CO_2). Aliquots of the mixture were removed at 0 and 4 hours, and acetonitrile was added. Samples were transferred to microcentrifuge tubes and centrifuged at 10,000g for 8 minutes to pellet the precipitated proteins. Supernatants were analyzed for amounts of the parent remaining and metabolites formed by LC-MS/MS. UV-visible light at 254 nm was used for semiquantitative analysis, wherein metabolites were measured as percentage of parent peak area. Analysis was by UV-visible light since metabolite standards had not been synthesized at the time of this experiment.

Determination of DIE for CTP-656 in HLM

Stock solutions (50 mM) of test compounds were prepared in DMSO. The 20 mg/ml HLM were diluted to 0.625 mg/ml in 0.1 M potassium phosphate buffer, pH 7.4, containing 3 mM MgCl_2 . Aliquots (375 μl) of the HLM and test compound mixtures were transferred to wells of a deep 96-well plate in triplicate and prewarmed for 7 minutes. Reactions were initiated by the addition of 125 μl of prewarmed NADPH solution. The final reaction volume was 0.5 ml and contained 0.5 mg/ml HLM; 1, 2, 5, 10, 20, 40, 60, 80, and 100 μM ivacaftor or CTP-656; 2 mM NADPH; and 3 mM MgCl_2 . The reaction mixtures were incubated at 37°C for 10 minutes, after which 50 μl aliquots were

removed and added to shallow 96-well plates. The reactions were stopped by the addition of 50 μ l of ice-cold acetonitrile with IS. The plates were stored at 4°C for 20 minutes, after which 100 μ l of water was added to the wells before centrifugation to pellet precipitated proteins. Supernatants were transferred to another 96-well plate and analyzed for parent and metabolite by LC-MS/MS using an AB Sciex API 4000 mass spectrometer.

The kinetic parameters K_m (concentration of substrate at one-half V_{max}) and V_{max} (maximum velocity) were calculated from the Michaelis-Menten equation. The enzyme velocity (enzyme velocity = concentration of hydroxyl methyl/time/protein concentration) from three separate experiments was analyzed by GraphPad Prism version 6.04 (GraphPad Software, San Diego, CA), and reported values for K_m and V_{max} were determined from the fit using the average value.

Preclinical PK Studies

All animal studies were carried out in accordance with the Guide for the Care and Use of Laboratory Animals, as adopted and promulgated by the US National Institutes of Health, and were approved by the National Institutes of Health Animal Care and Use Committee or the local equivalent.

PK of CTP-656, d_{18} -Ivacaftor and Ivacaftor in Rats. Male Sprague-Dawley rats (234–276 g) were obtained from Harlan Laboratories (Indianapolis, IN) and housed at VivoPath, LLC (Worcester, MA), where the studies were conducted. The PK of CTP-656, d_{18} -ivacaftor, and ivacaftor in rats were assessed as previously described (Morgan, 2015). The plasma PK parameters for the dosed compounds were calculated by noncompartmental analysis using Phoenix 64 WinNonlin software (Certara, Princeton, NJ).

PK of CTP-656, d_{18} -Ivacaftor, and Ivacaftor in Dogs. Four male beagle dogs were housed at Agilux Laboratories (Worcester, MA), where the studies were conducted. The dogs were administered 3 mg/kg each test compound orally in a discrete-dose crossover study with a 1-week washout period between the dosing phases. All dogs were fasted overnight before the administration of each test article. Each compound was formulated as a solution in 100% PEG400 at a concentration of 1.5 mg/ml. Blood samples were collected from each dog at 15 and 30 minutes, and at 1, 2, 4, 6, 8, 16, 24, 48, 72, 84, and 96 hours postdose. Blood samples were centrifuged to obtain plasma samples that were analyzed for concentrations of the dosed compound at each time point using LC-MS/MS. The limit of quantitation of each compound was 1 ng/ml. The plasma PK parameters for the dosed compounds were calculated by noncompartmental analysis using Phoenix 64 WinNonlin software (Certara).

Evaluation of PK Profiles of CTP-656 and d_{18} -Ivacaftor in Healthy Human Volunteers

The first-in-human study was a two-part open-label, randomized, sequential, crossover, single-dose study to evaluate the safety, tolerability, and PK of CTP-656 and d_{18} -ivacaftor amorphous dispersion aqueous suspension in healthy volunteers, with a PK comparison with ivacaftor. This article will report on the first part of this clinical study, which was a single-dose, randomized, two-period, two-treatment, crossover study conducted in healthy male and female volunteers to evaluate the safety, tolerability, and PK of CTP-656 and d_{18} -ivacaftor under fasted conditions. The study was performed at a

single center [CMAX (a division of IDT Australia Limited), Adelaide, SA, Australia] under the Clinical Trial Notification scheme through the Therapeutic Goods Administration. The study was conducted in accordance with the Declaration of Helsinki (e.g., the clinical study protocol, amendments, and informed consent documents), and any other study-related documents were reviewed and approved by the Bellberry Human Research Ethics Committee. Informed consent was obtained prior to any study-related procedures.

Six healthy volunteers were enrolled in this part of the study. On Day 1, three subjects received a single oral dose of 25 mg of CTP-656 and three subjects received a single oral dose of 25 mg of d_{18} -ivacaftor as an aqueous suspension. On Day 8 after the first dose, subjects crossed over and the dosing was repeated. A total of 21 PK blood samples (4 ml each) were collected for the assessment of plasma concentrations of CTP-656 and d_{18} -ivacaftor. Time points for collections were immediately prior to dosing (0 hour), and 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 20, 24, 36, 48, 60, 72, 84, and 96 hours postdose. Plasma levels of CTP-656 and d_{18} -ivacaftor were measured by LC-MS/MS. The plasma PK parameters for the dosed compounds were calculated by noncompartmental analysis using Phoenix 64 WinNonlin software (Certara). Each subject was monitored for safety throughout the study, including obtaining vital signs, performing physical examinations, obtaining clinical laboratory measurements, and performing 12-lead electrocardiograms.

Results

Deuterium Substitution Does Not Alter the Pharmacology of Ivacaftor and Its Major Metabolites. The pharmacological properties of CTP-656 and its major metabolites, d_8 -M1 and d_6 -M6, were compared with ivacaftor, M1, and M6 using a chloride conductance assay with fully differentiated HBE cells expressing mutant CFTR. It has been reported for the G551D CFTR mutation that M1 has approximately one-sixth the potency and M6 less than 1/50 the potency of ivacaftor (Kalydeco, 2015). In G551D/F508del HBE cells, ivacaftor and CTP-656 had similar potencies for potentiating forskolin responses, with EC_{50} values of 336 and 255 nM, respectively (Table 1). The potencies of the M1 and d_8 -M1 metabolites were also similar (0.96 and 1.35 μ M, respectively), and both metabolites were less potent than the parent: 3-fold for M1 and 5-fold for d_8 -M1. The EC_{50} values for M6 and d_6 -M6 were 11 and 15 μ M, respectively, which is in agreement with prior reports that the M6 metabolite does not contribute to the therapeutic efficacy of ivacaftor in CF patients (Kalydeco, 2015).

To compare the activities of CTP-656, ivacaftor, and their metabolites in the context of pharmacologically corrected F508del-CFTR, the potencies of all compounds for potentiating forskolin responses were tested in F508del/F508del HBE cells after a 24-hour incubation period with lumacaftor. Similar to results obtained with G551D/F508del HBE, the potencies of nondeuterated and deuterated parent and metabolite compounds were very similar (Table 2). d_{18} -Ivacaftor was also tested in G551D-CFTR-expressing FRT (Fischer rat thyroid)

TABLE 1

Potency of CFTR potentiation in G551D/F508del HBE cells by ivacaftor and deuterated ivacaftor analogs

Compound	EC_{50} , nM (95% CI) ^a	Compound	EC_{50} , nM (95% CI) ^a
Ivacaftor	336 (295–382)	CTP-656	255 (220–296)
M1	960 (756–1220)	d_8 -M1	1346 (1040–1743)
M6	11,320 (8906–14,380)	d_6 -M6	15,140 (10,390–22,050)

Current traces of representative data are shown in Supplemental Fig. 16.

^aCalculated using AUC potentiator I_{eq} (μ A/cm²) after forskolin addition, $n = 3$.

TABLE 2

Potency of CFTR potentiation in lumacaftor-corrected F508del/F508del HBE cells by ivacaftor and deuterated ivacaftor analogs

Compound	EC_{50} , nM (95% CI) ^a	Compound	EC_{50} , nM (95% CI) ^a
Ivacaftor	387 (275–545)	CTP-656	295 (151–575)
M1	390 (259–588)	d_8 -M1	286 (164–500)
M6	2410 (927–6269)	d_6 -M6	3048 (undetermined)

Current traces of representative data are shown in Supplemental Fig. 16.

^aCalculated using AUC potentiator I_{eq} (μ A/cm²) after forskolin addition, $n = 3$.

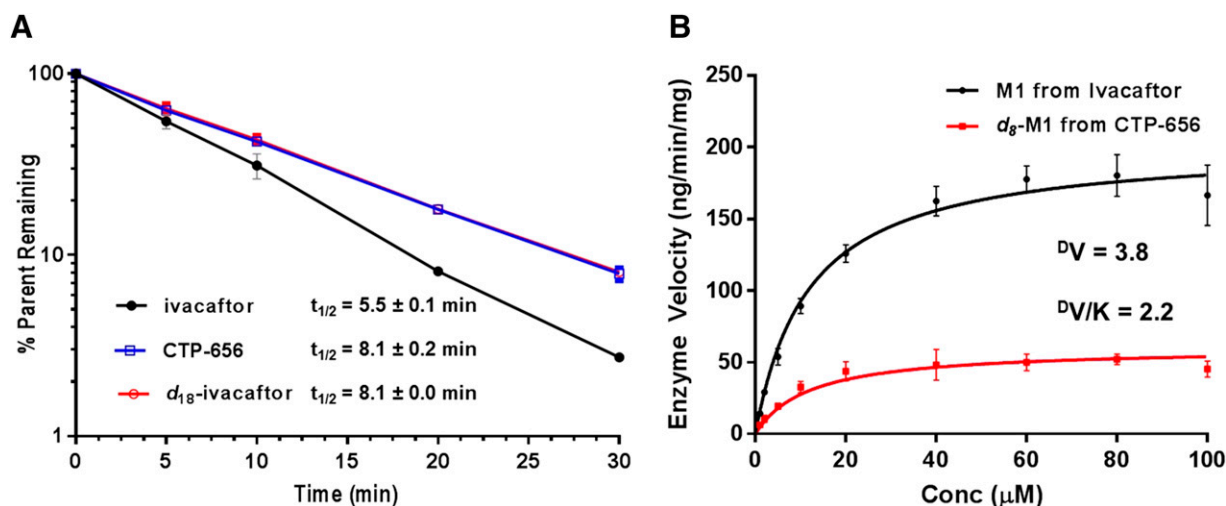


Fig. 3. In vitro metabolism. (A) $t_{1/2}$ values of ivacaftor and d -ivacaftor analogs in human CYP3A4 Supersomes. (B) Deuterium kinetic isotope effect as measured by the rate of formation of M1 and d_8 -M1 metabolites in HLM.

cells and lumacaftor-corrected F508del/F508del HBE cells; no significant differences in potentiation activity or potency were observed among d_{18} -ivacaftor, CTP-656, and ivacaftor (data not shown).

Deuterated Ivacaftor Analogs Show Increased Metabolic Stability in In Vitro Assays. Metabolic stability was initially determined by incubating each compound in the presence of CYP3A4 Supersomes for 30 minutes and monitoring the decrease in compound versus time. Additionally, compounds were incubated with human hepatocytes, and the amounts of parent remaining and metabolites formed were measured. In CYP3A4 Supersomes, the in vitro $t_{1/2}$ value for ivacaftor was 5.5 minutes, whereas the $t_{1/2}$ values for CTP-656 and d_{18} -ivacaftor were both 8.1 minutes (average of two experiments for the three compounds tested), which is a 47% increase in $t_{1/2}$ for CTP-656 and d_{18} -ivacaftor versus ivacaftor (Fig. 3A). The data from human hepatocytes showed an approximate 2-fold reduction of the M1 metabolites as a percentage of parent for both CTP-656 and d_{18} -ivacaftor with respect to ivacaftor (Table 3). Additionally, no new metabolites were identified in the human hepatocyte incubations of the two deuterium-modified compounds.

Deuterium-enhanced metabolic stability was also assessed by incubating increasing concentrations of ivacaftor and CTP-656 in the presence of HLM for 10 minutes and measuring the rate of formation for M1 and d_8 -M1, respectively. The data were then fit to the hyperbolic form of the Michaelis-Menten equation to derive the kinetic parameters K_m , V_{max} (enzyme velocity at saturating substrate: $[S] \gg K_m$), and V_{max}/K_m (enzyme velocity at low substrate concentrations: $[S] \ll K_m$). The ratios of the kinetic parameters V_{max} and V_{max}/K_m for

ivacaftor versus CTP-656 measure the deuterium kinetic isotope effects $^D V$ (V_{maxH}/V_{maxD}) and $^D V/K$ [$(V_{max}/K_m)H / (V_{max}/K_m)D$] for the CYP3A4-catalyzed formation of M1 in HLM (Northrop, 1975). Significant DIEs were measured for both kinetic parameters: $^D V = 3.8$ and $^D V/K = 2.2$ (Fig. 3B). The observed increase in $t_{1/2}$ for CTP-656 and d_{18} -ivacaftor versus ivacaftor in CYP3A4 Supersomes, the decreased metabolism in human hepatocytes, and the isotope effects observed in HLMs supported progression of the compounds to in vivo PK studies in rats and dogs.

Deuterated Ivacaftor Analogs Show Differentiated Nonclinical PK Upon Oral Dosing. The PK profiles of the three compounds were initially assessed in male Sprague-Dawley rats. Each compound was dosed at 10 mg/kg to each of three rats, with a total of nine rats in the study. The plasma levels of compound were measured over 72 hours. The plasma concentration versus time curves for drug exposure of the compounds are shown in Fig. 4A (only the 0 to 24-hour data are shown for clarity). Although d_{18} -ivacaftor showed greater exposure than either CTP-656 or ivacaftor, the error bars for the time points are overlapping, indicating modest differentiation for d_{18} -ivacaftor versus ivacaftor. This modest differentiation is supported by the PK parameters for the three compounds (Table 4). Across the parameters C_{max} , $AUC_{0-24 \text{ hour}}$, $C_{24 \text{ hour}}$ and $t_{1/2}$, d_{18} -ivacaftor showed a trend to larger exposures in rats compared with both CTP-656 and ivacaftor.

The PK profiles of the three compounds were also studied in male beagle dogs. Each compound was dosed by oral gavage at 3 mg/kg to each of four dogs in a crossover fashion with a 1-week washout period between doses, and the plasma levels

TABLE 3

Semiquantitative analysis of M1 metabolites versus parent after incubation of ivacaftor, CTP-656, and d_{18} -ivacaftor in human hepatocytes

Compound	Parent Area (mAU)	M1 Area (mAU)	M1 Formed as % of Parent
Ivacaftor	3.32	0.749	23
CTP-656	3.09	0.365	12
d_{18} -ivacaftor	3.28	0.298	9.1

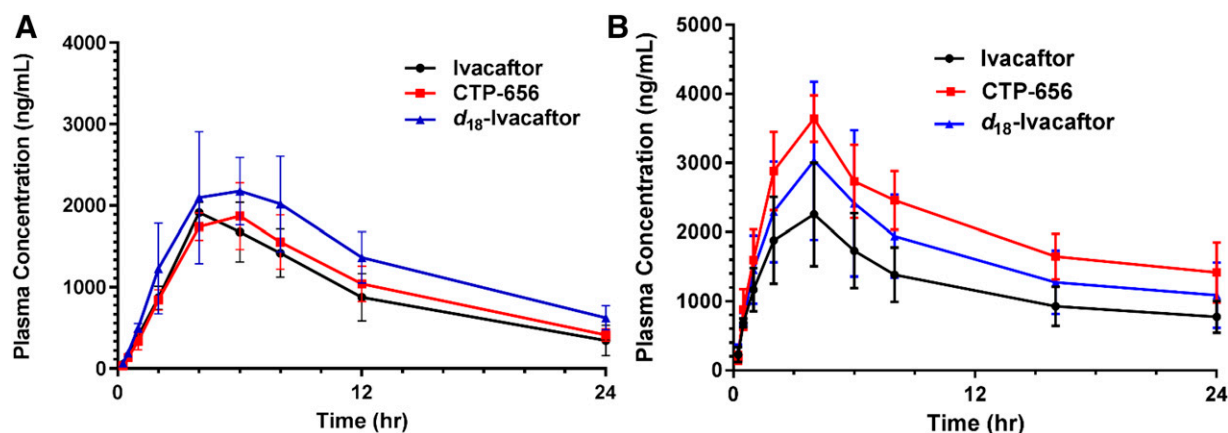


Fig. 4. In vivo metabolism. (A) Exposure profiles in male Sprague-Dawley rats ($n = 3/\text{group}$) after oral dosing of CTP-656, d_{18} -ivacaftor, and ivacaftor. (B) Exposure profiles in male beagle dogs ($n = 4$) after oral dosing of CTP-656, d_{18} -ivacaftor, and ivacaftor in a crossover study.

of compound were measured over 96 hours. As shown by the plasma concentration versus time profiles for each compound (Fig. 4B), CTP-656 had much greater exposure than ivacaftor and appeared to exhibit greater exposure than d_{18} -ivacaftor (only the 0 to 24-hour data are shown for clarity). The PK parameters for CTP-656 versus ivacaftor also supported the differentiated profile versus ivacaftor in dogs (Table 4). The C_{\max} , $AUC_{0-24\text{hr}}$, and $C_{24\text{hour}}$ values show 62%, 69%, and 83% increases, respectively, versus ivacaftor.

Although the PK data for CTP-656 and d_{18} -ivacaftor in rats and dogs demonstrated deuterium-mediated slowing of ivacaftor metabolism, the data did not clearly identify which compound should be advanced to human studies. The Pharmacology Review section of the Summary Basis of Approval for ivacaftor noted that exposure to ivacaftor was significantly greater than M1 and M6 in nonclinical test species (mouse, rat, and dog) (Center for Drug Evaluation and Research, Pharmacology Review(s): Ivacaftor (Application Number: 203188Orig1s000); http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/203188Orig1s000PharmR.pdf). However, in humans, the exposure to M1 and M6 is significantly greater than ivacaftor. In view of the extensive metabolism of ivacaftor that occurs in humans, but is not observed in either rats or dogs, both CTP-656 and d_{18} -ivacaftor were advanced to a first-in-human study to identify the preferred compound for continued clinical development.

CTP-656 Identified as the Deuterated Ivacaftor Analog for Clinical Advancement. This first-in-human study of CTP-656 and d_{18} -ivacaftor was designed as a two-part crossover study of a low (25 mg) single oral dose of the two compounds

as an aqueous suspension of their respective solid, amorphous dispersions. The baseline demographics of the study participants are summarized in Table 5. In the first part, six healthy volunteers (4 male and 2 female) were administered study drug on Day 1: three received CTP-656 and three received d_{18} -ivacaftor. On Day 8, the subjects were crossed over and were administered the other compound. Blood samples were collected over a 96-hour period on Days 1 and 8, and the PK parameters were determined (Table 6). The plasma concentration versus time curves (0–24 hours shown for clarity) for each compound are also shown (Fig. 5).

The PK parameters reflect the general trend shown in the exposure curves in that C_{\max} and AUC from time 0 extrapolated to infinity ($AUC_{0-\infty}$) values are greater for CTP-656 than for d_{18} -ivacaftor. The approximate 16-hour $t_{1/2}$ of CTP-656 is also compatible with identifying a dose that would enable once-daily dosing. PK data for a 25 mg dose of ivacaftor have been previously reported as part of a single-ascending dose (SAD) study (Center for Drug Evaluation and Research, Clinical Pharmacology and Biopharmaceutics Review(s): Ivacaftor (Application Number: 203188Orig1s000); http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/203188Orig1s000ClinPharmR.pdf). For comparative purposes, the $t_{1/2}$ for ivacaftor was 11.1 hours, and the exposure ($AUC_{0-\infty}$) was 1627 h*ng/mL. These data for ivacaftor suggest that at the same dose CTP-656 may have greater exposure and a longer $t_{1/2}$ versus ivacaftor in humans. In subsequent clinical studies, CTP-656 as a solid oral 150 mg dose was compared directly to a single dose of ivacaftor (150 mg tablet) and was shown to have an approximate 15-hour $t_{1/2}$ (Uttamsingh et al., 2016).

TABLE 4.

Mean PK parameters (%CV) for CTP-656, d_{18} -ivacaftor, and ivacaftor in male Sprague-Dawley rats (10 mg/kg) and male beagle dogs (3.0 mg/kg) after oral administration

Compound	C_{\max} (ng/ml)	$AUC_{0-24\text{ hr}}$ (h*ng/ml)	$C_{24\text{hr}}$ (ng/ml)	$t_{1/2}$ (h)
PK parameters in male Sprague-Dawley rats				
Ivacaftor	1913 (7%)	22,177 (24%)	346 (54%)	10.5 (17%)
CTP-656	1970 (15%)	24,260 (17%)	413 (19%)	13.2 (9%)
d_{18} -Ivacaftor	2460 (28%)	31,556 (20%)	623 (24%)	14.8 (9%)
PK parameters in male beagle dogs				
Ivacaftor	2255 (33%)	29,448 (29%)	774 (30%)	17.6 (6.9%)
CTP-656	3463 (9%)	49,782 (15%)	1418 (31%)	22.8 (62%)
d_{18} -Ivacaftor	3030 (38%)	40,019 (33%)	1089 (43%)	18.0 (26%)

TABLE 5

Subject demographics

	N	6
Sex, n (%)		
Male	4	(67)
Female	2	(33)
Race, n (%)		
Caucasian	4	(67)
Asian	1	(17)
Aboriginal/Torres Strait Islander	1	(17)
Age, mean year (range)	23.0	(18–31)
Weight, mean kg (range)	74.4	(62.8–91.4)
BMI, mean kg/m ² (range)	23.9	(18.8–27.0)

BMI, body mass index.

Discussion

This study reports the results of precision deuteration of ivacaftor (Kalydeco; Vertex Pharmaceuticals), which is the first approved CFTR modulator for the treatment of CF. Two deuterated analogs, *d*₉-ivacaftor (CTP-656) and *d*₁₈-ivacaftor were tested in vitro and in vivo to assess the pharmacology, PK, and metabolism of the compounds with respect to ivacaftor. The studies of the deuterated ivacaftor analogs were designed to assess whether a DIE could reduce metabolism in humans and provide an agent with a differentiated clinical profile. The data for the deuterated analogs of ivacaftor align with the previous article in this series (Uttamsingh et al., 2015) that assessed the effects of precision deuteration on paroxetine pharmacology and PK. Deuterated paroxetine (CTP-347) retained the intrinsic in vitro pharmacology profile of paroxetine with greatly reduced mechanism-based inactivation of CYP2D6 in in vitro studies. This preclinical profile of CTP-347 translated to a reduced drug-drug interaction liability with dextromethorphan in a clinical study. However, further clinical development of CTP-347 as a nonhormonal therapy for hot flashes was stopped for commercial reasons.

To confirm that deuterium substitution did not affect the intrinsic pharmacology of ivacaftor, in vitro studies were performed in HBE cells derived from CF patients. The potency as a potentiator of CFTR with a gating mutation was measured in G551D/F508del HBE cells. For homozygous F508del HBE cells, the cells were first corrected with lumacaftor prior to measuring the potentiation of conductance. Ivacaftor is extensively metabolized in humans to two major circulating metabolites, M1 and M6 (Zha et al., 2011). At steady state, the metabolites are the major circulating species in humans, with mean exposure ratios (metabolite/parent) of approximately 5-fold and 2-fold, respectively, for M1 and M6 (Center for Drug Evaluation and Research, Clinical Pharmacology and Biopharmaceutics Review(s): Ivacaftor (Application Number: 203188Orig1s000); http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/203188Orig1s000ClinPharmR.pdf).

These metabolites of ivacaftor and CTP-656 were, therefore, also evaluated in the HBE cells assays.

Overall, the in vitro pharmacology data confirmed that deuteration did not change the CFTR activity of ivacaftor and the major metabolites. The equivalent activity of CTP-656 and ivacaftor measured in G551D/F508del HBE cells supports the use of CTP-656 in the same patient population as ivacaftor. Likewise, the potentiation of CFTR function in lumacaftor-corrected F508del homozygous HBE cells supports the clinical use of CTP-656 in combination with lumacaftor and potentially other correctors currently in development.

Although precision deuteration of ivacaftor did not change the intrinsic pharmacology of the compound, decreases in metabolism for the deuterated analogs were shown both in vitro and in vivo. High protein binding, nonspecific binding, and low solubility prevented the metabolic stability assessment of ivacaftor in our standard HLM assay due to the slow apparent rate of metabolism (unpublished data). Since ivacaftor is metabolized primarily by CYP3A4 (Kalydeco, 2015), CYP3A4 Superosomes were used to assess metabolic stability. The two deuterated analogs exhibited *t*_{1/2} values approximately 50% greater than ivacaftor in this study, which signaled that precision deuteration could positively impact the PK of ivacaftor.

Once the respective M1 and *d*₈-M1 metabolites of ivacaftor and CTP-656 were synthesized, a more sensitive assay to measure metabolism in HLM was possible. By measuring the formation of metabolites instead of the loss of parent, a direct measurement of metabolite formation in HLM was possible and enabled calculation of the DIE using classic Michaelis-Menten kinetics. The measured ^DV/K and ^DV values for CYP3A4 catalyzed metabolism in HLM were large.

Although a ^DV/K isotope effect of 2.2 was measured, the literature reports that an isotope effect on V/K should not be observed unless it has been unmasked, which can occur under certain conditions (Korzekwa et al., 1989; Nelson and Trager, 2003). Unmasking could arise due to oxidation at a different site on CTP-656 to produce an alternate metabolite (“metabolic switching”) (Horning et al., 1975). Preclinical and clinical data, however, do not suggest metabolic switching. Incubations of CTP-656 and *d*₁₈-ivacaftor in human hepatocytes showed only reductions in the formation of the M1 metabolites versus ivacaftor; no other metabolites were detected. Clinical data also showed the same plasma metabolites for CTP-656 and ivacaftor in humans (manuscript in preparation). Another possible mechanism for unmasking the isotope effect is direct reduction of the activated enzyme-substrate complex to produce free enzyme, substrate, and water (Atkins and Sligar, 1987). Studies were not conducted to assess this mechanism, but it appears to be a reasonable explanation for the observed ^DV/K isotope effect for CTP-656 in the absence of metabolic switching.

A recent review (Guengerich, 2013) on the potential issues for deuterium in drug development notes that an isotope effect in vitro needs to translate to a PK effect in vivo to have a

TABLE 6

Mean PK parameters (%CV) for CTP-656 and *d*₁₈-ivacaftor in healthy males and females after oral administration of a single 25-mg dose

Compound	<i>C</i> _{max} (ng/ml)	<i>t</i> _{1/2} (h)	AUC _{0–inf} (h*ng/mL)	<i>C</i> _{24hr} (ng/ml)	CL/F (l/h)
CTP-656	270.5 (24%)	15.9 (18%)	3812 (26%)	52.6 (28%)	6.92 (25%)
<i>d</i> ₁₈ -Ivacaftor	232.7 (18%)	16.4 (16%)	3196 (15%)	42.3 (16%)	7.97 (15%)

CL/F; oral clearance.

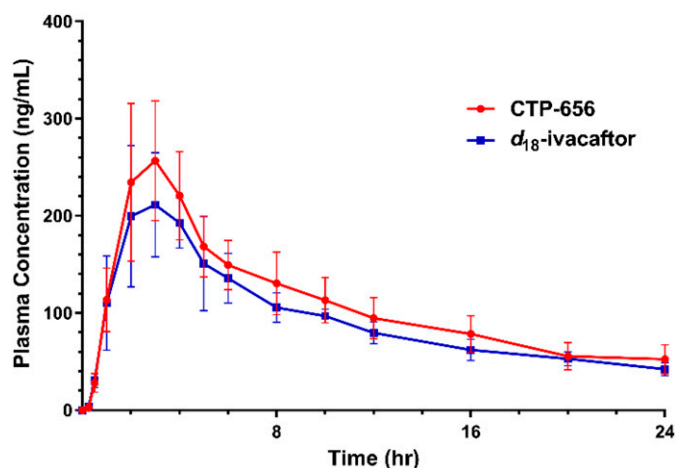


Fig. 5. Exposure profiles in healthy volunteers ($n = 6$) after oral dosing of CTP-656 and d_{18} -ivacaftor.

practical application to drug development. Although slower metabolism was observed *in vitro*, the translation to *in vivo* is unpredictable since non-cytochrome P450 clearance mechanisms may predominate *in vivo* and clearance mechanisms may vary between species. PK studies in rats and dogs were particularly challenging since neither rats nor dogs metabolize ivacaftor as extensively as humans; therefore, these preclinical species may not be adequate models for human metabolism (Center for Drug Evaluation and Research; Pharmacology Review(s): Ivacaftor (Application Number: 203188Orig1s000); http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/203188Orig1s000PharmR.pdf). The PK results in rats and dogs for CTP-656 were encouraging, with a longer $t_{1/2}$ and greater exposure versus ivacaftor in both species. d_{18} -Ivacaftor, however, showed increased exposure versus ivacaftor in rats, but not in dogs. These results emphasize the unpredictable effects of deuterium substitution. Although positive preclinical PK effects were very encouraging, these models were not sufficient to choose a preferred compound for clinical development. The more rigorous approach involved testing both compounds in humans and selecting a compound for advancement based upon human PK data.

The clinical crossover study of orally dosed CTP-656 and d_{18} -ivacaftor identified the preferred compound for further clinical development. CTP-656 exposure was only modestly increased versus d_{18} -ivacaftor; however, the increases were consistent across the PK parameters C_{max} , $t_{1/2}$, AUC_{0-inf} , C_{24hr} and oral clearance (CL/F). These data, coupled with the reduced amount of deuterium in CTP-656, were sufficient to identify CTP-656 for continued clinical development.

The potential of CTP-656 to be dosed once daily was predicted based on available information for ivacaftor, including SAD PK in healthy volunteers and PK/PD relationships in patients (Center for Drug Evaluation and Research, Clinical Pharmacology and Biopharmaceutics Review(s): Ivacaftor (Application Number: 203188Orig1s000); http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/203188Orig1s000ClinPharmR.pdf). In the SAD study of ivacaftor, which was conducted in the fasted condition, exposure increases were dose proportional from 25 to 375 mg. Additionally, the 150 mg twice-daily dose of ivacaftor was selected based on simulations that showed a plasma C_{min} at steady state of ~ 250 ng/ml achieved the predicted

EC_{90} for forced expiratory volume in 1 s and the EC_{84} for sweat chloride. The 25 mg dose of CTP-656 in the fasted state provided a C_{24hr} (C_{min}) of 53 ng/ml in plasma. Assuming dose-proportional increases in exposure for CTP-656, a once-daily dose of approximately 118 mg in the fasted condition would achieve the target C_{24hr} of 250 ng/ml even in the absence of accumulation on multiple dosing. Ivacaftor shows an increase in exposure of 2-fold to 3-fold when dosed with food; therefore, CTP-656 has the potential to exceed the 250 ng/ml C_{min} at steady state if a similar food effect is observed. Based on this analysis of the 25 mg dose, CTP-656 was projected to achieve the desired once-daily profile in future studies, which was confirmed in subsequent phase I studies.

Once-daily dosing may translate to improved adherence in real-world use. A small clinical study (Siracusa et al., 2015) assessed ivacaftor adherence in patients and found the adherence to be highly variable. The ivacaftor label (Kalydeco, 2015) specifies dosing every 12 hours; however, this study found that the median duration between doses was 16.9 hours, with a range of 12.1–43.7 hours. The overall adherence rate—calculated as (total doses taken)/(total days monitored \times 2 doses/d)—was only 61%. It is also noteworthy that the 10.4% improvement in absolute percent predicted forced expiratory volume in 1 s observed in the clinical study (≥ 12 years old) is much greater than the 5.4% improvement observed over 12 months in a non-clinical trial patient population (Fink et al., 2015). Although this may be due to a number of factors, the suboptimal adherence could be an important contributing factor, which may be positively impacted by a once-daily potentiator.

CTP-656 has completed three phase I studies in healthy volunteers, including SAD, multiple-ascending dose, and food-effect studies. The tablet formulation was reported to have an average $t_{1/2}$ of 15 hours after a single oral dose and an average $t_{1/2}$ of 18 hours in the multiple-ascending dose studies (Uttamsingh et al., 2016). Of particular note is the reported CTP-656/ d_8 -M1 AUC ratio of 1.5:1 after a single dose and also at steady state. This is much greater than ivacaftor, which has a parent/M1 AUC ratio of 1:5 at steady state. With a longer $t_{1/2}$ and greater exposure to the more active parent, the PK profile for CTP-656 may translate into an improved clinical profile in CF patients with gating mutations. A phase II safety and efficacy study in CF patients is currently underway (NCT02971839).

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Contributed new reagents or analytic tools: Morgan, Liu, Bridson, and Brummel.

Performed data analysis: Aslanian, Nguyen, Brummel, and Uttamsingh.

Wrote or contributed to the writing of the manuscript: Harbeson, Liu, Aslanian, Brummel, Tung, Braman, and Uttamsingh.

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