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ARTICLE

Assessment of Target Engagement in a First-in-Human Trial with Sinbaglustat, an Iminosugar to Treat Lysosomal Storage Disorders

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In this first-in-human study, the tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of single and multiple oral doses of sinbaglustat, a dual inhibitor of glucosylceramide synthase (GCS) and non-lysosomal glucosyl ceramidase (GBA2), were investigated in healthy subjects. The single-ascending dose (SAD) and multiple-ascending dose (MAD) studies were randomized, double-blind, and placebo-controlled. Single doses from 10 to 2,000 mg in men and multiple doses from 30 to 1,000 mg twice daily for 7 days in male and female subjects were investigated. Tolerability, PK, and PD data were collected up to 3 days after (last) treatment administration and analyzed descriptively. Sinbaglustat was well-tolerated in the SAD and MAD studies, however, at the highest dose of the MAD, three of the four female subjects presented a similar pattern of general symptoms. In all cohorts, sinbaglustat was rapidly absorbed. Thereafter, plasma concentrations decreased biphasically. In the MAD study, steady-state conditions were reached on Day 2 without accumulation. During sinbaglustat treatment, plasma concentrations of glucosylceramide (GlcCer), lactosylceramide, and globotriaosylceramide decreased in a dose-dependent manner, reflecting GCS inhibition. The more complex the glycosphingolipid, the more time was required to elicit PD changes. After treatment stop, GlcCer levels returned to baseline and increased above baseline at lowest doses, probably due to the higher potency of sinbaglustat on GBA2 compared to GCS. Overall, sinbaglustat was welltolerated up to the highest tested doses. The PK profile is compatible with b.i.d. dosing. Sinbaglustat demonstrated target engagement in the periphery for GCS and GBA2.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THIS TOPIC?

☑ Lysosomal storage disorders (LSDs) are caused by mutations in genes encoding lysosomal enzymes or transporters, leading to an intracellular accumulation of substrate and cellular dysfunction. Glucosylceramide synthase (GCS) inhibitors are approved treatments for two LSDs.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ This study investigated the safety, tolerability, and pharmacokinetics (PKs) of sinbaglustat, a GCS and non-lysosomal glucosyl ceramidase (GBA2) inhibitor. Additionally, the pharmacodynamic effects of the drug on plasma glycosphingolipids (GSLs) were investigated to support decisions for future clinical development.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Sinbaglustat was well-tolerated at all tested doses, with a PK profile compatible with b.i.d. dosing. Dose-dependent inhibition of GCS, including modulation of downstream GSLs, was demonstrated, and evidence of GBA2 target engagement was also gained. The study also provided an unprecedented understanding of GSL kinetics in humans.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ These data confirm that sinbaglustat can be further developed for the treatment of LSD.

Complex glycosphingolipids (GSLs) are synthesized ***de novo*** in the Golgi apparatus and then transported to the plasma membrane. GSLs are degraded in the late endosome/lysosome and can be recycled via the salvage pathway giving rise to new ganglioside synthesis or converted to sphingosine-1-phosphate and ultimately degraded[1] (**Figure 1**).

Lysosomal storage disorders (LSDs) are a group of ~ 60 rare diseases caused by deleterious mutations in genes encoding lysosomal enzymes or transporters.[2,3] In particular, many of these genes are involved in the degradation of GSLs. Their deficiency is associated with the toxic intracellular accumulation of GSLs, resulting in cellular dysfunction and organ damage. Some of the LSDs are exemplified in **Figure 1**.

LSDs caused by a primary dysfunction of GSLs catabolism include Gaucher disease (GD), Fabry disease (FD),

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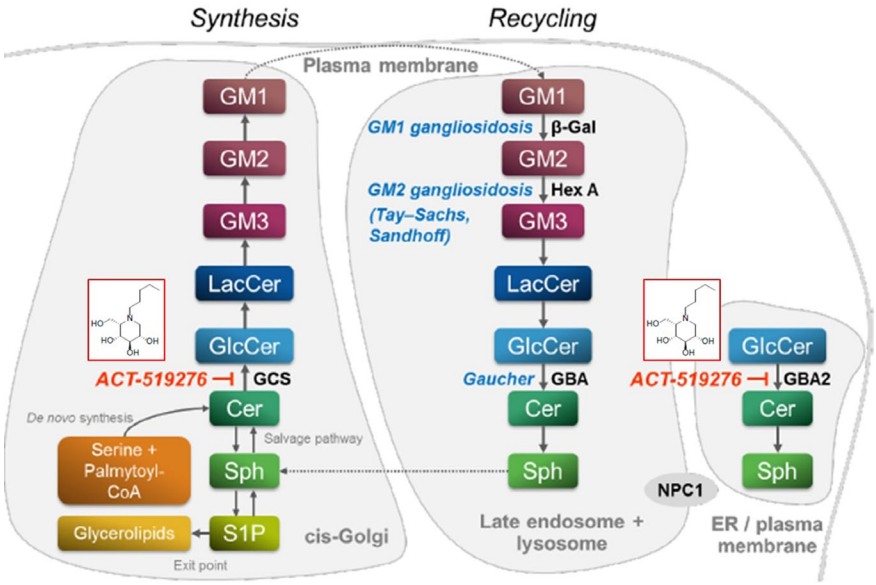

Figure 1 Sphingolipid metabolism and associated lysosomal storage disorders. Sphingolipid classes are shown in colored boxes. Lysosomal storage disorders (LSDs) are written in blue italics with their associated enzymes in black. β-Gal, β-galactosidase; Cer, ceramide; CoA, coenzyme A; ER, endoplasmic reticulum; GBA, lysosomal glucosylceramidase; GBA2, non-lysosomal glucosylceramidase; GCS, glucosylceramide synthase; GlcCer, glucosylceramide; GM1, monosialotetrahexosylganglioside; GM2, monosialotrihexosylganglioside or Tay-Sachs ganglioside; GM3, monosialodihexosylganglioside; Hex A, β-hexosaminidase A; LacCer, lactosylceramide; NPC1, Niemann-Pick C1 protein; S1P, sphingosine-1-phosphate; Sph, sphingosine.

and the gangliosidoses.[1] In Niemann-Pick type C (NPC) disease, cholesterol trafficking to lysosomes is disturbed, and is associated with a secondary accumulation of GSLs.[4] These diseases are life-threatening disorders with multiple organ defects that can affect children and adults. Most LSDs have neurological components and patients are affected, among other alterations, with motor and/or cognitive dysfunctions.

The most prominent treatment paradigm for the treatment of enzyme deficient LSDs has been to restore or enhance the activity of the affected enzyme, using enzyme replacement therapy (ERT) with recombinant enzymes.[5–7] However, systemically administered recombinant enzymes are unable to cross the blood-brain barrier. As a consequence, ERT has largely been restricted to LSDs with predominantly non-neurological pathophysiology.[8] ERT is available for the non-neuronopathic form of GD (type I) and FD, but the treatment is a heavy burden as it requires lifelong biweekly infusions. Other strategies have been developed, including chaperone molecules, such as migalastat[9] for FD and substrate reduction therapy (SRT), which uses small molecules to decrease the biosynthesis of the GSL to a level compatible with its residual clearance.[10,11] To date, inhibitors of the enzyme glucosylceramide synthase (GCS) have been developed as SRT in LSDs. GCS is responsible for the conversion of ceramide into glucosylceramide (GlcCer), which is then further glycosylated to form more complex GSLs (**Figure 1**). Inhibitors of GCS were shown effective in animal and cellular models not only of GD in which its direct GlcCer product is the accumulated lipid, but also in models of diseases in which more complex downstream GSLs accumulate, such as FD, Sandhoff disease, and GM1 gangliosidosis.[8,12–14]

Clinical experience with SRT indicates that this strategy could be effective for the management of LSDs.[10,11] Two GCS inhibitors, miglustat and eliglustat, have been approved as GD type I treatment.[15,16] Miglustat, combined with a low-carbohydrate ketogenic diet, was also associated with increased survival in infantile GM1 gangliosidosis as well as in infantile GM2 gangliosidosis but to a lesser extent.[17] Another GCS inhibitor, lucerastat, provided biomarker data[18] and is currently under investigation in a phase III study to determine its effect on neuropathic pain and gastrointestinal symptoms, two key clinical symptoms of FD (NCT03425539).

Sinbaglustat (ACT-519276, OGT2378) is an orally available N-alkyl iminosugar ((2S,3R,4R,5S)-2-(hydroxymethyl)-1-pentylpiperidine-3,4,5-triol) that crosses the blood-brain barrier.[19] It inhibits both GCS and the non-lysosomal glucosylceramidase (GBA2), an enzyme involved in the catabolism of GSLs (**Figure 1**). Sinbaglustat is 50-fold more potent in inhibiting GBA2 than GCS. Consequently, at low doses, sinbaglustat is expected to increase GlcCer levels by inhibiting GBA2 without inhibiting GCS and without altering the GlcCer downstream synthesis products: lactosylceramide (LacCer) and globotriaosylceramide (Gb3) produced in the Golgi (**Figure 1**). At higher doses, inhibition of GCS in the Golgi should first compensate GBA2 inhibition and then lead to a decrease in GlcCer levels. The latter will subsequently lead to a decrease in LacCer and Gb3. In the extralysosomal compartments, GlcCer can be degraded by GBA2 to ceramide and sphingosine, both of which act as pro-apoptotic triggers.[20] Genetic blockade of GBA2 activity has shown to be beneficial in GD type 1[21] and NPC[22] animal models. Pharmacological blockade extended survival of GM2 gangliosidosis[23] and NPC[22] mouse

models. In addition to reducing pro-apoptotic ceramide, GBA2 inhibition also appears to aid normalization of aberrant lysosomal pH.[24]

Sinbaglustat is being developed for the treatment of central neurodegenerative diseases associated with lysosomal dysfunctions. In preclinical neurodegenerative models, sinbaglustat has increased the survival of animals (Idorsia Pharmaceuticals, manuscript in preparation).

In these single-ascending dose (SAD) and multiple-ascending dose (MAD) studies in healthy subjects, tolerability and pharmacokinetics (PKs), including the effect of food, were assessed. Moreover, the pharmacodynamics (PD) of sinbaglustat on plasma GSLs were investigated to gain understanding of target engagement and of the wider clinical potential of sinbaglustat to treat LSDs.

METHODS

This study (NCT03372629) followed the principles of the Declaration of Helsinki and Good Clinical Practice. The protocol, its amendment, and any material provided to the subjects were reviewed and approved by North East-York Ethics Committee, UK. The study was conducted at Covance Clinical Research Unit, Leeds, UK.

Study design and dosing

The SAD and MAD studies were conducted following a single-center, randomized, double-blind, placebo-controlled design. Each dose level was investigated in a separate cohort of 8 subjects in the SAD study (n = 6 for sinbaglustat, n = 2 for placebo), and 10 subjects in the MAD study (n = 8 for sinbaglustat, n = 2 for placebo). In both studies, a staggered treatment administration scheme was applied within each dose level. Two subjects (1 active, 1 placebo) were initially investigated. After review of the interim tolerability data by the investigator without detection of any finding impacting the well-being of the first two subjects, the remaining subjects of the cohort were dosed. Escalation to the next dose was based on the available tolerability and PK data of the previous cohort.

The starting dose of 10 mg in the SAD study was supported by a safety margin of 114 for the most sensitive species (rats), using the human equivalent approach. The safety margin was obtained by dividing the no-observed-adverse-effect level exposure in rats by the predicted human exposure at the starting dose. Other single doses investigated in the SAD study were 30, 100, 300, 1,000, and 2,000 mg. The starting dose of 30 mg b.i.d. in the MAD study was selected on the basis of the blinded analysis of tolerability, safety, and PK data collected in the first three cohorts of the SAD study. The other doses tested in the MAD study were 100, 300, and 1,000 mg b.i.d., administered for 7 days. All morning drug administrations (for the SAD and MAD study) were performed after a 10-hour fast, except for the SAD 300 mg cohort in which subjects received the treatment first in fasted state and then again under fed conditions (30 minutes after a standardized high-fat and high-calorie breakfast) after a washout period of at least 7 days.[25] In the MAD study, the evening administration took place 2 hours after dinner.

Screening occurred from Day –28 to Day –2 (–10 for women of childbearing potential (WoCBP)). Subjects were admitted to the clinic in the afternoon of Day –1 and were confined to the clinic until 48 hours and 72 hours after (last) treatment administration for the SAD and MAD studies, respectively. End-of-study took place after the last PK sample was taken (48 and 72 hours after (last) treatment administration for the SAD and MAD studies, respectively). A telephone (or at-site for WoCBP) safety follow-up took place 29–34 days after (last) administration of the treatment.

Subjects

Each participating subject provided written informed consent. Healthy male subjects were enrolled in the SAD study and healthy male and female subjects were enrolled in the MAD study. In the latter, the male:female ratio was 1:1. Subjects were healthy based on medical history, physical examination, electrocardiogram (ECG), vital signs, and clinical laboratory tests. Subjects had to be between 18 and 55 years of age (included) and had to have a body mass index (BMI) between 18 and 30 kg/m^2. They could not participate if they smoked, had a history of drug or alcohol abuse, were allergic to any excipient of the drug formulation, or were using any medication (except for contraceptives). WoCBP had to have a negative pregnancy test at screening and on Day 1 predose, and had to agree to use for up to at least 30 days after the last treatment a highly effective method of contraception and ask their partner to either use condoms, or be sexually abstinent, or have a vasectomized partner. Male subjects had to agree to use condoms and spermicide and not to donate sperm for up to 90 days after the last treatment.

Pharmacokinetic assessments

Serial blood samples were collected for up to 48 hours postdose in the SAD study. In the MAD study, samples were collected for 12 hours on Day 1, for up to 72 hours following the last dose, and at trough on Days 2–6. Urine was collected in the 300 mg dose group in the SAD study (predose, 0–12, 12–24, and 24–48 hours after treatment) and in all cohorts of the MAD study (Day 1 predose and 0–12 hours on Day 7). Concentrations of sinbaglustat were measured in plasma and urine using validated chromatography coupled to liquid chromatography tandem mass spectrometry methods (LC-MS/MS) (**Supplementary Information S1**).

Plasma concentrations were subjected to noncompartmental analysis using Phoenix WinNonlin version 8.0 (Certara L.P., Mountain View, CA) in order to obtain the time to maximum concentration (T_{max}), the maximum concentration (C_{max}), the area under the curve (AUC) from 0 to time of the last measured concentration above the limit of quantification (AUC_{0-t}), AUC over a dosing interval (AUC_{τ}), AUC from zero to time 12 hour (AUC_{0-12}), the AUC from zero to infinity ($AUC_{0-\infty}$), the terminal half-life, the accumulation index, renal clearance (CL_R), and percentage of dose excreted unchanged in urine.

Pharmacodynamic assessments

GlcCer and its downstream synthesis products LacCer and Gb3, see **Figure 1**, were measured to investigate

the inhibitory potential of sinbaglustat on GCS. Serial blood samples were collected in each cohort of the MAD study, on Day 1 (0, 1, 2, 6, and 12 hours), Day 2 (0 hour), Day 4 (0 hour and 12 hours), and Day 7 (0, 1, 2, 6, and 12 hours), and 24, 48, and 72 hours after last treatment administration.

The concentration of GlcCer, LacCer, and Gb3 was measured in plasma using a validated LC-MS/MS method (**Supplementary Information S2**).

Tolerability/safety assessments

The safety and tolerability of sinbaglustat, as compared with baseline or placebo, were assessed by recording of adverse events (AEs), vital signs (blood pressure and pulse rate), ECG, physical examination, and clinical laboratory assessments. ECG was also monitored with Holter on Day 1 in the last cohort of the SAD and on Days 1 and 7 in the MAD study. For the cardiodynamic analysis, 10 ECG replicates were extracted from the Holter recordings from a 5-minute window at planned time points.

Data analysis

All PK, PD, and tolerability variables were analyzed descriptively. Differences in plasma PK variables AUC_{0-t} and C_{max} in the SAD study between the fed and the fasted (reference) states were investigated using a 2-sided 90% confidence interval (CI) of the the geometric mean ratio (GMR). Differences between fasted and fed T_{max} were explored using the median differences (nonparametric analysis) and their 90% CI using the fasted state as reference. A similar analysis was used to investigate the differences in AUC_{τ} and C_{max} between male and female subjects in the MAD study. Dose proportionality of the PKs of sinbaglustat was explored by comparing C_{max} and AUC values, corrected for dose and log transformed, using a power model described by Gough *et al*.[26] Attainment of steady-state conditions was determined by visual inspection of the trough plasma concentration-time profile and by analysis of variance of the plasma morning trough concentrations (from Day 2 to Day 7).

GlcCer, LacCer, and Gb3 concentrations were used to assess the percentage change from Day 1 predose to each postdose time point of measurement.

An exposure-response analysis was performed to investigate the potential effect of sinbaglustat on the QT interval. The relationship between sinbaglustat plasma concentration and change-from-baseline corrected QT Fridericia's formula (QTcF; ΔQTcF) was quantified using a linear mixed-effects model with ΔQTcF as the dependent variable, drug plasma concentration as continuous covariate, treatment (active or placebo) and time point as categorical factors, and a random intercept per subject. The degrees of freedom for the model estimates were determined by the Kenward–Roger method.[27] From the model, the slope (i.e., the regression parameter for the concentration) and the treatment effect (defined as the difference between active and placebo) were estimated together with the 2-sided 90% CI.

RESULTS

Demographics

In the SAD study, a total of 48 male subjects were enrolled. Of the 48 subjects, 40 were White, 7 were Black/African American, and 1 was Caucasian/Asian. Their median age was 30.5 years (range 19–54 years) and median BMI was 25.6 kg/m^2 (range 18.2–29.7). Median age and BMI were similar in all dose groups of the SAD study. In the MAD study, a total of 40 subjects were enrolled, 20 male and 20 female subjects; 36 were white, 3 were Black/African American, and 1 was Caucasian/Black. Their median age was 31.0 years (range 20–55 years) and median BMI was 23.5 kg/m^2 (range 19.5–29.5 kg/m^2). The median age, weight, height, and BMI were similar in all dose groups of the SAD and MAD studies. All subjects completed the study.

Single-dose pharmacokinetics

The plasma concentration-time profiles were characterized by a T_{max} between 0.9 and 2.0 hours and a biphasic disposition (**Figure 2a**). The distribution phase lasted ~ 24 hours and led to a decrease in sinbaglustat concentration of > 98% compared to the C_{max}. The second phase, used to characterize terminal half-life, could only be reliably assessed in 3 subjects dosed with 1,000 mg and in 1 subject dosed with 2,000 mg sinbaglustat and was about 12 hours (**Table 1**). Sinbaglustat PK were dose proportional up to 2,000 mg. The Gough test for dose proportionality yielded estimates of the slope for C_{max} and AUC_{0-t} of 0.96 and 1.10, respectively, and the corresponding 90% CIs, 0.92–1.00 and 1.08–1.13, were within the critical interval (0.87–1.13) for both parameters. About half of the 300 mg dose was excreted unchanged in urine and the CL_R was 225 mL/min.

After a high-fat, high-calorie standardized meal, the rate of absorption was slightly decreased (**Figure 2b**), as indicated by a median difference in T_{max} of 1 hour (90% CI 0.50–1.70) and a slight decrease in C_{max} (GMR of 0.81 (fed/fasted, 90% CI 0.66–0.99)). When compared with the fasted state, exposure as based on AUC_{0-t} remained unchanged: GMR (90% CI) 1.08 (1.00–1.16).

Multiple-dose pharmacokinetics

The plasma concentration-time profiles of sinbaglustat after multiple-dose administration were similar to those obtained after single doses (**Supplementary Information S3**). Steady-state concentrations of sinbaglustat were reached by Day 2 (**Supplementary Information S3**). Sinbaglustat did not accumulate after multiple doses (**Table 2**). The Gough test for dose proportionality yielded estimates of the slope (90% CI) for C_{max} and AUC_{τ} of 0.93 (0.86–1.00) and 1.06 (1.01–1.11), respectively, which were within the critical interval (0.80–1.20). At steady-state, the fraction of the dose excreted unchanged in urine over a dosing interval and the CL_R ranged from 55–46% and from 211–144 mL/min, respectively, for doses ranging from 30–1,000 mg b.i.d.

Overall, for all dose groups combined, at steady-state, female subjects showed an increased exposure of around 30% (compared to male subjects for C_{max} and AUC_{τ}).

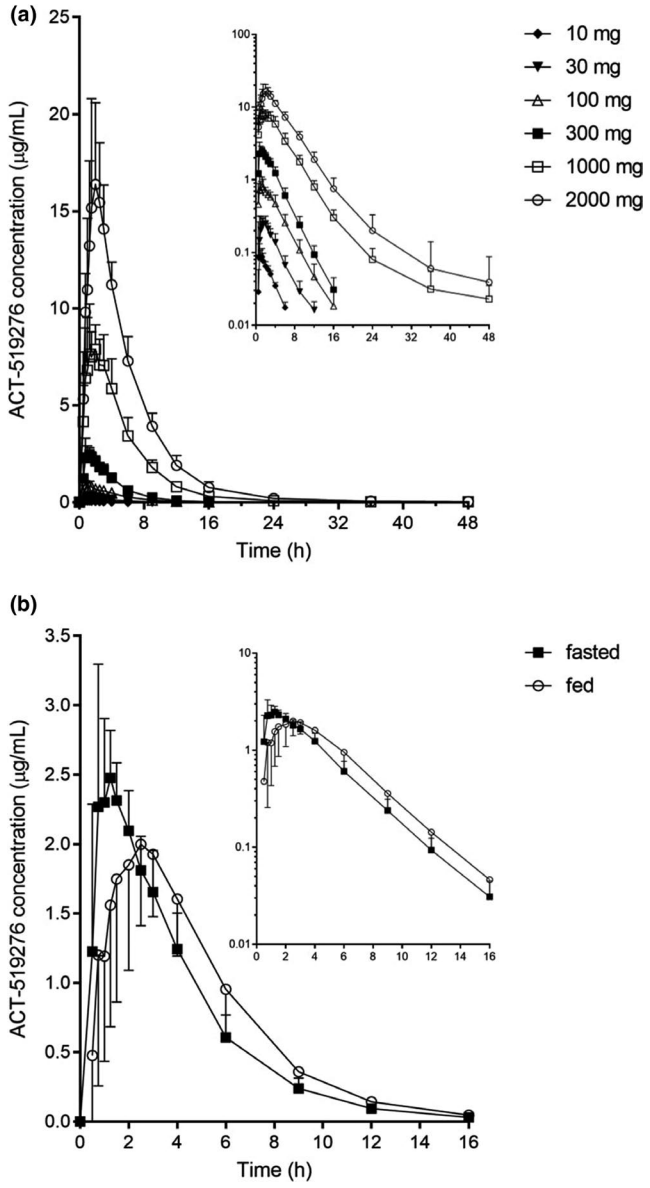

Figure 2 Sinbaglustat concentration-time profiles. (**a**) Arithmetic mean (±SD) plasma concentration-time profiles of sinbaglustat after single-dose administration in healthy male subjects in the fasted condition (n = 6) on linear and semilogarithmic scale. (**b**) Arithmetic mean (±SD) plasma concentration-time profiles of sinbaglustat after 300 mg single dose in fasted and fed conditions (n = 6) on linear and semilogarithmic scales.

Pharmacodynamics

A dose-dependent gradual decrease in GlcCer, LacCer, and Gb3 plasma concentrations was observed during repeated treatment with sinbaglustat (**Figure 3**). The 300 and 1,000 mg b.i.d. dose groups presented similarly decreased levels of the markers. With 30 mg sinbaglustat, the decrease was only observed for LacCer and Gb3 but not for GlcCer. The maximum decrease from baseline was observed on Day 7 for the 3 markers.

After the last dosing interval, GlcCer and LacCer concentrations gradually increased. The concentration of GlcCer returned to baseline in the 300 and 1,000 mg b.i.d. dose groups 72 hours after the last dose. The percentage change from baseline was –1 and –19% for 300 and 1,000 mg b.i.d., respectively. In the 100 mg b.i.d. dose group, the concentration of GlcCer returned to baseline 48 hours after the last dose (–7% change from baseline) and continued to increase above baseline 72 hours after last dose (+17% change from baseline). In the 30 mg b.i.d. dose group, for which GlcCer remained at baseline during the treatment period, GlcCer concentration started to gradually increase after the last dose of treatment and reached 32% increase from baseline concentration 72 hours after the last dose. LacCer concentrations tended to return to baseline (–8, –17, –26, and –33% change from baseline at 72 hours after the last dose in the 30, 100, 300, and 1,000 mg b.i.d. dose groups, respectively), but more slowly than GlcCer concentrations. Gb3 mean percentage change from baseline remained stable for 3 days after treatment stop.

In the placebo group, levels of GlcCer, LacCer, and Gb3 were stable across the 10 days of measurement, the mean percentage change from baseline ranged from –7 to +2%, –5 to +5%, and –3 to +3% for GlcCer, LacCer, and Gb3, respectively.

Tolerability

No serious AEs, severe AEs, or AEs leading to study or treatment discontinuation occurred in the study (up to end-of-study).

In the SAD study, 7 of the 36 subjects (19%) treated under fasted conditions with sinbaglustat reported a total of 13 AEs (**Table 3**). Two of the 12 subjects (17%) treated with placebo under fasted conditions reported a total of 3 AEs. All reported AEs were of mild intensity. The most frequently reported AE was headache, which was reported three times by two different subjects on active treatment with no relationship to dose. All other AEs were reported only once by one subject. All AEs resolved without sequelae. No AE was reported for the 30, 300, and 2,000 mg cohort and no AE was reported under fed conditions by any subject treated with sinbaglustat.

In the MAD study, 19 of 32 subjects (59%) treated with sinbaglustat reported a total of 66 AEs (**Table 3**). Five of 8 subjects (63%) treated with placebo reported a total of 10 AEs. The most frequently reported AEs were dizziness (10 subjects of 32 on sinbaglustat, and 2 subjects of 8 on placebo) and headache (8 subjects of 32 on sinbaglustat and none on placebo). Most subjects reported AEs of mild intensity and 7 subjects reported AEs of moderate intensity, including headache, nausea, dizziness, constipation, and arthralgia. All AEs resolved without sequelae at the time of the follow-up telephone call, except for two AEs of paresthesia and urticaria, each in one subject, for which the resolution information was missing. At 1,000 mg b.i.d. of sinbaglustat, all 4 female subjects reported headache and 3 of them reported a similar pattern of general symptoms that was of mild to moderate intensity: dizziness, nausea, decreased appetite, vomiting (2 subjects), fatigue (2 subjects), and diarrhea (2 subjects). None of the four male subjects reported these AEs.

No treatment-related pattern was detected that suggested an effect of single-dose or multiple-dose administration of sinbaglustat on blood pressure, clinical laboratory variables

Table 1 Geometric mean (95% CI) pharmacokinetic parameters of sinbaglustat following single-dose administration in healthy male subjects

Parameter	10 mg	30 mg	100 mg	300 mg fasted	300 mg fed	1,000 mg	2,000 mg
C_{max}, ng/mL	99.8 (65.2, 152.7)	311.4 (222.1, 436.5)	918.4 (724.8, 1,163.6)	2,819 (2,564, 3,099)	2,273 (1,818, 2,843)	8,590 (7,003, 10,536)	16,713 (13,171, 21,208)
T_{max}, hour[a]	1.00 (0.8, 2.5)	1.50 (0.8, 2.0)	1.40 (0.5, 4.0)	0.90 (0.8, 2.0)	2.25 (0.8, 4.0)	1.50 (0.8, 3.0)	2.00 (1.5, 2.5)
AUC_{0-t}, ng*hour/mL	282 (255, 311)	1,068 (857, 1,331)	3,946 (3,566, 4,366)	10,842 (9,288, 12,656)	11,701 (10,141, 13,501)	49,971 (44,442, 56,188)	101,360 (827,71, 124,125)
$t_{1/2}$, hour	–	–	–	–	–	11.57[b] (9.33, 14.34)	–[c]
UPE, %	–	–	–	48.74 (43.53, 54.59)	–	–	–
CL_R, mL/minutes	–	–	–	224.8 (203.1, 248.8)	–	–	–

N = 6 for each dose.
AUC_{0-t}, area under the plasma concentration-time curve from zero to time of the last measured concentration above the limit of quantification; CI, confidence interval; CL_R, renal clearance; C_{max}, maximum plasma concentration; $t_{1/2}$, terminal half-life; T_{max}, time to reach C_{max}; UPE, percentage of dose excreted unchanged in urine.
[a]T_{max} is presented as median (range). [b]*N* = 3. [c]Geometric mean value not calculated as assessed in only one subject.

Table 2 Geometric mean (95% CI) pharmacokinetic parameters on day 1 and day 7 of sinbaglustat following multiple-dose administration in healthy male and female subjects

Parameter	30 mg b.i.d.	100 mg b.i.d.	300 mg b.i.d.	1,000 mg b.i.d.
Day 1				
C_{max}, ng/mL	3,67.1 (2,81.3, 479.2)	1,345 (1,130, 1,602)	3,765 (2,778, 5,105)	10,582 (9,297, 12,043)
T_{max}, hour[a]	0.90 (0.8, 2.5)	0.90 (0.5, 2.6)	1.40 (0.8, 1.5)	1.45 (1.0, 2.5)
AUC_{0-12}, ng*hour/mL	1,205 (988, 1,468)	4,479 (3,971, 5,052)	15,219 (11,766, 19,686)	51,214 (44,119, 59,451)
Day 7				
C_{max}, ng/mL	413.4 (302.6, 564.8)	1,269 (1,171, 1,374)	3,993 (2,905, 5,489)	10,415 (8,686, 12,487)
T_{max}, hour[a]	0.90 (0.8, 1.3)	1.30 (0.8, 1.5)	0.90 (0.5, 2.5)	2.00 (1.3, 4.0)
AUC_{τ}, ng*hour/mL	1,306 (1076, 1585)	4,646 (4,051, 5,329)	16,774 (13,061, 21,541)	51,235 (44,190, 59,402)
$t_{1/2}$, hour	–	–	–	14.73[b] (9.93, 21.84)
AI	1.085 (0.993, 1.185)	1.037 (0.930, 1.156)	1.101 (1.012, 1.198)	1.001 (0.942, 1.064)
UPE, %	55.12 (52.08, 58.34)	53.55 (44.68, 64.19)	48.35 (40.84, 57.24)	45.54 (41.54, 49.92)
CL_R, mL/minutes	211.2 (172.5, 258.6)	192.2 (147.7, 250.0)	144.0 (113.7, 182.4)	148.2 (122.6, 179.3)

AI, accumulation index; AUC_{τ}, area under the concentration-time curve during a dose interval; AUC_{0-12}, AUC from zero to time 12 hour; CI, confidence interval; CL_R, renal clearance; C_{max}, maximum plasma concentration; $t_{1/2}$, terminal half-life; T_{max}, time to reach C_{max}; UPE, percentage of dose excreted unchanged in urine.
N = 8 for each dose.
[a]T_{max} is presented as median (range). [b]*N* = 7.

(including clinical chemistry, hematology, coagulation, and urinalysis), or ECG variables.

Cardiodynamic analysis

The cardiodynamic analysis of change-from-baseline and time-matched, placebo-adjusted change-from-baseline values revealed no clinically relevant effect of sinbaglustat on QTcF, HR, PR, and QRS intervals.

The results of the exposure-response analysis are presented in **Figure 4**. No concentration-dependent effect of sinbaglustat on ΔΔQTcF was identified, with a statistically nonsignificant slope of the relationship of 0.0002 ms per ng/mL (90% CI −0.00008 to 0.00056) and a nonstatistically significant treatment effect-specific intercept of −2.7 ms. Overall, the analysis showed that a QT effect (ΔΔQTcF) of sinbaglustat exceeding 10 ms can be excluded up to sinbaglustat plasma concentrations of ~ 22,000 ng/mL.

DISCUSSION

In this study, sinbaglustat, an inhibitor of GCS and GBA2, was administered for the first time as single and multiple doses to healthy male and female subjects.

After single-dose and multiple-dose administration, the absorption of sinbaglustat was rapid. The disposition of sinbaglustat was biphasic with rapidly decreasing concentrations in the distribution phase. The elimination phase started around 24 hours postdose and was observable for subjects dosed with ≥ 1,000 mg.

Food intake slightly decreased the rate of sinbaglustat absorption but did not change the extent of absorption. Therefore, sinbaglustat can be administered with or without food.

Overall, for all dose groups combined, female subjects showed an increased exposure of around 30% for C_{max}

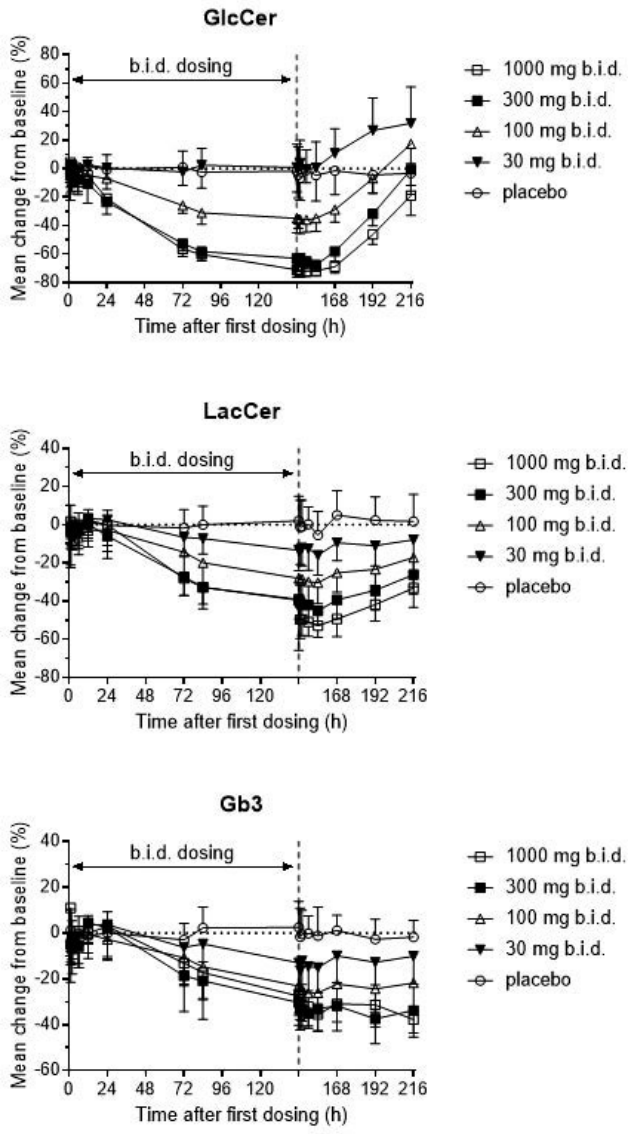

Figure 3 Arithmetic mean (±SD) percentage of change from baseline in GlcCer, LacCer, and Gb3 following multiple-dose administration of sinbaglustat or placebo b.i.d. for 7 days ($N = 8$). Gb3, globotriaosylceramide; GlcCer, glucosylceramide; LacCer, lactosylceramide.

and AUC_τ. This difference could not be entirely explained by a body weight difference, as it was still observed to some extent after body weight correction. The underlying mechanism for this difference, if not linked to intersubject variability, remains unknown.

As for miglustat and lucerastat, renal excretion of unchanged parent drug is a major elimination route for sinbaglustat, with ~ 50% of the dose excreted unchanged in urine for doses ranging from 30 to 1,000 mg b.i.d. CL_R was higher than the glomerular filtration rate, which suggests that CL_R includes active secretion of sinbaglustat into urine. The observation that CL_R decreases with dose in the MAD study could be explained either by variability, or by auto-inhibition of the organic cation transporter 2. Sinbaglustat has been characterized *in vitro* as a substrate and an inhibitor of this renal transporter (Idorsia Pharmaceuticals Ltd, unpublished data). Despite the observed decrease in CL_R, the PK parameters were dose proportional over the tested dose range in the SAD and MAD studies, pointing toward variability between subjects or suggesting that another elimination pathway might compensate for the lower CL_R.

Plasma sphingolipids are part of lipoprotein complexes which are exocytosed from the liver.[28,29] During repeated treatment with sinbaglustat, plasma GlcCer, LacCer, and Gb3 concentrations decreased in a dose-dependent manner. The decrease in plasma GSLs is attained through inhibition of GCS. The highest tested dose of sinbaglustat resulted in a reduction in GlcCer of 72%, which is in line with the data published for other GCS inhibitors.[30] The GCS selective inhibitor venglustat decreased plasma GlcCer in healthy subjects by up to 80%.[31] The dose-dependent reduction of GlcCer demonstrates target engagement (i.e., inhibition of peripheral GCS by sinbaglustat). The effect of GCS inhibition appeared to propagate down the GSL pathway, with LacCer and Gb3 also being reduced. The rate of GSL change appeared to be slower the more complex the GSL, both during sinbaglustat administration and after cessation of drug treatment, indicating that the more complex the GSLs the longer their plasmatic half-life. As larger net decreases in plasma LacCer and Gb3 have been observed in longer studies with other GCS inhibitors, such as lucerastat[18] and venglustat (NCT02228460), it is likely that a maximal effect was not reached for these GSLs after 1 week of treatment. This study is the first to provide a high-time resolution of the GSLs kinetics in humans and showed that the daily variation in drug exposure had no impact on the GSL levels.

Although the lowest dose of 30 mg b.i.d. did not lead to a decrease in plasmatic GlcCer, the downstream GSLs LacCer and Gb3 were still decreased suggesting effective GCS inhibition. The absence of GlcCer decrease in plasma might be explained by GBA2 inhibition in the endoplasmic reticulum, which would lead to an increased GlcCer concentration in this compartment resulting in a null net effect. The peripheral net effect of sinbaglustat on GlcCer is driven by GCS inhibition, which masks the GBA2 inhibition component at doses higher than 30 mg b.i.d. A similar bell-shaped GlcCer dose-response curve has been observed for the dual GBA2, GCS inhibitor miglustat in mouse liver.[32] After cessation of sinbaglustat administration, GlcCer concentration increased rapidly, which led to an increase exceeding the baseline in the 30 and 100 mg b.i.d. groups. This observation is consistent with the higher potency of sinbaglustat for GBA2 inhibition compared with GCS and suggests GBA2 target engagement in the periphery. A quantitative analysis of the effect of sinbaglustat on GCS and GBA2 would improve the understanding of the sinbaglustat dose-response relationship. However, to develop this model, a quantification of the GBA2 contribution independently from GCS contribution would be required. Lower sinbaglustat doses than those administered in the study and/or longer post-treatment PD sampling time would be necessary and will be the aim of future studies.

Table 3 Overview of reported main AEs by treatment after SAD and MAD of sinbaglustat

	SAD					MAD				
	Treatment (mg)					Treatment (mg) b.i.d. for 7 days				
	10 (*n* = 6)	100 (*n* = 6)	1,000 (*n* = 6)	Placebo fasted (*n* = 12)	Placebo fed (*n* = 2)	30 (*n* = 8)	100 (*n* = 8)	300 (*n* = 8)	1,000 (*n* = 8)	Placebo (*n* = 8)
Number of subjects with at least 1 AE	1	3	3	2	1	5	5	4	5	5
Total number of AEs	1	3	9	3	1	13	8	8	37	10
Headache	–	–	2	1	1	2	–	2	4	–
Dizziness	1	–	–	–	–	4	2	1	3	2
Nausea	–	–	1	–	–	–	–	1	3	–
Fatigue	–	–	–	–	–	–	1	–	2	–
Decreased appetite	–	–	–	–	–	–	–	–	3	–
Vomiting	–	–	1	–	–	–	–	–	2	–
Muscle twitching	–	–	–	–	–	1	–	–	1	–
Constipation	–	–	–	–	–	–	–	–	1	1
Diarrhea	–	–	–	–	–	–	–	–	2	–
Pain in extremity	–	–	–	1	–	1	–	–	–	–
Palpitations	–	–	1	–	–	–	–	–	1	–
Viral upper respiratory tract	–	–	1	–	–	1	–	–	–	–
Rash	–	1	–	–	–	–	–	1	–	–

AE, adverse event; MAD, multiple-ascending dose; SAD, single-ascending dose.
Only AEs reported by more than one subject in the study are reported in the table. AEs reported more than once by the same subject were counted only once. No AEs were reported in the 30, 300, and 2,000 mg cohorts in the SAD study.

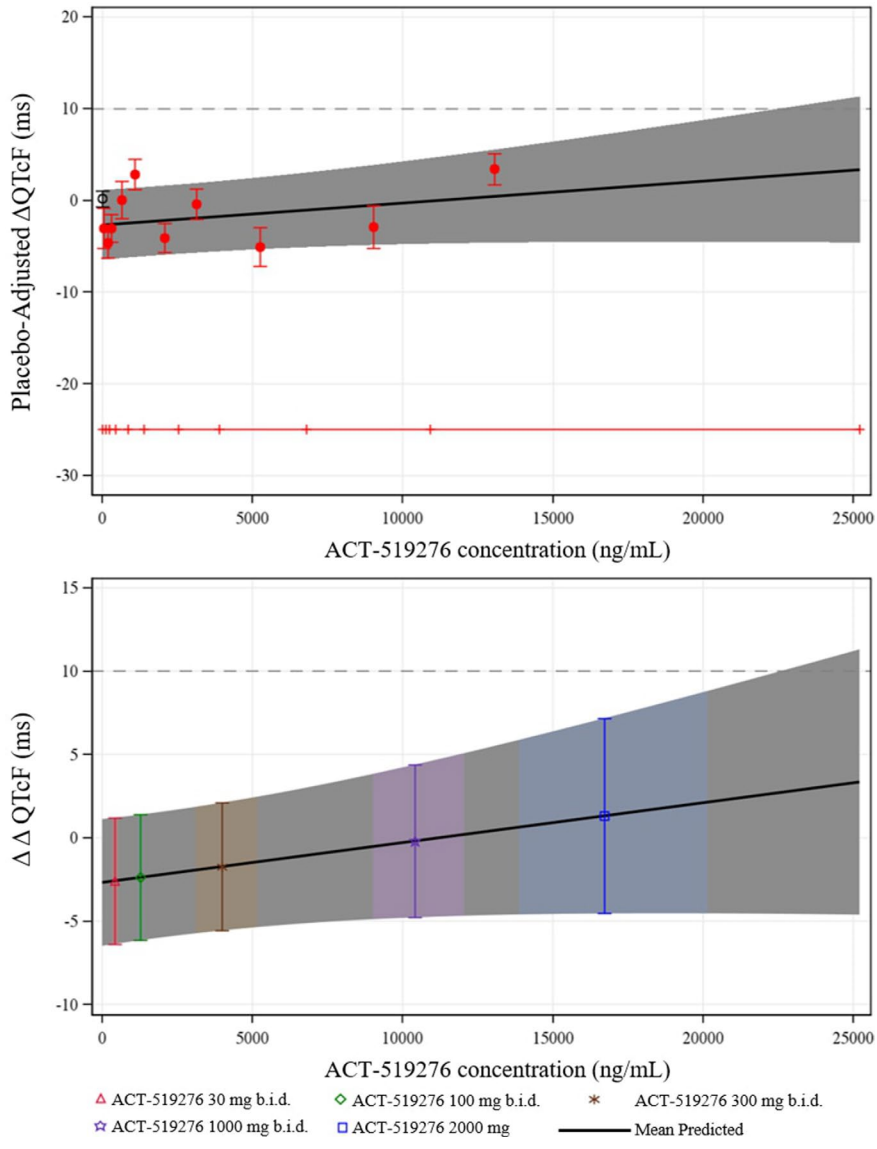

Figure 4 Model-predicted and observed baseline corrected QT Fridericia's formula (ΔΔQTcF; mean and 90% confidence interval (CI)) across deciles of sinbaglustat plasma concentrations (upper panel). Mean predicted (grey area) ΔΔQTcF (± 90% CI) interval at geometric mean peak sinbaglustat concentrations (lower panel). In the graphs, the 10 ms threshold of regulatory concern (for the upper bound of the CI around the mean effect on QTc) is indicated, as referred to in the ICH E14 guideline.[38]

Inhibition of GCS has a proven beneficial effect in the treatment of GD type 1.[15,16,33–35] The beneficial effects of GBA2 inhibition have been demonstrated in various animal models.[21–23] In a model of NPC disorder, treatment with the dual GBA2 GCS inhibitor miglustat led to an improvement of behavioral symptoms and survival, despite an observed increase in GlcCer levels in the brain.[36] This effect seems to be associated with the ability of miglustat to inhibit GBA2.[37] This hypothesis is further supported by the results obtained in the GD type I and NPC mouse models, which indicate that the beneficial effects were purely obtained via GBA2 inhibition.[21,22] To ensure that sinbaglustat also has the potential to become a new treatment for neuronopathic LSDs, further studies are needed to demonstrate target engagement at the site of action and improvement of the neurological symptoms of the diseases.

Single-dose and multiple-dose administrations of sinbaglustat were overall safe and well-tolerated. However, at the highest dose of the MAD study, all four female subjects reported AEs (3 of them presented a similar pattern of general symptoms, such as dizziness and nausea) whereas none of the male subjects reported AEs. This observation might, at least in part, be explained by the slightly higher sinbaglustat exposure (around 30%) in female than in male subjects. However, geometric mean C_{max} in male subjects of the SAD 2,000 mg dose group was higher than in these female subjects (16,713 ng/mL in the SAD vs. 12,024 ng/mL in women in the 1,000 mg b.i.d. cohort on day 7 in the MAD) and none of the male subjects reported any AE. Therefore, the increased incidence of AEs in female subjects compared to male subjects in the 1,000 mg b.i.d. cohort is unlikely to be a result of PK differences.

Cardiac evaluation of Holter data did not highlight any sinbaglustat effects on the ECG variables. The exposure–response analysis showed that it is unlikely that sinbaglustat causes any QT liability up to a dose > 2,000 mg. In summary, single-dose and multiple-dose administration of sinbaglustat was well-tolerated up to 2,000 mg and 1,000 mg b.i.d. for 7 days, respectively, in healthy male and female subjects. The tolerability, safety, and PK/PD profile of sinbaglustat is compatible with its further clinical development in LSDs.

Supporting Information. Supplementary information accompanies this paper on the *Clinical and Translational Science* website (www.cts-journal.com).

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Conflict of Interest. All authors are employees of Idorsia Pharmaceuticals Ltd.

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