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ORIGINAL ARTICLE

# Evaluation of the effects of an oral notch inhibitor, crenigacestat (LY3039478), on QT interval, and bioavailability studies conducted in healthy subjects

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## Abstract

**Purpose** Crenigacestat (LY3039478) is a Notch inhibitor currently being investigated in advanced cancer patients. Conducting clinical pharmacology studies in healthy subjects avoids nonbeneficial drug exposures in cancer patients and mitigates confounding effects of disease state and concomitant medications.

**Methods** Three studies were conducted in healthy subjects, assessing safety, pharmacokinetics, effect on QT interval, and relative and absolute bioavailability of crenigacestat. Crenigacestat was administered as single 25, 50, or 75 mg oral doses or as an intravenous dose of 350 µg $^{13}C^{15}N^{2}H$-crenigacestat. Electrocardiogram measurements, and plasma and urine samples were collected up to 48 h postdose, and safety assessments were conducted up to 14 days postdose.

**Results and conclusions** Exposures were dose proportional in the 25 to 75 mg dose range and mean elimination half-life was approximately 5–6 h. The exposure achieved from the new formulated capsule was approximately 30% and 20% higher for area under the plasma concentration time curve from time zero to infinity [AUC(0–∞)] and maximum plasma concentration ($C_{max}$), respectively, compared to the reference drug in capsule formulation. The geometric least-squares mean [90% confidence interval (CI)] absolute bioavailability of crenigacestat was 0.572 (0.532, 0.615). The regression slope (90% CI) of placebo-adjusted QTcF against crenigacestat plasma concentration was −0.001 (−0.006, 0.003), suggesting no significant linear association. Thirty-nine subjects completed the studies and the majority of adverse events were mild. Single oral doses of 25 to 75 mg crenigacestat and an IV dose of 350 µg $^{13}C^{15}N^{2}H$-crenigacestat were well tolerated in healthy subjects.

**Keywords** LY3039478 · Crenigacestat · Notch · QT interval · Bioavailability · Pharmacokinetics

## Introduction

Notch signaling is an evolutionary conserved pathway that plays an integral role in development and tissue homeostasis [1]. Crenigacestat [LY3039478; (4,4,4-trifluoro-N-[(1S)-2-[[(7S)-5-(2-hydroxyethyl)-6-oxo-7H-pyrido[2,3-d] [3] benzazepin-7-yl]amino]-1-methyl-2-oxo-ethyl]butanamide)] is an orally available, small molecule, potent Notch inhibitor that prevents the release of the Notch intracellular domain, thereby decreasing Notch signaling and its downstream biologic effects. Crenigacestat has been shown to inhibit Notch signaling in cell lines and xenograft models representing a number of tissues such as human ovary, colon, and nonsmall-cell lung cancers [2]. In the first-in-human study, patients with advanced or metastatic cancer were given crenigacestat over the dose range of 2.5–100 mg three times a week (TIW), using a drug in capsule formulation. The most common study drug-related toxicities included gastrointestinal-related symptoms, asthenia, and hypophosphatemia [3]. The recommended phase 2 dose of crenigacestat was 50 mg TIW. Crenigacestat was rapidly absorbed with peak concentrations occurring within 1 to 2 h and an elimination half-life of approximately 6 h, with little-to-no accumulation

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upon TIW dosing. Apparent clearance of crenigacestat was approximately 15 L/h, and renal clearance contributed to approximately 20% of the apparent plasma clearance. 80% of maximal biomarker effects [plasma amyloid beta (Aβ) inhibition and inhibition of notch-regulated genes] were obtained after approximately 15–50 mg TIW doses of crenigacestat [4].

Since the release of International Conference on Harmonisation (ICH) E14 in 2005, regulatory agencies have required drug sponsors to conduct thorough QT (TQT) studies to inform the extent of electrocardiogram (ECG) monitoring in Phase 3 [5]. However, it has been suggested that high-quality data collected in Phase 1 may be sufficient to inform and potentially obviate the TQT study [6, 7]. Thus, dense sampling with time-matched pharmacokinetics (PK) and ECG collections in healthy subjects can inform the potential risk for QT interval increase with crenigacestat in a clinically relevant dose range that can be translated to cancer patients. Conducting studies in healthy subjects mitigates the potential confounding effects of the disease state and concomitant medications, and avoids nonbeneficial drug exposures in cancer patients.

A change in the formulation of crenigacestat, from the drug in capsule formulation to a formulated capsule, has occurred since the start of the first-in-human study. This is common in the drug development process, where non-formulated drug products may be used in early clinical trials, with subsequent changes to formulated products once safety and other drug characteristics have been evaluated. It was, therefore, relevant to conduct a pilot relative bioavailability study to evaluate the differences between the two formulations, and to inform if any dose adjustments were required for future clinical trials when switching between formulations.

Absolute bioavailability data are required by regulatory agencies in some geographical areas [8]. Knowledge of absolute bioavailability is also helpful in the design of future clinical studies and in the interpretation of PK data. The conventional oral absolute bioavailability study design requires 2 periods for separate administrations of oral and IV drug formulations. In contrast, incorporation of stable isotopically labelled drug to the IV formulation allows concurrent administration of oral and IV formulations in a single period, which eliminates day-to-day PK variability and ensures that the systemic clearance is equivalent for the IV and oral doses [9].

Three clinical pharmacology studies were conducted in healthy subjects: Study 1 assessed safety, tolerability, and the effect of crenigacestat on QT interval following single ascending doses. PK and pharmacodynamics (PD) were also characterized as secondary and exploratory objectives in this study. Study 2 was a pilot relative bioavailability study of crenigacestat administered as drug in capsule versus formulated capsule, and Study 3 was conducted to estimate the absolute bioavailability of crenigacestat following a single oral administration of crenigacestat and an intravenous (IV) administration of 350 µg $^{13}C^{15}N^{2}H$-crenigacestat (a stable isotopically labelled crenigacestat).

## Materials and methods

### Study designs

In all studies, overtly healthy males and females not of childbearing potential, aged 18–65 years, with a body mass index of 18–32 kg/m$^2$, were eligible for entry into the studies. Subjects were admitted to the clinical research unit (CRU) the day before the start of each period. Following an overnight fast of at least 10 h, crenigacestat, placebo, or $^{13}C^{15}N^{2}H$-crenigacestat was administered according to study protocol. Subjects resided in the CRU for up to 48 h postdose, with additional blood sample collections and adverse event (AE) assessments conducted 7 days and approximately 14 days after dosing. Where there was more than 1 period in the study, washout between periods was at least 14 days. Safety assessments performed during the studies included the recording of AEs, clinical laboratory evaluations, vital signs, 12-lead ECGs, and physical examinations. Sample sizes used in all studies were typical of Phase 1 studies and not intended to meet any priori statistical requirement. All procedures performed in all studies were in accordance with the ethical standards of the institutional and/or national research committee, and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Study 1 and 2 were performed at the same site.

Study 1 was a 3-period crossover, subject- and investigator-blind study conducted in a single center. Approximately 15 healthy subjects were enrolled in order that 12 complete the study. Subjects were randomized to one of three treatment sequences, and were administered placebo or single oral doses of 25, 50, or 75 mg crenigacestat (formulated capsules) in each period. Each subject received two of the three crenigacestat doses and one dose of placebo over the course of the study. Clinical laboratory sample collection and collection of serial crenigacestat PK samples that were time-matched to triplicate Holter ECG extractions were conducted for up to 48 h postdose.

Study 2 was a 2-period, open-label, crossover design study, which aimed to enroll approximately 14 healthy subjects to ensure 12 completed. Subjects were randomized to one of two treatment sequences. In each period, subjects were administered a single oral dose of 50 mg crenigacestat as a formulated capsule (test), or as drug in capsule (reference). The formulated capsule consisted of crenigacestat blended with starch and silicone oil and filled into a gelatin

capsule, while the drug in capsule formulation consisted of crenigacestat in a hydroxypropyl methylcellulose capsule. Blood sample collection and safety observations were conducted up to 48 h postdose, to characterize PK and safety of crenigacestat.

Study 3 was a single-center, open-label, single-period study. Up to 12 subjects were enrolled, so that at least 8 completed the study. Subjects received a single oral dose of 75 mg crenigacestat (formulated capsule), and approximately 15 min later, an IV infusion of duration 45 min containing approximately 350 µg $^{13}C^{15}N^{2}H$-crenigacestat was given. Blood and urine samples were collected up to 48 h after the start of dosing to quantify the concentrations of crenigacestat and $^{13}C^{15}N^{2}H$-crenigacestat in plasma and of crenigacestat in urine.

## Bioanalytical methods

Human plasma samples were analysed for crenigacestat and $^{13}C^{15}N^{2}H$-crenigacestat using two different validated liquid chromatographies with tandem mass spectrometric (LC/MS/MS) methods at $Q^2$ Solutions (Ithaca, New York, USA). The lower and upper limits of quantification were 0.1 ng/mL and 100 ng/mL, respectively, for crenigacestat and 0.005 ng/mL and 5 ng/mL, respectively, for $^{13}C^{15}N^{2}H$-crenigacestat. In Studies 1 and 2, for crenigacestat, the inter-assay accuracy (% relative error) during validation ranged from − 3.75 to 2.00%. The inter-assay precision (% relative standard deviation) during validation was ≤ 7.14%. Crenigacestat was stable for up to 634 days when stored at approximately − 20 °C and for up to 911 days when stored at approximately − 70 °C. In Study 3, the inter-assay accuracy (% relative error) during validation ranged from − 8.93 to 2.81% for crenigacestat, and from − 10.21 to − 4.67% for $^{13}C^{15}N^{2}H$-crenigacestat. The inter-assay precision (% relative standard deviation) during validation was ≤ 4.35% for crenigacestat and ≤ 5.21% for $^{13}C^{15}N^{2}H$-crenigacestat. Crenigacestat and $^{13}C^{15}N^{2}H$-crenigacestat in plasma were stable for up to 61 days when stored at approximately − 20 °C and for up to 61 days when stored at approximately − 70 °C.

Plasma samples were also analysed for Aβ peptide concentrations using the INNO-BIA plasma Aβ forms assay at Innogenetics (Ghent, Belgium). Further details of the assay can be found elsewhere [10], but, briefly, the quantification range was 7.6–1545 ng/L, with an inter-assay accuracy range (% relative error) of -17 to 8% and inter-assay precision (% coefficient of variation) of 5–17% during validation. Analyte stability was demonstrated in plasma at 2–8 °C for up to 6 h and frozen storage up to 12 months.

Urine samples, where collected, were analysed for crenigacestat using a validated LC/MS/MS method. The lower and upper limits of quantification were 1 ng/mL and 1000 ng/mL, respectively. In Studies 1 and 2, the inter-assay accuracy (% relative error) during validation ranged from 3.59 to 6.00%. The inter-assay precision (% relative standard deviation) during validation was ≤ 7.55%. In Study 3, the inter-assay accuracy (% relative error) during validation ranged from − 3.59 to 6.00%. The inter-assay precision (% relative standard deviation) during validation was ≤ 7.55% for crenigacestat. In all three studies, crenigacestat in urine was stable for up to 336 days when stored at approximately − 20 °C, and for up to 1581 days when stored at approximately − 70 °C.

## Pharmacokinetic and pharmacodynamic analysis methods

In all three studies, venous blood samples were collected up to 48 h postdose to determine plasma concentrations of crenigacestat and $^{13}C^{15}N^{2}H$-crenigacestat (only in Study 3). Urine samples up to 48 h postdose were also collected in Studies 1 and 3. PK analyses were conducted on subjects who received at least one dose of crenigacestat and had sufficient samples collected to allow the estimation of PK parameters, which were determined using noncompartmental methods (Phoenix WinNonlin version 6.4; Certara, Princeton, New Jersey, USA). Actual sampling times were used in the analysis of PK parameters and predose samples that were below the limit of quantification (BQL) were set to zero, whilst postdose BQL samples were treated as missing.

Exploratory assessment of dose proportionality was conducted in Study 1, based on the PK parameters of area under the plasma concentration time curve from time zero to infinity [AUC(0–∞)] and maximum plasma concentration ($C_{max}$). A power model [11] was used, fitting log (PK parameter) against log (dose) with a random effect for subject.

To delineate the effects of crenigacestat formulation, log-transformed AUC(0–∞) and $C_{max}$ estimates were evaluated in a mixed-effects model with fixed effects for formulation, period, and a random effect for subject. Predose samples were checked to confirm that there was no evidence of carryover effects.

The absolute bioavailability of crenigacestat was calculated using crenigacestat AUC(0–∞) estimates, after oral dosing of crenigacestat and after IV administration of $^{13}C^{15}N^{2}H$-crenigacestat, with adjustment for dose. A mixed-effects analysis of variance model was applied to the log-transformed dose-adjusted AUC(0–∞)s.

For PD analyses, venous blood samples were collected up to 48 h postdose to determine plasma concentrations of Aβ. The percentage (%) inhibition of plasma Aβ was calculated at each time point, and plasma Aβ concentrations below the limit of quantification were replaced with a value of half the lower limit of quantification for calculation of % inhibition. PD parameters for % inhibition of Aβ were determined using

noncompartmental methods (Phoenix WinNonlin version 6.4; Certara, Princeton, New Jersey, USA).

### Statistical analysis methods for TQT and safety

Triplicate QT corrected for heart rate using Fridericia's equation (QTcF) from Study 1 was averaged prior to analysis. Time-matched placebo-adjusted QTcF (ΔQTcF) for each time point was calculated by subtracting each subject's time-matched placebo QTcF from their QTcF results after receiving crenigacestat. The relationship between plasma concentrations of crenigacestat and ΔQTcF was evaluated using a linear mixed-effects modeling approach. Crenigacestat was judged not to cause clinically significant QTcF prolongation if the upper bound of the two-sided 90% confidence interval (CI) of the predicted mean ΔQTcF was below 10 ms [12] at the highest clinically observed plasma concentrations of crenigacestat.

Across all studies, safety parameters assessed included safety laboratory, vital sign, and ECG parameters. The frequencies of treatment-emergent adverse events (TEAEs) were summarized by treatment.

## Results

### Subject disposition

Forty-one healthy subjects were enrolled across the three studies, with 14, 13, and 12 subjects completed in Studies 1, 2, and 3, respectively. All subjects were males, with the exception of two females each in Study 1 and 2. The average age across all three studies was approximately 39–41 years, and average weight of subjects was approximately 81 to 83 kg with body mass index of approximately 26–27 kg/m$^2$.

### Pharmacokinetics

The plasma concentration versus time profiles in healthy subjects after single 25, 50, or 75 mg crenigacestat doses

**Table 1** Summary of pharmacokinetic and pharmacodynamic parameter estimates of crenigacestat following single oral doses of 25, 50, or 75 mg in healthy subjects in Study 1

| Pharmacokinetic parameter | Geometric mean (CV%) | | |
|---|---|---|---|
| | 25 mg crenigacestat Drug in capsule formulation ($N=10$) | 50 mg crenigacestat Drug in capsule formulation ($N=9$) | 75 mg crenigacestat Drug in capsule formulation ($N=10$) |
| AUC(0–∞) (ng h/mL) | 711 (39) | 1400 (21) | 2090 (41) |
| $C_{max}$ (ng/mL) | 158 (43) | 296 (31) | 461 (44) |
| $t_{max}$[a] (h) | 1.58 (0.55–2.10) | 1.05 (1.05–4.10) | 2.10 (0.55–2.10) |
| $t_{1/2}$[b] (h) | 5.63 (4.67–7.06) | 5.47 (4.48–6.44) | 5.71 (5.03–6.60) |
| CL/F (L/h) | 35.2 (39) | 35.7 (21) | 35.9 (41) |
| $V_z$/F (L) | 285 (43) | 282 (16) | 296 (48) |
| CLr (L/h) | 7.92 (30) | 7.61 (17) | 6.44 (28) |
| Fe(0–48) (%) | 22.5 (24) | 21.3 (13) | 18.0 (30) |

| Pharmacodynamic parameter | Arithmetic mean (SD) | | | |
|---|---|---|---|---|
| | Placebo ($N=14$) | 25 mg crenigacestat Drug in capsule formulation ($N=10$) | 50 mg crenigacestat Drug in capsule formulation ($N=9$) | 75 mg crenigacestat Drug in capsule formulation ($N=10$) |
| AUEC (h%) | − 98.6 (491) | 1240 (436) | 1690 (465) | 2110 (702) |
| $E_{max}$ (%) | 16.8 (10.2) | 81.7 (16.5) | 91.8 (2.79) | 87.6 (10.6) |
| $T_{Emax}$[a] (h) | 24.1 (0.00–48.0) | 6.21 (4.10–8.10) | 6.10 (6.10–8.10) | 6.10 (3.10–12.1) |

*AUC(0–∞)* area under the concentration versus time curve from time zero to infinity, *CL/F* apparent total body clearance of drug calculated after extravascular administration, *CLr* renal clearance, $C_{max}$ maximum observed drug concentration, *CV* coefficient of variation, $F_{e(0-48)}$ fraction of oral dose excreted unchanged in urine over 48 hours, *N* number of subjects, $t_{1/2}$ half-life associated with the terminal rate constant, $t_{max}$ time of maximum observed drug concentration, $V_z/F$ apparent volume of distribution during the terminal phase, *AUEC* area under the effect curve from time zero up to 48 h postdose, $E_{max}$ maximum observed effect, *SD* standard deviation, $T_{Emax}$ time of maximum observed effect

[a]Median (range)

[b]Geometric mean (range)

in Study 1 were characterized by a rapid absorption phase and biphasic elimination. Median time at which maximum plasma concentrations were reached ($t_{max}$) ranged from approximately 1 to 2 h, and mean terminal elimination half-life ($t_{1/2}$) ranged from 5.5 to 5.7 h (Table 1). Dose proportionality was assessed using the ratio of dose normalized geometric means at the highest dose (75 mg) compared to the lowest dose (25 mg). Both estimates, 0.95 [90% CI (0.86, 1.05)] for AUC(0–∞) and 0.96 [90% CI (0.82, 1.13)] for $C_{max}$, were close to the dose proportional value of one and had 90% CIs entirely contained within the region (0.8, 1.25) [11]. These findings support dose proportionality of exposures for the specified dose range of 25–75 mg crenigacestat in healthy subjects.

Intra-individual variation in AUC(0–∞) and $C_{max}$ ranged between 11 and 18%, and inter-individual variation ranged between 32 and 34%. The majority of drug excreted in the urine was recovered within the first 6 h postdose. Mean renal clearance (CLr) values ranged from 6.4 to 7.9 L/h, and represented approximately 20% of apparent plasma clearance (CL/F).

## Relative bioavailability

In Study 2, the estimated geometric means of AUC(0–∞) and $C_{max}$ were higher for the formulated capsule (test) than the drug in capsule (reference) (Table 2). The estimated ratios of geometric mean exposures were greater than 1 (test/ref); with values estimated at 1.31 (90% CI [1.23, 1.40]) for AUC(0–∞) and 1.20 (90% CI [1.07, 1.35]) for $C_{max}$. The exposure achieved from the new formulated capsule was approximately 30% and 20% higher for AUC(0–∞) and $C_{max}$, respectively, compared to the reference drug in capsule formulation.

**Table 2** Summary of PK parameter estimates following single oral doses of 50 mg crenigacestat as drug in capsule formulation (reference) or formulated capsule (test) in healthy subjects (Study 2)

| Parameter | Geometric mean (CV%) | |
|---|---|---|
| | 50 mg crenigacestat formulated capsule, test | 50 mg crenigacestat drug in capsule, reference |
| *N* | 14 | 13 |
| AUC(0–∞) (ng h/mL) | 1750 (19) | 1340 (22) |
| $C_{max}$ (ng/mL) | 384 (21) | 322 (25) |
| $t_{max}$[a] (h) | 2.00 (1.00–4.00) | 1.00 (1.00–3.00) |
| $t_{1/2}$[b] (h) | 5.74 (5.16–7.85) | 6.42 (5.66–7.86) |
| CL/*F* (L/h) | 28.6 (19) | 37.2 (22) |
| $V_z/F$ (L) | 237 (24) | 344 (22) |

*AUC(0–∞)* area under the concentration versus time curve from zero to infinity, *CL/F* apparent total body clearance of drug calculated after extravascular administration, $C_{max}$ maximum observed drug concentration, *CV* coefficient of variation, *N* number of subjects, $t_{1/2}$ half-life associated with the terminal rate constant in noncompartmental analysis, $t_{max}$ time of maximum observed drug concentration, $V_z/F$ apparent volume of distribution during the terminal phase

[a]Median (range)

[b]Geometric mean (range)

## Absolute bioavailability

Following oral administration of a single dose of 75 mg crenigacestat in Study 3, median $t_{max}$ of 1.5 h (range 1.0–3.5 h) was achieved (Table 3). After oral crenigacestat administration, plasma concentrations appeared to decline in a biphasic manner after $t_{max}$, and the resulting geometric

**Table 3** Summary of PK parameter estimates following a single 75 mg oral dose and $^{13}C^{15}N^{2}H$-crenigacestat following a 350 µg IV dose in healthy subjects in Study 3

| Parameter | Geometric mean (CV%) | |
|---|---|---|
| | 75 mg crenigacestat oral | 350 µg 13C15N2H-crenigacestat IV[a] |
| *N* | 12 | 12 |
| AUC(0–∞) (ng h/mL) | 1920 (23) | 15.6 (16) |
| AUC(0–∞)/dose[b] (ng h/mL) | 25.6 (23) | 44.8 (16) |
| $C_{max}$ (ng/mL) | 444 (23) | 7.24 (15) |
| $t_{max}$[c] (h) | 1.50 (1.00–3.50) | 0.75 (0.75–0.75) |
| $t_{1/2}$[d] (h) | 5.42 (4.93–6.88) | 3.31 (2.84–3.75) |
| CL/*F* [CL[e]] (L/h) | 39.1 (23) | 22.3 (16) |
| $V_z/F\left[V_z^e\right]$ (L) | 306 (27) | 107 (20) |
| *F* | 0.572 (14) | – |
| CLr (0–48 h) (L/h) | 8.41 (20) | – |
| CLr/CL (%) | – | 37.7 (10) |
| CLr/CL/*F* (%) | 21.5 (21) | – |

*AUC(0–∞)* area under the concentration versus time curve from time zero to infinity, *AUC(0–∞)/dose* dose-normalized area under the concentration versus time curve from time zero to infinity, *CL* total body clearance of drug calculated after IV administration, *CL/F* apparent total body clearance of drug calculated after extravascular administration, $C_{max}$ maximum observed concentration, *CV* coefficient of variation, *F* bioavailability of drug, *N* number of subjects studied, $t_{1/2}$ half-life associated with the terminal rate constant in noncompartmental analysis, $t_{max}$ time of maximum observed concentration, $V_z$ volume of distribution during the terminal phase after IV administration, $V_z/F$ apparent volume of distribution during the terminal phase after extravascular administration

[a]For $^{13}C^{15}N^{2}H$-crenigacestat the units for $C_{max}$, AUC(0–∞), and AUC(0–∞)/dose are ng equiv/mL, ng equiv.h/mL, and ng equiv.h/mL/mg, respectively

[b]AUC(0–∞)/dose was used to calculate the absolute bioavailability of crenigacestat

[c]Median (range)

[d]Geometric mean (range)

[e]Equivalent parameter after IV administration

mean $t_{1/2}$ based on the terminal elimination phase was 5.4 h. For IV $^{13}C^{15}N^{2}H$-crenigacestat, the geometric mean $t_{1/2}$ appeared to be shorter (3.3 h); as concentrations were only quantifiable until 24 h postdose, it is likely that the terminal elimination phase for the IV dose had not been fully defined.

The geometric least-squares mean (90% CI) absolute bioavailability of crenigacestat after oral administration was 0.572 (0.532, 0.615). For individual subjects, absolute bioavailability ranged from 0.469 to 0.676.

## Pharmacodynamics

Administration of single doses of crenigacestat across the 25–75 mg dose range resulted in rapid inhibition of Aβ concentrations, which was not observed following administration of placebo (Fig. 1).

Mean maximum observed effect ($E_{max}$) was similar across the dose levels ranging from 81.7 to 91.8%. The median time of maximum observed effect was also similar across the dose levels and occurred at approximately 6 h postdose. Although $E_{max}$ was similar across the 25 to 75 mg dose range, the mean area under the effect curve from time zero up to 48 h postdose (AUEC) values increased with dose, from minimal change after placebo (− 98.6 h%) to 1240 h% after 25 mg crenigacestat dosing and 2110 h% after 75 mg crenigacestat dosing.

## TQT and safety

The analysis was conducted using time-matched placebo-adjusted QTcF at post-treatment time points, across the 25–75 mg crenigacestat dose range in Study 1. All matched PK and ECG samples were taken within 30 min of each other. A scatter plot of placebo-adjusted QTcF against crenigacestat plasma concentration with the fitted regression line and 90% confidence band overlaid is shown in Fig. 2. The regression slope did not significantly differ from zero [− 0.001 90% CI (− 0.006, 0.003)] suggesting no significant linear association between placebo-adjusted QTcF and crenigacestat plasma concentration.

The upper 90% CI of the predicted mean placebo-adjusted QTcF was below 10 msec even at the highest observed concentrations; therefore, there was no evidence of any significant QTcF prolongation effect from crenigacestat dosing for this plasma concentration range.

Of the 41 subjects across all three studies who received one or more doses of placebo, crenigacestat, or $^{13}C^{15}N^{2}H$-crenigacestat, 18 treatment-emergent adverse events

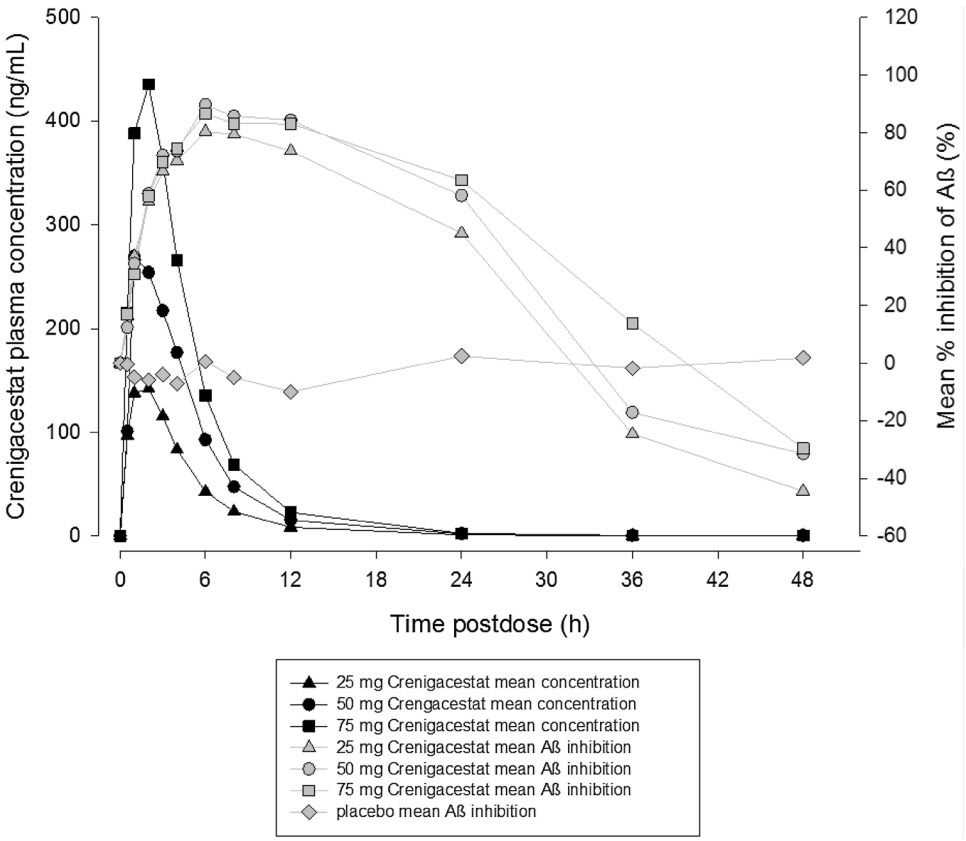


**Fig. 1** Mean plasma crenigacestat concentration and Aβ inhibition versus time profiles following single oral doses of 25, 50, or 75 mg crenigacestat or placebo in healthy subjects in Study 1

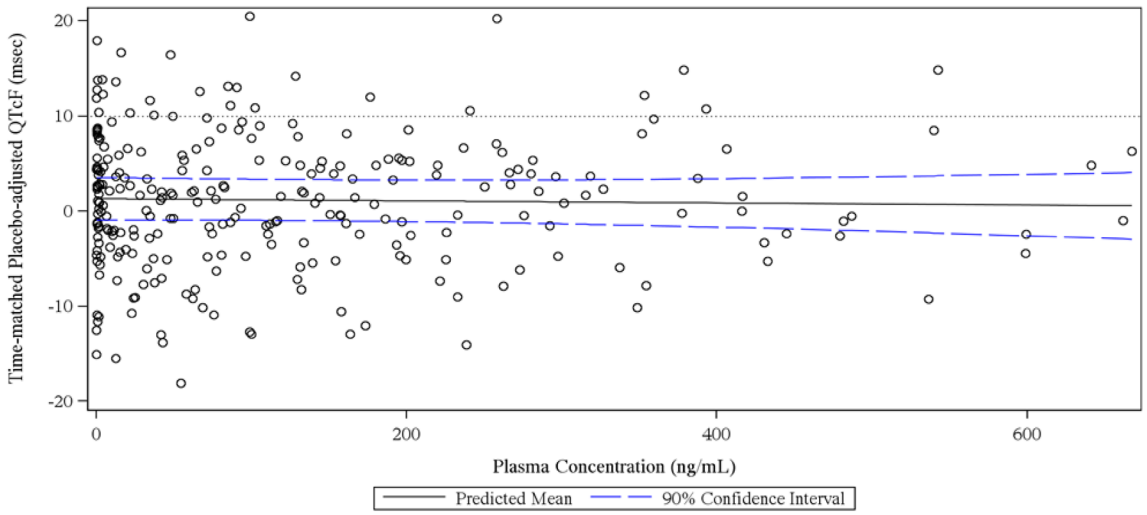


**Fig. 2** Time-matched placebo-adjusted QTcF versus crenigacestat plasma concentrations following single oral doses of 25, 50, or 75 mg crenigacestat in healthy subjects in Study 1

**Table 4** Frequency of treatment-emergent adverse events (related to study treatment)

| Treatment group | *N* | Number of AEs [number of subjects with AEs] | | | | | |
|---|---|---|---|---|---|---|---|
| | | Diarrhoea | Oral Herpes | Headache | Arthralgia | Dysgeusia | Cough |
| Study 1 | | | | | | | |
| Placebo | 14 | 1 [1] | | | | | |
| 25 mg crenigacestat | 10 | | | 1 [1] | | | |
| 50 mg crenigacestat | 9 | | 1 [1] | | | | |
| 75 mg crenigacestat | 10 | | | | | | |
| Study 2 | | | | | | | |
| 50 mg crenigacestat drug in capsule | 13 | | | | | | |
| 50 mg crenigacestat formulated capsule | 14 | | | | | | |
| Study 3 | | | | | | | |
| 75 mg crenigacestat oral + 350 µg $^{13}C^{15}N^{2}H$-crenigacestat IV | 12 | 1 [1] | | | 2 [2] | 1 [1] | 1 [1] |
| Overall | 41 | 2 [2] | 1 [1] | 1 [1] | 2 [2] | 1 [1] | 1 [1] |

*AE* adverse effect

(TEAEs) were reported by 12 subjects. Of these, eight TEAEs, reported by eight subjects, were considered related to study treatment as judged by the investigator (Table 4). The majority of AEs were of mild severity, and none were considered severe. There were no safety concerns in terms of clinical laboratory evaluations, vital signs, and 12-lead ECGs across all studies.

## Discussion

Three clinical pharmacology studies were conducted in healthy subjects using single oral doses of LY30397478 and one micro-dose of IV $^{13}C^{15}N^{2}H$-crenigacestat. The first study investigated the safety, tolerability, effect on QT interval, and PK and PD effects of single 25, 50, and 75 mg oral doses of crenigacestat compared to placebo. The second study was a pilot relative bioavailability study using 50 mg oral doses of crenigacestat formulated as drug in capsule or formulated capsule, and the third study was an absolute bioavailability study with an oral dose of 75 mg crenigacestat followed by an IV infusion of 350 µg $^{13}C^{15}N^{2}H$-crenigacestat in the same period.

These clinical pharmacology studies were conducted in healthy subjects instead of cancer patients, to mitigate the potential confounding effects of disease state and use of concomitant medications, as well as to avoid nonbeneficial drug exposures in cancer patients. In 1-month nonclinical

toxicology studies in rats and dogs using TIW dosing, the primary target organ was the gastrointestinal (GI) tract, with reversibility of mucoid enteropathy demonstrated in the 1-month rat study (data on file). Mucoid enteropathy is a known target-mediated toxicity of Notch inhibitors [13]. There was no expected risk of QT prolongation based on human ether-à-go-go-related gene (hERG) in-vitro assay results, and the most common TEAEs in the first-in-human trial considered related to the study treatment were GI events [3]. There were no reported TEAEs of Grade 3 severity or higher (regardless of relationship to study drug) on the first day of treatment in patients receiving 75 mg of crenigacestat (data on file). Taken together, the in-vitro, nonclinical and initial clinical data supported the conduct of single-dose (or single dose over repeated periods with sufficient washout) crenigacestat clinical pharmacology studies in healthy subjects.

Crenigacestat exposure following administration of the formulated capsule was dose proportional in the 25–75 mg dose range tested. The fraction of drug excreted unchanged in the urine over the first 48 h across the same dose range was approximately 20%, with the majority of drug recovered in the urine within the first 6 h postdose. Renal clearance values represented approximately 20% of the apparently plasma clearance following oral dosing and approximately 40% of the total body clearance following IV dosing. Observed apparent plasma clearances in healthy subjects after a dose of 50 mg crenigacestat in Study 1 were approximately 2.5 times higher than those observed in patients with advanced cancer [4] after receiving the same formulation, resulting in lower exposures in healthy subjects. Clinical and preliminary in-vitro data have shown that crenigacestat is cleared by a mixture of routes involving both the kidney and hydrolases in the red blood cells (data on file). The differences seen in CL/F between healthy subjects and patients with advanced cancer may in part be explained by the differences in renal and hematologic function between these two populations, where patients with advanced cancer tend to have poorer renal function and lower red blood cell counts due to disease state and multiple pretreatment regimens. The requirements for fasting around dosing were also less rigorous for patients compared to HVs, which may have also contributed to differences in exposures. However, based on preliminary in-vitro data, food is not expected to influence PK. Renal clearance values also represented approximately 20% of the apparent plasma clearance in patients, suggesting that elimination pathways did not differ between the two populations. The inter-individual variabilities in exposure were lower in healthy subjects (range 16–34%) compared to patients with advanced cancer (up to 95%) [4].

Since crenigacestat prevents release of the Notch Intracellular Domain (NICD) by inhibiting proteolytic activity of the gamma (γ)-secretase complex, and γ-secretase is also responsible for the cleavage of amyloid precursor proteins, plasma Aβ levels can be used as biomarkers for PD effects of Notch activity. In Study 1, administration of single 25–75 mg doses of crenigacestat, but not placebo, had inhibitory effects on plasma Aβ concentrations. $E_{max}$ was achieved in the majority of subjects even at the lowest dose of crenigacestat administered (25 mg); however, the area under the effect curve increased with crenigacestat dose, as a result of the response being sustained for longer as the dose was increased. In patients with advanced cancer, 80% inhibition of plasma Aβ occurred at approximate doses of 45 to 100 mg [3], which were slightly higher than that observed in healthy subjects, where approximately 80% inhibition occurred after doses of 25 mg (also exposures in healthy subjects were lower than patients with advanced cancer at similar doses). In addition to the differences in PK between healthy subjects and patients as discussed, there may also be differences in the PK–Aβ relationships between these two populations. A population PK/PD model combining patient and healthy subject data will help to identify the covariates and to quantify the differences in PK and PD between the two populations, but is outside the scope of this report.

Analysis of placebo-adjusted QTcF revealed no evidence of significant prolongation in the crenigacestat plasma concentration range achieved in healthy subjects in the 25–75 mg dose range. These findings were supported by those of the 1-month dog toxicology study in which no drug-related effects were observed in heart rate, RR interval, PR interval, QRS duration, QT, or corrected QT intervals (data on file). High-quality data collected in Phase 1 studies such as in Study 1 may be sufficient to inform the extent of ECG monitoring in Phase 3 and potentially obviate the TQT study [6, 7]. Although there have been PK differences noted between patients and healthy subjects with the latter exhibiting faster drug clearances and, therefore, lower plasma concentrations, the maximum tolerated dose in patients was 50 mg TIW [3], whereas the highest dose tested in Study 1 was 75 mg. PK and ECG data from the first-in-human study may also be pooled with the healthy subject data to widen the concentration range for further analyses.

Study 2 demonstrated that, in healthy subjects, administration of single 50 mg oral doses of crenigacestat as formulated capsules resulted in approximately 30–20% higher exposure, as measured by AUC(0–∞) and $C_{max}$, respectively, compared to crenigacestat as drug in capsule formulation. Formulated capsules are easier to scale up and are closer to the market-image formulation compared to the drug in capsule formulation, and, thus, a relative bioavailability study was conducted to ensure that PK profiles do not change significantly and no change to dosing regimen is required in future and current clinical trials when the formulation is switched over. Although the formulated capsules resulted in higher exposures, no changes to the dosing

regimen were instituted when formulations were switched, since the observed patient variability was over 90% [4], and no concentration threshold had been previously identified for safety reasons from the patient data.

In Study 3, subjects received an oral dose of 75 mg crenigacestat followed 15 min later by an IV infusion of 350 µg $^{13}C^{15}N^{2}H$-crenigacestat of duration 45 min to evaluate absolute bioavailability. Maximum concentrations of crenigacestat for the oral dose were achieved at a median time of 1.5 h postdose, which was close to the end of the IV infusion of $^{13}C^{15}N^{2}H$-crenigacestat. There are several advantages to the use of this method to estimate bioavailability. First, by administering an IV dose in the same period as an oral dose, day-to-day variation of systemic clearances is avoided, and thus, more accurate bioavailability readouts can be obtained. Second, the use of a micro-dose allows the absolute bioavailability study to be conducted without the need for a local tolerability toxicity study, and avoids solubility issues for compounds with lower solubility. In addition, the use of a stable isotope instead of radioisotope labels eliminates the safety concerns to the subject and staff involved with the collection of radioactive material.

Single oral doses of crenigacestat and IV doses of 350 µg $^{13}C^{15}N^{2}H$-crenigacestat were well tolerated by healthy subjects across all three studies. Eight TEAEs that were considered related to study drug were reported by 8 out of a total of 41 subjects. Of the 8 TEAEs, there were only two incidences related to the gastrointestinal tract, which nonclinical and clinical studies suggest is the target organ for crenigacestat toxicity. There were no safety concerns following administration of oral doses of 25, 50, and 75 mg crenigacestat and IV doses of 350 µg $^{13}C^{15}N^{2}H$-crenigacestat based on clinical laboratory evaluations, vital signs, and 12-lead ECG assessments performed during the studies.

## Conclusions

Following single oral doses of 25 to 75 mg crenigacestat administered to healthy subjects, there was no evidence of any QTcF prolongation. Single oral doses of 25 to 75 mg crenigacestat and an IV dose of 350 µg $^{13}C^{15}N^{2}H$-crenigacestat were generally well tolerated in healthy subjects. There were no other safety concerns in terms of clinical laboratory evaluations, vital signs, and 12 lead ECGs during the studies. The AUC(0–∞) and $C_{max}$ of crenigacestat administered as the formulated capsule (test) was approximately 30% and 20% higher, respectively, than the drug in capsule formulation (reference) in healthy subjects. The geometric mean (90% CI) absolute bioavailability of a 75 mg oral dose of crenigacestat was 0.572 (0.532, 0.615). Crenigacestat PK was characterized by a rapid absorption phase and biphasic elimination with an elimination half-life of approximately 6 h. Exposure to crenigacestat in healthy subjects was dose proportional in the 25 to 75 mg range tested, and the cumulative fraction of the dose excreted unchanged over 48 h postdose was approximately 20%. Administration of 25–75 mg crenigacestat had an inhibitory effect on plasma Aβ concentrations, with the maximum inhibition achieved in the majority of healthy subjects even at the lowest dose of 25 mg crenigacestat; however, the inhibition of Aβ was sustained for a longer period of time as the dose increased.

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## Compliance with ethical standards

**Conflict of interest** All authors are employees of Eli Lilly and Company.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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