Pharmacokinetics, Pharmacodynamics, Safety, and Tolerability of Oral Venglustat in Healthy Volunteers

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Abstract

Venglustat is a small-molecule glucosylceramide synthase (GCS) inhibitor designed to reduce the production of glucosylceramide (GL-1) and thus is expected to substantially reduce formation of glucosylceramide-based glycosphingolipids. Because of its effect on glycosphingolipid formation, GCS inhibition has therapeutic potential across many disorders affecting glycosphingolipid metabolism. Therefore, venglustat is under development for substrate reduction therapy in multiple diseases, including Gaucher disease type 3, Parkinson’s disease associated with GBA mutations, Fabry disease, GM2 gangliosidosis, and autosomal dominant polycystic kidney disease. Phase 1 studies were conducted in healthy volunteers to determine venglustat pharmacokinetics, pharmacodynamics, safety, and tolerability and to assess food effects on pharmacokinetics (single-dose and food-effect studies: NCT01674036; repeated-dose study: NCT01710826). Following a single oral dose of venglustat L-malate (2, 5, 15, 25, 50, 100, or 150 mg), venglustat demonstrated linear pharmacokinetics, rapid absorption (median $t_{\text{max}}$, 3.00–5.50 hours), systemic exposure unaffected by food, low apparent total body clearance (mean CL/F, 5.18–6.43 L/h), and pooled geometric mean $t_{1/2}$ of 28.9 hours. Following repeated once-daily oral doses of venglustat L-malate (5, 10, or 20 mg) for 14 days, apparent steadystate occurred within 5 days of repeated dosing, with pooled accumulation ratios of 2.10 for $C_{\text{max}}$ and 2.22 for $AUC_{0–24}$, and no statistically significant effect of dose or sex on accumulation. The mean fraction of dose excreted unchanged in urine ($f_{\text{U}}$) was 26.3% to 33.1%. Plasma GL-1 and GM3 decreased time- and dose-dependently. Venglustat demonstrated a favorable safety and tolerability profile.

Keywords

glucosylceramide (GL-1), globotriaosylceramide (GL-3), glucosylceramide synthase (GCS) inhibition, glucosylsphingosine (lyso-GL-1), monosialodihexosylganglioside (GM3), substrate reduction therapy, venglustat (GZ/SAR402671; Genz-682452)

Glycosphingolipids are essential for diverse cellular functions, and abnormalities of glycosphingolipid metabolism can have important consequences for health and disease.1,2 Glucosylceramide (GL-1), formed from ceramide and uridine diphosphate-glucose by glucosylceramide synthase (GCS), is the central building block for more complex glycosphingolipids such as globotriaosylceramide (GL-3), monosialodihexosylganglioside (GM3), disialotetrahexosylganglioside (GM2), and monosialotetrahexosylganglioside (GM1 ganglioside).3 Inherited deficiencies of enzymes acting on substrates downstream of GCS can cause severe glycosphingolipid metabolic dysregulation in Gaucher disease (GD),4 Fabry disease (FD),5 and GM2 gangliosidosis,6 as well as in Parkinson’s disease in patients carrying GBA mutations (GBA-PD).7 GL-1 and GM3 dysregulation also

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occurs in autosomal dominant polycystic kidney disease (ADPKD) as GCS activity increases, resulting in pathogenic glycosphingolipid accumulation.\textsuperscript{1,8–11} In all these cases, GCS inhibition may provide therapeutic benefit by reducing formation of the upstream glycosphingolipid building block GL-1.\textsuperscript{12}

Substrate reduction therapy (SRT) relies on small-molecule inhibition of an upstream enzyme to reduce pathogenic substrate accumulation. Clinical precedent exists for SRT of a glycosphingolipid-related disorder by GCS inhibition.\textsuperscript{12} For example, eliglustat, an oral GCS inhibitor, is approved as first-line therapy for adults with GD type 1 with extensive, intermediate, or poor cytochrome P450 (CYP) 2D6-metabolizer phenotypes.\textsuperscript{13} However, eliglustat is subject to P-glycoprotein-mediated efflux from the blood-brain barrier,\textsuperscript{14} making it unsuitable for treatment of overt central nervous system (CNS) manifestations in neuronopathic (types 2 and 3) GD, GM2 gangliosidosis, or \textit{GBA}-PD. Sanofi Genzyme is developing venglustat (GZ/SAR402671, PubChem CID 60199242; Figure 1A), a novel brain-penetrant GCS inhibitor, as an investigational disease-modifying therapy for multiple disorders associated with GL-1-related glycosphingolipids. Based on nonclinical studies (absorption, distribution, metabolism, and elimination; pharmacodynamics; and disease-model efficacy) showing availability and efficacy in proposed target organs including the CNS,\textsuperscript{11,15–17} venglustat is under investigation for treatment of FD, ADPKD, GD type 3, \textit{GBA}-PD, and GM2 gangliosidosis.

As determined from unpublished in vitro studies using human biomaterial, CYP3A4 was found to be the predominant (approximately 80\%) drug-metabolizing CYP450 isozyme contributing to the metabolism of venglustat. Clinical significance of impact of CYP3A inhibitors and inducers on venglustat pharmacokinetics still needs to be evaluated. Further, based on in vitro CYP inhibition, CYP induction, and P-glycoprotein inhibition data, the likelihood for drug-drug interactions because of inhibition or induction of the major CYP450-metabolizing enzymes and inhibition of P-glycoprotein by venglustat is considered remote. In vitro assessment for additional potential transporter-mediated drug-drug interactions is ongoing.

Venglustat can correct the aberrant flux of glycosphingolipids, which are key pathogenic contributors in \textit{GBA}-PD\textsuperscript{18} and ADPKD in mouse models.\textsuperscript{1} Heterozygous \textit{GBA} mutations are the most common genetic risk factor for Parkinson’s disease and dementia with Lewy bodies,\textsuperscript{19} and lipid substrate accumulation has been reported in \textit{GBA}-PD cultured neurons and patient brain tissue.\textsuperscript{7} Pharmacologic GL-1 reduction in 2 mouse models of synucleinopathies decreased \textalpha-\textit{synuclein} pathology and improved behavioral outcomes, indicating that GCS inhibition can modulate \textalpha-\textit{synuclein} processing and potentially reduce Parkinson’s disease progression\textsuperscript{15}; thus, venglustat is being studied in human \textit{GBA}-PD.\textsuperscript{20} In ADPKD, GCS-mediated glycosphingolipid accumulation contributes to cystogenesis in humans and mice. GCS inhibition has been shown to reduce cystogenesis,
improve urine concentration, and reduce the rate of renal function decline in 3 genetic models of polycystic kidney disease including the orthologous ADPKD mouse model,\textsuperscript{11} supporting investigation of venglustat in ADPKD.\textsuperscript{1} Venglustat has been shown to reduce CNS histopathological changes, improve behavioral responses, and increase life span in a mouse model of neuronopathic GD\textsuperscript{16} and is being studied in human adult GD3.\textsuperscript{18} In a mouse FD model, venglustat alone reduced GL3 accumulation and histopathologic changes and in combination with agalsidase beta augmented peripheral efficacy of enzyme replacement.\textsuperscript{17} Venglustat may in principle be applicable to treatment of GM2 gangliosidosis via its ability to reduce GM2 synthesis and CNS availability.\textsuperscript{6}

In view of venglustat’s proposed mechanism of action, preclinical pharmacology, and safety profile, phase 1 clinical studies were conducted to evaluate its pharmacokinetics, pharmacodynamics, safety, and tolerability to support future drug development. This report summarizes the results from 3 phase 1 studies in healthy volunteers (single ascending-dose study and effect of food on venglustat pharmacokinetics following a single dose, both NCT01674036, and multiple ascending-dose study NCT01710826).

**Methods**

**Ethics and Study Designs**

All 3 studies received institutional review board approvals (IntegReview, Austin, Texas), complying with the Declaration of Helsinki (1964) and applicable amendments, and were conducted according to Good Clinical Practice. All subjects gave written informed consent before participating. The studies were conducted by PPD Development, LLC, at a single center in Austin, Texas. TDU12766-FED12767 (NCT01674036) was a 2-part single-center trial in healthy adult male volunteers. Study TDU12766 was a double-blind, randomized, placebo-controlled sequential ascending single-dose study of venglustat safety, tolerability, and pharmacokinetics. Study FED12767 was an open-label, randomized, crossover 2-sequence, 2-period, 2-treatment study of venglustat pharmacokinetics with and without a high-fat meal, with a minimum 7-day washout period between treatment conditions. TDR12768 (NCT01710826) was a single-center, double-blind, randomized, placebo-controlled, sequential ascending repeated-dose study of the safety, tolerability, pharmacokinetics, and pharmacodynamics of venglustat in healthy adult male and female volunteers.

**Subjects**

Inclusion criteria defined eligible subjects as men (TDU12766-FED12767 and TDR12768) and women (TDR12768 only) aged 18–45 years, weighing 50–100 kg (men) or 40–90 kg (women), with normal-range vital signs, normal 12-lead electrocardiogram (ECG) results (after 10 minutes supine), and normal laboratory parameters. Women participants had to test negative for pregnancy. Sexually active subjects and their partners (except women ≥1 year postmenopausal or sterilized ≥3 months earlier) had to use double contraception methods on-study and for 2 months after final dosing.

Key exclusion criteria included:

- History or presence of clinically relevant cardiovascular, pulmonary, gastrointestinal, hepatic, renal, metabolic, hematological, neurological, musculoskeletal, psychiatric, systemic, ocular, or infectious disease (including hepatitis B or C or human immunodeficiency virus 1 or 2), or signs of acute illness;
- Frequent headaches and/or migraines, recurrent nausea and/or vomiting;
- Blood donation (>100 mL) within 2 months before the study; symptomatic postural hypotension;
- Presence or history of drug hypersensitivity, allergic disease, or substance abuse; smoking (>5 cigarettes/day equivalent, if unable to stop on-study); any medication (including St. John’s wort) within 14 days before inclusion or within 5 elimination or pharmacodynamic half-lives of study medication; any vaccination within the last 28 days; citrus fruit or juice consumption within 5 days before or during the study.

**Study Drug**

Venglustat L-malate (venglustat A, PubChem 60199241) and placebo were provided as capsules (venglustat L-malate: 5 mg [3.7 mg venglustat base], 25 mg, or 250 mg); 250-milligram capsules were used to prepare a solution for a 2-mg liquid dose. Starting doses were based on FDA guidance, predicted human pharmacokinetics, and the pharmacologically active dose. Dose escalation was based on adequate exposure margins from preclinical toxicology and supported by monitoring of potential safety signals and exposure (not to have exceeded 27 900 ng·h/mL, the exposure observed at the first toxic dose in male rats [45 mg/kg]).

In TDU12766 (single ascending dose), subjects were randomized to receive 2, 5, 15, 25, 50, 100, or 150 mg of venglustat L-malate (providing 1.5, 3.7, 11.2, 18.6, 37.2, 74.4, or 112 mg of free venglustat) or matching placebo in the morning of Day 1 after a ≥10-hour fast. In FED12767 (food effect), subjects were randomized to receive a single oral dose of 5 mg venglustat L-malate (3.7 mg free venglustat) either while fasting (≥10 hours before and 4 hours after administration) or 30 minutes
after a standardized high-fat breakfast (~815 kcal). After a 7-day washout period, participants crossed over to the second period/other condition.

In TDR12768 (multiple ascending doses), subjects were randomized to receive 14 days of once-daily doses of venglustat t-malate 5, 10, or 20 mg (provided as 5-mg capsules; free venglustat doses 3.7, 7.44, or 14.9 mg), or placebo after a ≥10-hour fast.

Assessments
In the single ascending-dose study (TDU12766), blood samples for analysis of plasma venglustat concentrations were collected at 0 (predose), 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 48, 72, and 96 hours postdose. Urine samples were collected for analysis of venglustat concentrations beginning 2 hours before study drug administration through 48 hours afterward.

In the food-effect study (FED12767), blood samples for analysis of plasma venglustat concentrations were collected at 0 pre-dose), 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, and 48 hours postdose.

In the repeated ascending-dose study (TDR12768), blood samples for analysis of plasma venglustat concentrations were collected as follows: Day 1: 0 (predose), 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, and 16 hours postdose; Days 2–5, 8, 11, and 13: 0 hour (predose); Day 14: 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 48, and 72 hours postdose. Urine samples were collected for analysis of venglustat concentrations beginning 2 hours before study drug administration through 48 hours afterward.

Venglustat measurement in urine used 2 methods with different sample volumes and detection ranges. The first (5.0 to 500 ng/mL) used an internal standard of Genz-684010 at 20 ng/mL, with the same solid-phase extraction, HPLC column, and mobile phase as the plasma assay, m/z monitoring at 390.2→220.0 for venglustat and 396.2→226.0 for internal standard. The quantitation range was 0.500 to 500 ng/mL using a 100-μL sample volume. At 0.500 ng/mL, intraday % coefficient of variation (CV) ranged from 3.8 to 11.6, and intraday % relative error (RE) ranged from –1.4 to –7.6. Between-day variability in LLOQ precision was –2.4% RE and in LLOQ precision was 6.7% CV.

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The second urine assay (limit of sensitivity, 5.0–5000 ng/mL) used an internal standard of Genz-684010 at 200 ng/mL and the same solid-phase extraction procedure as for the plasma venglustat assay. The Acquity UPLC (Waters) method, used until 2014, utilized the same HPLC column, mobile phase, and MS settings as for the plasma venglustat assay; m/z ratios monitored were 390.2→220.0 for venglustat and 396.2→226.0 for the internal standard. The post-2014 Nexera UHPLC (Shimadzu) method was partially validated from the Acquity method and kept its parameters. Both had limits of sensitivity of 5.00 to 5000 ng/mL and intraday % RE of –2.2.

The plasma 4β-OH cholesterol assay (used as a marker of CYP3A induction or inhibition21,22) has an LLOQ of 4 ng/mL and was performed by Bioanalytical Services, Covance Laboratories Ltd., Alnwick, UK. Plasma concentrations and relative actual times were used to calculate pharmacokinetic parameters using validated software (PKDM5, version 2.0 with WinNonlin Professional, version 5.2.1, Certara USA, Inc., Princeton, New Jersey).

The following pharmacokinetic parameters were determined in TDU12766-FED12767: maximum plasma concentration (Cmax); time to Cmax (tmax); area under the time-concentration curve from t = 0 to last measurable concentration (AUClast); AUC extrapolated to infinity (AUCinf); apparent total clearance from plasma...
(CL/F); and terminal half-life (t_{1/2}). The fraction excreted unchanged in urine through 48 hours (f_{0-48}) was evaluated in TDU12766 only. Time of last plasma concentration above LLOQ (t_{last}) and time between administration and the sampling time preceding the first concentration above LLOQ (t_{lag}) were determined in FED12767 only. In TDR12768 the following were determined: C_{max}, trough plasma concentration predadministration (C_{trough}); t_{max}, AUC over a 24-hour dosing interval (AUC_{0-24}); t_{1/2} (assessed on Day 14); t_{last}; CL/F at steady state on Day 14 (CL_{ss}/F); fraction excreted unchanged in urine from t = 0 to 24 hours after venglustat administration (f_{0-24}); cumulative amount excreted in urine from t = 0 to 24 hours after venglustat administration (A_{C_{0-24}}); and renal clearance from t = 0 to 24 hours after venglustat administration, CL_{R(0-24)}.

Pharmacodynamic assessments are described in Supplementary Methods, including details of a plasma GM3 method not previously published (briefly, total GM3 in plasma was quantitated using a protein precipitation procedure followed by LC-MS/MS analysis).

In all studies, safety was evaluated by assessing treatment-emergent adverse events (TEAEs) from first dose through 10 Days after the last dose of study medication, as well as ECG parameters, clinical laboratory values, vital signs, and physical examinations.

### Statistical Analyses

Sample sizes for all studies reflected empirical considerations (no sample size calculations were performed). All subjects free of major administration-related deviations (eg, vomiting) were analyzed for pharmacokinetics (TDU12766-FED12767 and TDR12768) and pharmacodynamics (TDR12768). All subjects receiving ≥1 dose of venglustat were included in safety analyses. Demographic and baseline characteristics and pharmacokinetic parameters were summarized using descriptive statistics.

For pharmacokinetics, dose effects, food effects, occurrence of steady state, and accumulation were assessed using linear-effects models (Supplementary Methods). The pharmacokinetic parameters shown in Figures 1, 2, and S1 were graphed by regarding values below the LLOQ as zero. For pharmacodynamics, for each parameter (GL-1, GL-3, and GM3), percent reduction from baseline was calculated as (baseline – value/baseline) × 100. Any pharmacodynamic value below the LLOQ was imputed as LLOQ/2 for consistency with the method used for regulatory-submitted data (and the LLOQ/2 method was used only for pharmacodynamic data). For GL-1, the relationship between Day 15 percent reduction from baseline and Day 15 trough concentration was modeled via a sigmoid E_{max} model using the SAS (Statistical Analysis Software, Cary, North Carolina) NLMIXED procedure,

\[
\% \text{reduction} = E_0 + (E_{\text{max}} \times C^\gamma)(EC_{50}^\gamma + C^\gamma)^{-1}
\]

where C is the trough concentration, E_{max} is the maximal percent reduction from baseline, E_0 is the percent reduction from baseline with placebo, EC_{50} is the trough concentration producing half-maximal percent reduction from baseline attributable to venglustat, and \gamma is a slope parameter.

Trough concentrations for all placebo subjects were set to zero. Model adequacy was assessed graphically by scatterplot of percent reduction from baseline versus trough concentration overlaid with a fitted sigmoid E_{max} model. For each venglustat dose level, the estimated mean percent reduction from baseline attributable to venglustat (with 90% confidence interval [CI]) was calculated from the fitted sigmoid E_{max} model at the corresponding observed geometric mean trough value.

### Results

#### Subject Disposition and Demographics

TDU12766 enrolled and randomized 55 healthy men in the single ascending-dose study (placebo, n = 14; 2-, 5-, 15-, 25-, 50-, and 100-mg venglustat L-malate, n = 6 each; 150-mg venglustat L-malate, n = 5). Eight healthy men participated in the FED12767 food-effect study. TDR12768 (repeated ascending-dose study) enrolled and randomized 36 healthy adults (19 men and 17 women; 4 groups of 9 subjects received placebo or venglustat L-malate 5, 10, or 20 mg). All subjects completed each study; Supplementary Table S1 presents demographics.

#### Pharmacokinetic Analysis

In the single ascending-dose study (TDU12766), across the single oral doses of venglustat L-malate evaluated (2–150 mg), venglustat was absorbed with a median t_{max} of 3.00 to 5.50 hours and eliminated with a geometric mean t_{1/2} of 28.9 hours (Figure 1B). On Day 14, mean CL/F ranged from 5.18 to 6.43 L/h across the dose groups. Table 1 presents pharmacokinetic parameters following single ascending venglustat doses. Exposure increased close to dose-proportionally throughout the dose range: a 75-fold dose increase resulted in 97.3-, 89.2-, and 85.9-fold increases in geometric mean C_{max}, AUC_{last}, and AUC_{inf}, respectively (Supplemental Table S2). Mean 48-hour urinary excretion fractions were 14.7% to 23.5% over the 2- to 150-mg venglustat L-malate dose range.

In the preliminary assessment of the effect of food on venglustat pharmacokinetics, administration of 5 mg venglustat L-malate with a high-fat meal had no
Figure 2. Mean (+ standard deviation) venglustat plasma concentrations on Day 1 (A) and Day 14 (B), and trough concentrations from Day 1 to Day 14 in the repeated ascending-dose study (C). Values below the lower limit of quantitation (LLOQ) were considered zero for this graph.
Table 1. Pharmacokinetic Parameters in Single Ascending-Dose Study

| Parameter                        | 2 mg (n = 6) | 5 mg (n = 6) | 15 mg (n = 6) | 25 mg (n = 6) | 50 mg (n = 6) | 100 mg (n = 6) | 150 mg (n = 5) |
|---------------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| $C_{\text{max}}$, ng/mL        |             |             |             |             |             |             |             |
| Mean (SD)                       | 5.7 (1.2)   | 14.7 (1.61) | 53.0 (16.7) | 84.4 (31.8) | 181 (56)    | 374 (38)    | 529 (109)   |
| Geometric mean (CV)             | 5.6 (21.4)  | 14.6 (10.9) | 50.7 (31.5) | 79.9 (37.7) | 173 (31)    | 372 (10.3)  | 520 (21)    |
| $t_{\text{max}}$ (hours), median (range) | 3.50 (3.00–8.00) | 5.50 (4.00–8.00) | 3.50 (2.00–5.00) | 5.00 (4.00–8.00) | 4.00 (3.00–6.00) | 3.00 (2.00–4.00) | 4.00 (1.00–8.00) |
| AUClast, ng·h/mL                |             |             |             |             |             |             |             |
| Mean (SD)                       | 214 (52)    | 560 (71)    | 1830 (520)  | 3380 (1100) | 6310 (1880) | 13 000 (2330) | 18 600 (5480) |
| Geometric mean (CV)             | 209 (24.3)  | 556 (12.7)  | 1760 (29)   | 3240 (33)   | 6070 (30)   | 12 800 (18)  | 18 000 (30)  |
| AUCinf, ng·h/mL                 |             |             |             |             |             |             |             |
| Mean (SD)                       | 243 (61)    | 652 (122)   | 2070 (600)  | 3810 (1080) | 7130 (2320) | 14 400 (3010) | 20 600 (6640) |
| Geometric mean (CV)             | 237 (25)    | 643 (19)    | 1990 (29)   | 3690 (28)   | 6800 (33)   | 14 100 (21)  | 19 900 (32)  |
| $t_{1/2}$ (hours)               |             |             |             |             |             |             |             |
| Mean (SD)                       | 29.2 (43)   | 33.3 (8.1)  | 29.7 (7.1)  | 30.2 (5.5)  | 28.9 (5.3)  | 27.8 (3.6)   | 26.9 (5.7)   |
| Geometric mean (CV)             | 28.9 (14.8) | 32.5 (24.4) | 29.0 (24.0) | 29.8 (18.1) | 28.5 (18.4) | 27.6 (12.8)  | 26.4 (21.3)  |
| CL/F, L/h                       |             |             |             |             |             |             |             |
| Mean (SD)                       | 6.43 (1.41) | 5.86 (1.01) | 5.85 (1.89) | 5.18 (1.31) | 5.75 (2.01) | 5.38 (1.25)  | 5.80 (1.55)  |
| Geometric mean (CV)             | 6.3 (22.0)  | 5.8 (17.3)  | 5.6 (32.2)  | 5.0 (25.3)  | 5.5 (34.9)  | 5.3 (23.4)   | 5.6 (26.7)   |

$AUC_{\text{inf}}$, area under the time-concentration curve extrapolated to infinity; $AUC_{\text{last}}$, AUC from $t = 0$ to last measurable concentration; $C_{\text{max}}$, maximum plasma concentration; CL/F, apparent total clearance from plasma; CV, coefficient of variation; SD, standard deviation; $t_{1/2}$, terminal half-life; $t_{\text{max}}$, time to $C_{\text{max}}$. 
Table 2. Pharmacokinetic Parameters on Days 1 and 14 in the Repeated Ascending-Dose Study

| Parameter                        | 5 mg (n = 9) | 10 mg (n = 9) | 20 mg (n = 9) |
|----------------------------------|--------------|---------------|--------------|
| C<sub>max</sub>, ng/mL           |              |               |              |
| Day 1                            |              |               |              |
| Mean (SD)                        | 18.5 (3.2)   | 38.5 (7.4)    | 68.0 (15.7)  |
| Geometric mean (CV)              | 18.2 (17.3)  | 37.8 (19.3)   | 66.5 (23.1)  |
| t<sub>max</sub> (hours), median (range) | 5.00 (2.00–8.17) | 3.00 (2.00–5.00) | 3.07 (2.00–6.00) |
| AUC<sub>0–24</sub>, ng·h/mL      |              |               |              |
| Mean (SD)                        | 296 (54)     | 635 (132)     | 1100 (211)   |
| Geometric mean (CV)              | 292 (18)     | 623 (21)      | 1080 (19)    |
| Day 14                           |              |               |              |
| C<sub>max</sub>, ng/mL           |              |               |              |
| Mean (SD)                        | 37.0 (6.4)   | 89.7 (29.1)   | 142 (40)     |
| Geometric mean (CV)              | 36.5 (17.2)  | 86.0 (32.5)   | 137 (28.3)   |
| t<sub>max</sub> (hours), median (range) | 3.00 (2.00–6.00) | 2.00 (2.00–6.00) | 3.00 (2.00–8.00) |
| AUC<sub>0–24</sub>, ng·h/mL      |              |               |              |
| Mean (SD)                        | 642 (121)    | 1550 (464)    | 2420 (705)   |
| Geometric mean (CV)              | 632 (19)     | 1490 (30)     | 2340 (29)    |
| C<sub>trough</sub> (ng/mL)       |              |               |              |
| Mean (SD)                        | 19.4 (4.0)   | 49.9 (19.3)   | 73.3 (24.4)  |
| Geometric mean (CV)              | 19.0 (20.5)  | 47.5 (38.7)   | 69.9 (33.2)  |
| t<sub>1/2</sub> (hours)          |              |               |              |
| Mean (SD)                        | 29.3 (4.6)   | 31.3 (3.3)    | 35.0 (6.3)   |
| Geometric mean (CV)              | 29.0 (15.8)  | 31.2 (10.5)   | 34.5 (18.0)  |
| CL<sub>L/F</sub> (L/h)           |              |               |              |
| Mean (SD)                        | 5.98 (1.17)  | 5.13 (1.25)   | 6.58 (1.70)  |
| Geometric mean (CV)              | 5.9 (19.5)   | 5.0 (24.4)    | 6.4 (25.8)   |
| CL<sub>R(0–24)</sub> (L/h)       |              |               |              |
| Mean (SD)                        | 1.55 (0.68)  | 1.49 (0.41)   | 2.07 (0.58)  |
| Geometric mean (CV)              | NA<sup>a</sup> | 1.4 (27.7)    | 2.0 (28.0)   |

AUC<sub>0–24</sub>, area under the time-concentration curve over the 24-hour dosing interval; C<sub>max</sub>, maximum plasma concentration; C<sub>trough</sub>, trough plasma concentration; AUC<sub>0–24</sub>, renal clearance of venglustat from t = 0 to 24 hours after venglustat dose; CL<sub>L/F</sub>, apparent total clearance from plasma at steady state; CV, coefficient of variation; SD, standard deviation; t<sub>1/2</sub>, terminal half-life; t<sub>max</sub>, time to C<sub>max</sub>

<sup>a</sup>Geometric mean not calculated because of venglustat concentration below the lower limit of quantification in Day 14 urine sample of 1 subject.

effect on venglustat exposure compared with fasting conditions (Supplemental Tables S3 and S4, Supplemental Figure S1). Fed/fasted geometric mean ratios were 0.92 and 0.91 for C<sub>max</sub> and AUC<sub>last</sub>, respectively. Within-subject variability (ie, fed vs fasted) accounted for less than half the total subject variability (Supplemental Table S4). Median t<sub>max</sub> was 6.00 hours whether fed or fasting (Supplemental Table S3).

In the repeated ascending-dose study (TDR12768) in subjects receiving 5, 10, or 20 mg venglustat l-malate once daily for 14 days, venglustat was absorbed with a median t<sub>max</sub> of 2.00 to 5.00 hours postdose on Days 1 and 14 (Figure 2A,B). Steady state appeared to be reached within 5 days of repeated dosing (Figure 2C). Table 2 presents pharmacokinetic parameters for all dose groups on Days 1 and 14. Venglustat exposure increased close to dose-proportionally over the dose range of 5–20 mg venglustat l-malate: this 4-fold dose increase resulted in 3.76- and 3.69-fold increases in geometric mean C<sub>max</sub> and AUC<sub>0–24</sub> values, respectively, on Day 14 (Supplemental Table S5). Median and 90th percentile times to steady state are given in Supplemental Table S6. After 14 days of administration, pooled venglustat accumulation ratios were 2.10 for C<sub>max</sub> and 2.22 for AUC<sub>0–24</sub>, independent of dose and sex (Supplemental Table S7 and data not shown). Dose and sex also had no effect on t<sub>1/2</sub> (Supplemental Table S8 and data not shown). Point estimates for within-subject variability were approximately 14% for C<sub>max</sub> and 13% for AUC<sub>0–24</sub>. After 14 once-daily doses of venglustat l-malate, the 24-hour unchanged urinary excretion fraction of venglustat (mean fe<sub>0–24</sub>) ranged between 26.3% and 33.1% (Supplemental Table S9). Mean CL<sub>R(0–24)</sub> ranged between 1.49 and 2.07 L/h, approximately 3.18- to 3.86-fold lower than observed plasma CL/F. The geometric mean plasma Day 14/Day 1 ratios...
of 4β-hydroxycholesterol showed no marked difference between placebo and venglustat-treated groups, indicating minimal induction of CYP3A4 (Supplemental Table S10).

**Pharmacodynamic Analyses and Pharmacokinetic/Pharmacodynamic Relationship**

In the repeated ascending-dose study (TDR12768), plasma GL-1, GL-3, and GM3 in placebo recipients remained similar to baseline throughout, whereas plasma GL-1 and GM3 levels decreased from baseline time- and dose-dependently across the 3 venglustat dose groups (Table 3, Figure 3).

Maximal sustained effects on GL-1 occurred on Day 11 in the 5- and 10-mg dose groups and by Day 8 in the 20-mg dose group. Mean calculated GL-1 reductions from baseline on Day 15 were 41.9%, 69.6%, and 74.6% in the 5-, 10-, and 20-mg dose groups, respectively. However, these values are mathematically limited by the assay LLOQ and by low baseline GL-1 values relative to the LLOQ, as may be expected in healthy volunteers, who have normal GL-1 levels in plasma. GL-1 values were below the LLOQ at baseline in 1 subject in the 5-mg venglustat L-malate dose group and on Day 15 in 3, 5, and 9 subjects in the 5-, 10-, and 20-mg dose groups, respectively.

Maximal sustained GM3 decreases occurred across all venglustat L-malate dose groups starting on Day 13. Mean Day 15 plasma GM3 levels were 42.7%, 49.4%, and 57.8% of baseline for the 5-, 10-, and 20-mg dose groups, respectively. GM3 was below LLOQ on Day 15 in 1 and 2 subjects in the 10- and 20-mg dose groups, respectively.

Plasma GL-3 also decreased with time in all venglustat L-malate dose groups; however, mean reductions in calculated GL-3 levels are not useful, as GL-3 levels were below the LLOQ at baseline in 11 of 36 subjects enrolled, as may be expected in healthy volunteers who exhibit normal GL-3 levels in plasma.

Mean estimated plasma GL-1 reductions from baseline (90% CI) attributable to venglustat C_trough in the 5-, 10-, and 20-mg dose groups (19.0, 47.5, and 69.9 ng/mL, respectively) were 67.0% (54.4%–79.7%), 74.4% (63.7%–85.2%), and 76.3% (64.8%–87.8%), respectively. An E_max model adequately described the relationship between venglustat C_trough and percent GL-1 reduction (Supplemental Figure S2).

**Safety and Tolerability Profile**

There were no deaths, serious adverse events (SAEs), severe TEAEs, or TEAEs leading to participant discontinuation reported in any of the 3 studies.

In the single ascending-dose study (TDU12766), 1 subject in the 50-mg dose group and 2 subjects in the 100-mg dose group reported a total of 4 mild TEAEs (Table 4), of which only a headache in 1 subject in the 100-mg dose group on Day 3 was considered study drug-related. In the food-effect study (FED12767), 1 subject reported a mild TEAE in the fed phase of the study (contact dermatitis, reported as “skin irritation at ECG site”), which was considered unrelated to study drug (Table 4).

In the multiple ascending-dose study (TDR12768), 17 subjects reported a total of 32 mild TEAEs, including 10 TEAEs in 6 of 9 subjects (67%) in the placebo group and 22 TEAEs in 11 of 27 total subjects (41%) in the 5-, 10-, and 20-mg dose groups (Table 5). In the venglustat L-malate dose groups, the TEAEs reported by the investigator as study drug-related were constipation, diarrhea, dry mouth, flatulence, pruritus, and fatigue.

No clinically relevant hematologic or biochemical abnormalities were reported in any of the studies. Vital signs and ECG parameters showed no relevant changes from baseline in any of the studies. In the multiple ascending-dose study (TDR12768), no ECG parameters changed statistically significantly from average baseline in any venglustat dose group versus placebo (data not shown).
Figure 3. Mean (SEM) remaining of glucosylceramide (GL-1; A), globostriaosylceramide (GL-3; B), and GM3 ganglioside (GM3; C) in placebo, 5-, 10-, and 20-mg dose groups (venglustat L-malate, GZ402671) from Day 1 to Day 15 in the repeated ascending-dose study
### Table 4. Treatment-Emergent Adverse Events (TEAEs) in the Single Ascending-Dose and Food-Effect Studies

| Venglustat L-Malate Dose Groups | Single Ascending-Dose Study | Food-Effect Study |
|---------------------------------|-----------------------------|------------------|
| TEAE, n (%)                     | Placebo (n = 14)            | 2-25 mg \(^b\) (n = 24) | 50 mg (n = 6) | 100 mg (n = 6) | 150 mg (n = 5) | 5 mg fed (n = 8) | 5 mg fasted (n = 8) |
| Primary System Organ Class\(^a\) | Preferred Term\(^a\) | | | | | | |
| Any TEAE                        | 0 | 0 | 1 (16.7) | 2 (33.3) | 0 | 1 (12.5) | 0 |
| Skin and subcutaneous tissue disorders | 0 | 0 | 0 | 1 (16.7) | 0 | 1 (12.5) | 0 |
| Dermatitis contact             | 0 | 0 | 0 | 1 (16.7) | 0 | 1 (12.5) | 0 |
| Infections and infestations   | 0 | 0 | 1 (16.7) | 0 | 0 | 0 | 0 |
| Rhinitis                        | 0 | 0 | 1 (16.7) | 0 | 0 | 0 | 0 |
| Nervous system disorders       | 0 | 0 | 0 | 1 (16.7) | 0 | 0 | 0 |
| Headache                       | 0 | 0 | 0 | 1 (16.7) | 0 | 0 | 0 |
| General disorders and administration-site conditions | 0 | 0 | 1 (16.7) | 0 | 0 | 0 | 0 |
| Feeling cold                   | 0 | 0 | 1 (16.7) | 0 | 0 | 0 | 0 |

\(^a\)Per the Medical Dictionary for Regulatory Activities, version 15.1.

\(^b\)Pooled data for dose groups.

### Table 5. Treatment-Emergent Adverse Events (TEAEs) in the Repeated Ascending-Dose Study

| Venglustat L-malate Dose Groups | Placebo (n = 9) | 5 mg (n = 9) | 10 mg (n = 9) | 20 mg (n = 9) |
|---------------------------------|----------------|-------------|--------------|--------------|
| TEAE, n (%)                     |                |             |              |              |
| Primary System Organ Class\(^a\) | Preferred Term\(^a\) | | | |
| Any TEAE                        | 6 (66.7) | 3 (33.3) | 4 (44.4) | 4 (44.4) |
| Skin and subcutaneous tissue disorders | 1 (11.1) | 2 (22.2) | 3 (33.3) | 1 (11.1) |
| Dermatitis contact             | 1 (11.1) | 0 | 1 (11.1) | 1 (11.1) |
| Pruritus                        | 0 | 1 (11.1) | 0 | 0 |
| Skin irritation                 | 0 | 2 (22.2) | 2 (22.2) | 0 |
| Gastrointestinal disorders      | 1 (11.1) | 1 (11.1) | 1 (11.1) | 3 (33.3) |
| Diarrhea                        | 1 (11.1) | 0 | 1 (11.1) | 1 (11.1) |
| Dry mouth                       | 0 | 0 | 0 | 1 (11.1) |
| Flatulence                      | 0 | 0 | 0 | 1 (11.1) |
| Constipation                    | 1 (11.1) | 1 (11.1) | 1 (11.1) | 0 |
| Respiratory, thoracic and mediastinal disorders | 1 (11.1) | 1 (11.1) | 0 | 2 (22.2) |
| Nasal congestion                | 0 | 0 | 0 | 2 (22.2) |
| Oropharyngeal pain              | 1 (11.1) | 0 | 0 | 0 |
| Rhinitis allergic               | 0 | 1 (11.1) | 0 | 0 |
| Nervous system disorders        | 4 (44.4) | 0 | 0 | 1 (11.1) |
| Headache                        | 3 (33.3) | 0 | 0 | 1 (11.1) |
| Dizziness postural              | 1 (11.1) | 0 | 0 | 0 |
| Infections and infestations     | 2 (22.2) | 0 | 0 | 1 (11.1) |
| Upper respiratory tract infection | 0 | 0 | 0 | 1 (11.1) |
| Nasopharyngitis                 | 1 (11.1) | 0 | 0 | 0 |
| Sinusitis                       | 1 (11.1) | 0 | 0 | 0 |
| Musculoskeletal and connective tissue disorders | 0 | 0 | 1 (11.1) | 0 |
| Neck pain                       | 0 | 0 | 1 (11.1) | 0 |
| General disorders and administration-site conditions | 0 | 0 | 0 | 1 (11.1) |
| Fatigue                         | 0 | 0 | 0 | 1 (11.1) |

\(^a\)Per the Medical Dictionary for Regulatory Activities, version 15.1.
Discussion

Phase 1 studies were conducted with venglustat in healthy male and female volunteers. The objectives of these studies were to determine the pharmacokinetics, and pharmacodynamics, safety tolerability of orally administered venglustat l-malate and to assess the effect of food on venglustat pharmacokinetics. The main goal of the studies was to determine doses of venglustat with favorable safety and tolerability that could be tested in subsequent trials.

In these phase 1 studies, venglustat demonstrated linear pharmacokinetics (when administered as single venglustat l-malate doses ranging from 2 to 150 mg or as repeated once-daily doses ranging from 5 to 20 mg for 14 days), rapid absorption, systemic exposure unaffected by food, low apparent total body clearance, and mean fraction of dose excreted unchanged in urine (fe0–24) ranging from 26.3% to 33.1%.

Plasma pharmacodynamic evaluation following repeated once-daily doses of venglustat demonstrated reduction of plasma concentrations of GL-1 and GM3 in a time- and dose-dependent manner, consistent with its proposed mechanism of action: venglustat-mediated GCS inhibition. The effect of venglustat on GL-3 was not testable in this study, as the baseline levels of GL-3 in healthy volunteers were below the LLOQ. The dose- and time-dependent GL-1 and GM3 reductions corroborated the intended mechanism of action of venglustat: decreased GL-1 formation via inhibition of GCS and the resulting decreased downstream GM3 formation.

Modeling of pharmacokinetic-pharmacodynamic relations indicated an Emax of 80% for venglustat, which was closely approached following administration of 20 mg venglustat l-malate in the repeated-dose study (mean GL-1 reduction from baseline on Day 15, 74.6%).

At the doses and dosing regimen tested, venglustat showed a favorable safety and tolerability profile. First-in-human healthy-volunteer studies support the conduct of subsequent studies in patients with targeted diseases, but do not necessarily predict their safety or pharmacokinetic results. Nonetheless, in a 2019 presentation to the American Association of Neurology, MOVES-PD Part 1 participants with GBA-PD receiving venglustat for 26 weeks demonstrated no SAEs or deaths, near-dose-proportional plasma and cerebrospinal fluid pharmacokinetics, and dose-dependent GL-1 and GL-3 reduction.20,25 A full article on that study is in preparation.

Conclusions

In 3 phase 1 studies in healthy subjects, at the doses and dosing regimen tested, venglustat showed a safety and tolerability profile without SAEs or severe adverse events. Repeated once-daily venglustat dosing up to 20 mg venglustat l-malate for 2 weeks (corresponding to 15 mg venglustat) reduced plasma GL-1 concentrations in a time- and dose-dependent manner. Steady-state plasma concentrations of venglustat occurred within 5 days of repeated dosing, with an approximately 2-fold accumulation ratio for Cmax and AUC0–24 and a near-dose-proportional increase in exposure.

The pharmacokinetic, pharmacodynamic, and safety and tolerability results of the phase 1 studies support clinical development of venglustat as an oral GCS inhibitor in phase 2 and phase 3 studies designed to test the hypothesis that GL-1 is a key node for pathophysiology in diverse diseases involving the glycosphingolipid pathway.

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Conflicts of Interest

M.J.P., S.J.M.G., A.J.J., and J.S. are full-time employees of Sanofi Genzyme. N.P.S.C. was a Sanofi employee at the time of the studies. T.T.P. is a full-time employee of PPD Development, LLC, contracted by Sanofi Genzyme to perform the studies.

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Data Sharing

Qualified researchers may request access to patient-level data and related study documents including the clinical study report, study protocol with any amendments, blank case report form, statistical analysis plan, and data set specifications. Patient-level data will be anonymized, and study documents will be redacted to protect the privacy of trial participants. Further details on Sanofi’s data sharing criteria, eligible studies and process for requesting access can be found at: https://www.clinicalstudydatarequest.com.

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Supplemental Information

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