SYNOPSIS

Name of Sponsor/Company	Janssen Research & Development*
Name of Investigational Product	JNJ-28431754 (Canagliflozin)

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Title of Study: A Double-Blind, Placebo-Controlled, Randomized, Parallel Groups, Multicenter Study to Investigate the Effects of Canagliflozin on Insulin Sensitivity, Hepatic Fat Content and Beta Cell Function in Subjects with Type 2 Diabetes Mellitus

NCT No.: NCT02009488

Clinical Registry No.: CR103062

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Study Centers: USA (2 centers)

Publication (Reference): None

Study Period: 08 September 2014 (Date first subject signed informed consent) to 05 March 2017 (Date of last observation for last subject recorded as part of the database)

Phase of Development: 1

Objectives: The objectives of this study were to assess the change from baseline as compared to placebo treatment in subjects with type 2 diabetes mellitus (T2DM), with inadequate glycemic control on metformin monotherapy or on combination therapy with a dipeptidyl peptidase-4 (DPP-4) inhibitor, after approximately 24 to 25 weeks of treatment with canagliflozin (titrated to 300 mg/day):

- Hepatic insulin sensitivity (IS), determined during a tracer-labeled euglycemic, hyperinsulinemic clamp (first-step clamp with low insulin infusion rate)
- Peripheral tissue IS, determined during the tracer-labeled, euglycemic, hyperinsulinemic clamp (second-step clamp with high insulin infusion rate)
- Liver fat content, determined using ^{1}H-nuclear magnetic resonance spectroscopy (MRS)
- Insulin secretion rate (ISR) at 9 mmol/L plasma glucose, during a mixed-meal tolerance test (MMTT)
- β-cell glucose sensitivity (slope of ISR vs. plasma glucose concentration during the MMTT)

- Insulin clearance during the euglycemic, hyperinsulinemic clamp ($CL_{insulin,\ clamp}$) and MMTT ($CL_{insulin,\ MMTT}$).
- Glucose and fat oxidation rates (G_{ox}, F_{ox}) determined using indirect calorimetry during the MMTT and the euglycemic, hyperinsulinemic clamp
- Overall safety and tolerability

Methodology: Phase 1, randomized, double-blind, placebo-controlled, parallel-group study conducted at 2 clinical research centers (CRCs) in the United States. Approximately 56 men and women, ages 25 to 75 years, with T2DM inadequately controlled on either metformin monotherapy or combination therapy with metformin and a DPP-4 inhibitor, were planned to be enrolled in this study.

The study consisted of 3 phases:

- Pre-treatment phase included the screening visit (Week -5), 14-day (Day -28 to Day -15) single-blind placebo run-in period (Start Visit at Week -4), and 14-day (Day -14 to Day -1) single-blind placebo baseline period (Start Visit at Week -2). On Day -14, after completion of all eligibility assessments, subjects were randomized (1:1) to one of two treatment groups, either canagliflozin or placebo. Liver fat content was then measured by MRS. There were 2 inpatient stays during this baseline period: (1) Day -11 to Day -10: On Day -10, an MMTT included baseline assessments of β-cell function and substrate oxidation (by indirect calorimetry); and (2) Day -5 to Day -4: On Day -4, subjects underwent baseline assessment of IS using a tracer-labeled 2-step hyperinsulinemic euglycemic clamp and substrate oxidation (by indirect calorimetry).
- Double-blind treatment phase: Began on Day 1, and ended at approximately Week 25; during the 25-week treatment phase, subjects were assessed at least biweekly at outpatient visits or by telephone contact. At the end of this phase, subjects repeated the same procedures conducted during the baseline period: MRS (Day 168), MMTT (Day 172), including indirect calorimetry, and tracer-labeled 2-step hyperinsulinemic euglycemic clamp including indirect calorimetry (Day 178).
- Post-treatment phase: A follow-up visit occurred within approximately 28 days after the last dose of study agent.

After providing the written informed consent, subjects underwent screening evaluations within 5 weeks prior to the planned first double-blind study agent dose.

At Week -4, eligible subjects returned to the CRC in the fasting condition. Subject eligibility was re-assessed, including a fasting plasma glucose (FPG) ≥100 mg/dL and ≤240 mg/dL. Eligible subjects began a 14-day single-blind placebo run-in period at the Week -4 visit and were provided with instructions for self-monitoring of blood glucose (SMBG), and study agent administration at home.

On Day -14 (Week -2, single-blind placebo baseline period start visit), eligible subjects returned to the CRC in the morning after an overnight fast. Study eligibility was assessed, including a fasting fingerstick glucose ≥100 mg/dL and ≤240 mg/dL. Eligible subjects were randomized (1:1) to 1 of 2 treatment groups, either canagliflozin or placebo. Randomization was stratified by glycated haemoglobin (HbA_{1c}) values (7.0% to 8.0% or >8.0% to 9.5%) determined at screening. On Day -14, baseline liver fat content was measured by MRS.

On Day -11, subjects were admitted to the CRC in the afternoon, received a standard dinner and fasted overnight for at least 8 hours. On Day -10, a MMTT included baseline assessments of β-cell function and substrate oxidation (by indirect calorimetry). A single-blind placebo dose was given ~30 minutes before start of the MMTT (Time 0).

On Day -5, subjects were admitted to the CRC in the afternoon, received a standard dinner and then fasted overnight for at least 8 hours. On Day -4, subjects underwent baseline assessment of IS using a tracer-labeled 2-step hyperinsulinemic euglycemic clamp and substrate oxidation (by indirect

calorimetry). A single-blind placebo dose was given ~30 minutes before the insulin infusion (Time 0) for the clamp procedure began.

On Day 1, subjects received the first dose of double-blind study agent on Day 1 at the CRC approximately 30 minutes before the breakfast. Canagliflozin treatment was initiated at 100 mg/day, with up-titration to 300 mg/day. To maintain the treatment blind and allow blinded canagliflozin dose titration, study agent was supplied in 2 dose levels: Dose Level 1 (canagliflozin 100 mg or matching placebo) and Dose Level 2 (canagliflozin 300 mg once daily or matching placebo).

On Day 1, subjects received the Dose Level 1 of canagliflozin or placebo as per the randomization schedule. Subjects were to return to the CRC at Week 3 for safety assessments, including laboratory tests. Subjects who had tolerated Dose Level 1 and whose estimated glomerular filtration rate (eGFR) was ≥60 mL/min/1.73 m^2 (based on the result at Week 3), were up-titrated to Dose Level 2 at the Week 4 visit.

Subjects with an eGFR <60 mL/min/1.73 m^2 at Week 3 were to remain on Dose Level 1, and had to have serum creatinine measured weekly until Week 11. Titration to Dose Level 2 could occur within the first 12 weeks. The Dose Level 2, once achieved, was continued during the remainder for the double-blind treatment phase, unless study withdrawal criteria were met.

After Week 12, subjects who did not meet the up-titration criteria were to remain on Dose Level 1 until the end of the double-blind treatment phase. Subjects were instructed not to take the morning study agent dose and not to eat or drink (except water) at home prior to all the CRC visits during the double-blind treatment phase. The study agent doses were to be administered at CRC on these days.

During the 25-week double-blind treatment phase, subjects were contacted by telephone or visited the CRC at 2- to 4-week intervals, for the assessment of safety and study compliance. During the dose titration period (Day 1 to Week 12), some subjects could visit the CRC more frequently if needed.

At the end of the double-blind treatment phase, subjects were required to repeat the same procedures conducted during the baseline period (ie, MRS, MMTT including indirect calorimetry, and tracer labeled 2-step hyperinsulinemic euglycemic clamp including indirect calorimetry) at Weeks 24 to 25.

A final safety follow-up visit occurred within approximately 28 days after the last dose of study agent.

During the study, subjects remained on their stable dose regimens of metformin or combination metformin and DPP-4 inhibitor therapy, unless the investigator considered dose modification was medically necessary.

Venous blood samples (approximately 2 mL) for pharmacokinetic (PK) assessments were collected from all subjects prior to study agent dose, in the morning and after an overnight fast of at least 8 hours at each scheduled timepoint as specified in the Time and Events Schedule in the protocol.

Safety assessment was based on the incidence and type of treatment-emergent adverse events (TEAEs), hypoglycemic events, vital signs (blood pressure and pulse rate), 12 lead electrocardiogram (ECG), changes in clinical laboratory test results (including chemistry, hematology, urinalysis, lipids) and self-monitored blood glucose (SMBG).

Number of Subjects (planned and analyzed): A total of 56 subjects (28 subjects per treatment group) were planned to be enrolled in this study.

A total of 56 subjects (26 subjects in the canagliflozin group and 30 subjects in the placebo group) were treated and analyzed in this study. The randomization was stratified by the HbA_{1c} value at screening: (1) 7.0% to 8.0%: canagliflozin (n=20) and placebo (n=19); (2) >8.0% to 9.5%: canagliflozin: (n=9) and placebo (n=11).

Fifty-six subjects were included in the safety analysis set (ie, subjects who received at least 1 dose of study agent during the double-blind treatment phase) and 51 subjects were included in the pharmacodynamic (PD) analysis set (subjects who had taken at least 1 dose of double-blind study agent and had both baseline and post-baseline PD measurement).

Diagnosis and Main Criteria for Inclusion: Subjects with inadequate glycemic control (ie, HbA_{1c} 7.0% to 9.5%, inclusive) on metformin monotherapy at a stable dose of ≥1,000 mg/day, or on combination therapy with metformin ≥1,000 mg/day and a DPP-4 inhibitor at stable daily doses for at least 12 weeks prior to screening were eligible for enrolment.

Subjects were eligible for randomization if they had inadequate glycemic control (ie, FPG ≥100 mg/dL and ≤240 mg/dL) at the Week -4 visit, and met all other eligibility criteria (including Day -14 fasting finger stick glucose ≥100 mg/dL and ≤240 mg/dL).

Test Product, Dose and Mode of Administration, Batch No.: Canagliflozin, was provided in 100- and 300-mg capsules, which were taken orally with 240 mL of water.

Test Product	Batch nos.	Expiration Dates
Canagliflozin	HG-13F031 HG-13F027	02-2016
	HG-14A004 HG-14A007	10-2016
	HG-13L048 HG-14A002	10-2016
	HG-15D025 HG-15E032	01-2018

Reference Therapy, Dose and Mode of Administration, Batch No.: Placebo capsules matched canagliflozin capsules in appearance, which were taken orally with 240 mL of water.

Reference Therapy	Batch nos.	Expiration Dates
Placebo	HG-13F017	02-2016
	HG-14B012	10-2016
	HG-14D021	10-2016
	HG-15C016	01-2018

Duration of Treatment:

The total study duration for each subject participating in this study was up to approximately 34 weeks.

Pre-treatment phase: Single-blind placebo capsules for approximately 28 days, including 14 days during the single-blind run-in period and 14 days during the baseline period.

Double-blind treatment phase: Subjects were randomly assigned to canagliflozin or placebo starting at a dose of 100 mg once daily and were titrated to the 300-mg dose once daily. Starting on Day 1, subjects received the first dose of double-blind study agent, canagliflozin or placebo for approximately 178 days.

Criteria for Evaluation:

Pharmacokinetic Evaluations:

Blood samples for measurements of trough plasma canagliflozin concentrations were collected at specified time points at Weeks 8, 12, 20, and 24 of the double-blind treatment phase, to confirm PK exposure of canagliflozin.

Pharmacodynamic Evaluations

Most of the PD evaluations were made during the 6,6-$^2H_2$-tracer-labeled 2-step hyperinsulinemic, euglycemic clamp (first clamp step with a low insulin infusion rate of 20 mU/min/m^2 body surface area (BSA) followed by the second clamp step with a high insulin infusion rate of 120 mU/min/m^2 BSA) and MMTT procedures. Each subject underwent both of these procedures at baseline (prior to treatment initiation) and again after approximately 6 months of treatment (during Week 24 to 25). Liver fat content was measured at baseline (Day -14) and Week 24 using MRS images on a 3T magnetic resonance imaging (MRI) whole body scanner.

Indirect calorimetry was performed to measure gas exchange so that energy expenditure and substrate utilization rates could be determined. Measurements were taken at specified intervals during the MMTT (pre-meal, approximately 75 to 105 minutes after ingestion, and approximately 195 to 225 minutes after ingestion) and during the clamps (in the basal state [ie, no insulin infusion] and at the end of the low and high insulin infusion periods).

The measures for PD evaluation were:

- Basal endogenous glucose production (EGP)
- % Insulin mediated suppression of EGP, determined during the first-step of the tracer-labeled 2-step hyperinsulinemic (calculated as $100\times[EGP_{basal}–EGP_{low}]/EGP_{basal}$, where EGP_{low} is the rate during the low insulin infusion)
- Basal and insulin-stimulated peripheral IS, determined as the mean tissue glucose disposal rate (Tissue R_d, calculated as total R_d – urinary glucose excretion [UGE] rate) during basal and each clamp step divided by the mean plasma glucose concentration divided by the mean plasma insulin concentration
- % Insulin suppression of plasma free fatty acid (FFA) concentrations during each clamp step
- Non-oxidative and oxidative glucose disposal in the basal state and under hyperinsulinemic conditions (low- and high-dose insulin infusions)
- Insulin clearance during the clamp ($CL_{insulin,\ clamp}$) and MMTT ($CL_{insulin,\ MMTT}$). Insulin clearance during the clamp was estimated by dividing the insulin infusion rates at each clamp step by steady-state plasma insulin concentrations. Insulin clearance during the MMTT was estimated by dividing the area under the plasma concentration-time curve (AUC) for the ISR (calculated from deconvolution of C-peptide values) by the AUC of plasma insulin.
- Plasma glucagon concentrations in the basal state and under hyperinsulinemic conditions
- Liver fat content (absolute %, defined as ratio of corrected fat signal to the sum of corrected fat and water signals) determined using ^1H MRS
- Insulin secretion rate (ISR) at 9 mmol/L plasma glucose concentration during the MMTT using a model-based method based on deconvolution of plasma C-peptide concentrations to obtain ISR at each time point. The value of ISR at 9 mmol/L plasma glucose was obtained using the linear regression relationship between ISR and plasma glucose values
- β-cell glucose sensitivity (slope of ISR vs. plasma glucose plot during the MMTT) using the model-based method for ISR calculation and the regression relationship between ISR and plasma glucose
- Insulin sensitivity index from the clamp ($S_{IP,clamp}$) calculated as the change in tissue glucose R_d (which is calculated as total R_d–UGE) from the basal period to the high insulin infusion period divided by the change in insulin and the plasma glucose concentration and the disposition index calculated as $S_{IP,clamp}\times$β-cell glucose sensitivity.

- Basal and postprandial glucose oxidation rate (G_{ox} = 4.55 rate of elimination of carbon dioxide [V_{CO2}] – 3.21 oxygen uptake/consumption [V_{O2}] – 2.87 N), and fat oxidation rate (F_{ox} =1.67 V_{O2} - 1.67 V_{CO2} – 1.92 N), and protein oxidation rate (P_{ox} = 6.25 N, and Energy Production rate (EPR) = 1.11 V_{CO2} + 3.91 V_{O2} – 3.34 N, where V_{O2} is the O_2 consumption rate and V_{CO2} is the CO_2 production rate, and N is the urinary nitrogen excretion)
- Basal and postprandial plasma glucagon, FFAs and β-hydroxybutyrate during the MMTT
- Renal threshold for glucose (RT_G) estimated using an MMTT-based method
- $HbA_{1c,}$ FPG, and body weight (BW)

Biomarker Evaluations

Venous blood samples (4 mL each) were collected under fasting conditions at baseline and Week 24. Plasma samples (1 mL aliquots) were to be archived (at ≤-80° C) for potential future analysis of inflammatory markers (eg, interleukin-6 [IL-6]; tumor necrosis factor alpha [TNF-α]; monocyte chemoattractant protein-1 [MCP-1]), cardiovascular markers (eg, adiponectin; C-reactive protein [CRP]; fibrinogen; von Willebrand factor; plasminogen activator inhibitor-1 [PAI-1]) or markers of hepatic fibrosis.

Safety Evaluations

Safety evaluations included the collection of adverse events (AEs), hypoglycemic events, 12-lead ECGs, vital signs (blood pressure and pulse rate), physical examinations, SMBG, and clinical laboratory tests (including chemistry, hematology, urinalysis, lipids).

Statistical Methods:

Sample Size Determination

Many patients with T2DM have non-alcoholic fatty liver disease (NAFLD) and a proportion of them have non-alcoholic steatohepatitis. Based on published data on liver fat content in subjects with non-alcoholic steatohepatitis measured using MRS, the estimated standard deviation (SD) for the change from baseline in liver fat content using MRS is 7% (absolute value). Using a SD of 7%, a sample size of 25 completers per treatment group in the present study was determined to be sufficient for estimating the mean change from baseline in liver fat content between treatment groups to be within ±4% with 95% confidence. For canagliflozin 300 mg, an approximate 4% mean reduction from baseline in BW was observed in Phase 3 studies. The data from the published study showed that BW reduction is correlated with the reduction in liver fat content measured using MRS. Based on an unpublished evaluation of the placebo data from that study, for a 4% reduction from baseline in BW, the predicted reduction in liver fat content was estimated to be 7%. With a sample size of 25 completers per treatment group and using a 1-sided test with alpha=2.5%, the present study would have >90% power for detecting a mean reduction of 7% in liver fat in the canagliflozin group compared to the placebo group.

Based on a published study in T2DM patients, the estimated SDs for the change from baseline in the R_d is 8.2 mg/kg/min. Using a SD for R_d of 8.2 mg/kg/min, a sample size of 25 completers per group in the present study would be sufficient to estimate the mean change from baseline in R_d between treatment groups to be within ±4.7 mg/kg/min with 95% confidence. With a sample size of 25 completers per treatment group and using a 1-sided test with alpha=2.5%, the present study would have 80% power for detecting a mean difference between canagliflozin and placebo groups of 6.5 mg/kg/min.

Based on data from previous canagliflozin clinical studies, the mean changes from baseline in β-cell function (ie, ISR at 9mM glucose) and the SD of the mean changes from baseline for the canagliflozin 300 mg and placebo treatments are summarized in the table below:

Baseline values of the ISR at 9mM glucose are ~109 pmol/min/m^2.

	Mean Change from Baseline	SD for Change from Baseline
Placebo	0.7	73
Canagliflozin	74	108

Assuming a SD of 92 pmol/min/m^2 (ie, the pooled variance estimator arising from 2-sample t-tests) for change from baseline in the ISR at 9 mM glucose, a sample size of 25 completed subjects per treatment group was determined to be sufficient to estimate the difference in mean change from baseline in the ISR at 9 mM glucose between canagliflozin 300 mg and placebo to within ±52.3 pmol/min/m^2 with 95% confidence. With a sample size of 25 completers per treatment group and using a 1-sided test with alpha=2.5%, the present study should have 80% power for detecting a mean difference between canagliflozin and placebo groups of 73 pmol/min/m^2.

To increase the probability of 25 subjects completing per treatment group, 28 subjects per treatment group (56 total) were planned to be enrolled in this study. A drop-out rate of 10% was assumed.

Pharmacokinetic Analysis

Pharmacokinetic analyses were done by Kinesis Pharma BV, Breda, The Netherlands, using SAS (version 9.3, SAS Institute Inc., Cary, NC, USA) for the creation of PK tables and figures.

No PK parameters were calculated. Descriptive statistics were used to summarize canagliflozin plasma concentrations prior to study agent dose (trough plasma concentrations) at each sampling time point, including arithmetic mean, SD, coefficient of variation, median, minimum, and maximum for each treatment group.

Data were listed for all subjects with available plasma concentrations per treatment group. All concentrations below the lowest quantifiable concentration or missing data were labeled as such in the concentration database. All subjects and samples excluded from the analysis are clearly documented in the study report.

Pharmacodynamic Analysis

All the PD endpoints were summarized using descriptive statistics by treatment group [N, mean, SD, median, range, and 95% confidence interval (CI)].

Statistical analysis was performed for the PD variables using mixed model for the design. The mixed model for change and percentage change from baseline values included site, HbA1c category, baseline and treatment as fixed effects and subjects as random effect. The LS mean change and percentage change from baseline between canagliflozin and placebo treatment groups and the corresponding 95% CIs were presented for all PD variables.

Safety Analysis:

Safety was evaluated by examining the incidence and type of AEs, hypoglycemic events, changes in clinical laboratory test results, ECGs, physical examinations, and vital signs.

Interim Analysis:

An interim analysis of unblinded data (study endpoint for liver fat content measured by MRS) from approximately 50% of the subjects who completed the study was performed.

RESULTS:

STUDY POPULATION:

A total of 59 subjects were randomized (n=29 in the canagliflozin group and n=30 in the placebo group), 3 subjects were withdrawn from the study. A total of 56 subjects (n=26 in the canagliflozin group and n=30 in the placebo group) received at least 1 dose of study agent during the double-blind treatment phase and were included in the safety analysis. Three subjects randomized to the canagliflozin group were withdrawn from the study prior to Day 1 (1 subject each for withdrawal of consent, AE of urticaria, and history of alcohol abuse before screening). All available PD data from randomized subjects who had taken at least 1 dose of double-blind study agent and had both baseline and post-baseline PD measurement were included in the PD analysis set (n=24 in the canagliflozin group and n=27 in the placebo group). The number of subjects in the PD analysis set varied based on the PD analysis population and available data for various calculated PD parameters.

Of the total 56 subjects who received at least 1 dose of study agent during the double-blind treatment phase, 51 subjects (91.1%) completed the study (24 subjects [92.3%] in the canagliflozin group and 27 subjects [90.0%] in the placebo group) and 5 subjects (8.9%) did not complete the study (2 subjects [7.7%] in the canagliflozin group and 3 subjects [10.0%] in the placebo group). The reasons for study discontinuation were: AEs (1 subject each in the canagliflozin and placebo groups), lost to follow-up (2 subjects [6.7%] in the placebo group), and other (1 subject [3.8%] in the canagliflozin group had history of alcohol abuse before screening).

All treated subjects were titrated from Dose Level 1 (canagliflozin 100 mg or matching placebo) to the Dose Level 2 (canagliflozin 300 mg once daily or matching placebo).

The median age was 59.0 years (range: 28 to 72 years) and the median body mass index (BMI) was 31 kg/m^2 (range: 23.4 to 40.4 kg/m^2). The majority of subjects were white (38 subjects [67.9%]). Demographic and baseline characteristics were generally similar between the 2 treatment groups.

PHARMACOKINETIC AND PHARMACODYNAMIC RESULTS:

Pharmacokinetic Results

From the mean and overlay of individual trough plasma concentration-time plots for Days 56, 84, 140, and 168; it appeared that for most subjects steady-state conditions were achieved and maintained. Canagliflozin mean trough plasma concentrations ranged between 375 ng/mL and 457 ng/mL. The range of C_{trough} concentrations for the 300 mg dose observed in this study are consistent with the range of C_{trough} values reported in an earlier multiple dose canagliflozin study. The intersubject variability, expressed as % coefficient of variation (CV), associated with the trough plasma concentrations was moderate to high, with values ranging between 49.8% and 133.8%.

Pharmacodynamic Results

HbA1c, FPG, and Body Weight

Treatment with canagliflozin lowered HbA1c, FPG, and BW, results were consistent with previously conducted canagliflozin studies.

The placebo-subtracted least square mean change (ΔLSM) values were -0.77% for HbA1c (95% CI: -1.13%, -0.42%), -30 mg/dL (95% CI: -46.5, -14.2) for FPG (-1.7 mmol/L with 95% CI: -2.6;-0.8 mmol/L), and -3.3 kg (95% CI: -5.1 kg;-1.5 kg) for BW.

Liver Fat Content

The mean values of the liver fat content at the baseline visit were approximately 50% higher in the placebo group than in the canagliflozin group. As changes in liver fat tend to be related to baseline liver fat (with subjects having higher baseline liver fat tending to get larger reductions in liver fat), this baseline imbalance could affect the between-group comparisons for changes in liver fat. The analysis of covariance (ANCOVA) models used in the LS Mean analyses included baseline liver fat as a covariate and this provided some adjustment for the baseline differences.

In both placebo and canagliflozin groups, mean liver fat content was decreased from baseline to Week 24. The LS mean absolute reductions in the liver fat content were 2.2% to 3.1% greater with canagliflozin compared with placebo in the full set of subjects and the subset with elevated liver fat content (>5.5%) at baseline, respectively, but the 95% CIs for these differences included 0 for both data sets. In terms of relative percent changes in the liver fat content, the LS Mean changes were -6% in placebo and -16% with canagliflozin in the full set of subjects and -20% in placebo and -38% with canagliflozin in the subset of subjects with elevated liver fat content (>5.5%) at baseline, and the 95% CIs for these differences also included 0. In both the full set of subjects and the subset with elevated liver fat content (>5.5%) at baseline, there was a strong correlation between weight loss and reductions in liver fat.

Responses During the 2-step Hyperinsulinemic Euglycemic Clamps

Plasma Glucose and Insulin and Glucose Infusion Rate:

Subjects underwent a 2-step hyperinsulinemic euglycemic clamp at baseline and post-treatment at Week 25. The plasma glucose concentrations were similar in both treatment groups during the last hour of each clamp step and plasma insulin concentrations were well matched between groups. The measured values of plasma glucose, [6,6-$^2H_2$] glucose enrichment, insulin during the 2-step clamp, and the glucose infusion rate did not show notable changes between baseline and Week 25 in the placebo group. In the canagliflozin group, the pre-insulin infusion plasma glucose concentrations were lower at Week 25 than at baseline as expected, due to the glycemic lowering effect with canagliflozin treatment and the glucose infusion rate was higher at Week 25 than at baseline, indicating greater IS.

Endogenous Glucose Production (EGP), Glucose Disposal (R_d), and Tissue R_d:

No notable changes in EGP, R_d, and Tissue R_d measures between baseline and Week 25 were observed in the placebo group. In the canagliflozin group, the basal (pre-insulin infusion) EGP was increased at Week 25 compared to baseline, although the increase compared to placebo was not statistically significant (the 95% CI for the placebo-subtracted difference included 0). Similarly, the increase in total R_d throughout the clamp period at Week 25 compared to baseline was not statistically significant relative to placebo (the 95% CIs included 0). The pre-insulin infusion value for Tissue R_d (which is calculated as total R_d–UGE) was decreased from baseline with canagliflozin treatment compared to placebo. During the clamps, modest numerical increases in mean Tissue Rd were observed with canagliflozin treatment, however, the numerical values for LS mean changes from baseline to Week 25 in Tissue R_d were considerably smaller than the changes in total R_d and none of the changes were significant compared to placebo as evidenced by the 95% CIs. The values for Tissue R_d/(Glucose×Insulin), the insulin-stimulated peripheral IS, were significantly higher for canagliflozin than placebo in each of the clamp steps (the 95% CIs exclude 0).

Insulin Sensitivity for Glucose Production and Disposal:

The relative suppression of EGP during the low insulin infusion (a measure of hepatic IS) and tissue glucose disposal during the high insulin infusion (a measure mainly of muscle IS) were calculated from the clamp data. Both indices of IS were increased with canagliflozin treatment compared to placebo. The percentage suppression of EGP during the low insulin infusion increased from 43% at baseline to 55% at

Week 25 with canagliflozin compared to little change in the placebo group (39% at baseline and 42% at Week 25); the placebo-subtracted LS Mean change from baseline (95% CI) was 10.8% (2.9%; 18.8%). The IS index for glucose disposal during the high insulin infusion (S_{IP},clamp) increased by approximately 30% with canagliflozin compared to placebo.

Insulin Clearance During the High Insulin Infusion:

Consistent with the similar plasma insulin concentrations seen at baseline and Week 25, no change in insulin clearance for infused insulin was seen in either the placebo or canagliflozin groups.

Plasma Glucagon and Free Fatty Acid:

Plasma glucagon concentrations were generally similar at baseline and Week 25 in the canagliflozin group, whereas, they were modestly lower numerically at Week 25 compared to baseline in the placebo group. No between-treatment differences in glucagon concentrations were observed prior to the insulin infusion and at each of the clamp steps. Fasting FFA levels were also similar at baseline between groups, but basal plasma FFA concentrations were modestly increased from baseline to Week 25 in the canagliflozin group compared to placebo, but no between-group differences in the suppression of FFA during the insulin infusions were observed.

Calorimetry Results During Clamps:

Energy Production Rate: A small decrease in the EPR was observed in the canagliflozin group during the pre-insulin infusion and low insulin infusion periods, whereas no difference was observed during the high insulin infusion period.

Carbohydrate and Fat Oxidation Rates: In the basal (pre-insulin infusion) state, canagliflozin treatment led to a shift in substrate utilization, and a decrease in the respiratory quotient, indicating a decreased proportion of energy coming from carbohydrate oxidation and an increased proportion from fat oxidation. The rate of carbohydrate oxidation was decreased with canagliflozin treatment compared to placebo, whereas fat oxidation was unchanged. During the euglycemic clamps (when circulating concentrations of glucose and FFA were similar between groups), no notable between-group differences in fat and carbohydrate oxidation were observed.

Oxidative and Non-Oxidative Glucose Disposal Rates:

The rates of oxidative and non-oxidative glucose disposal during the clamps were calculated from the measured R_d and carbohydrate oxidation rates. The oxidative R_d (calculated in mg/kg/min) were consistent with the values calculated in mg/min, with canagliflozin treatment leading to a reduction in basal oxidative glucose disposal compared to placebo, but no difference in oxidative disposal during the clamp periods. The non-oxidative R_d were increased during the basal period and during both clamp steps with canagliflozin compared to placebo. Some of the increase in non-oxidative glucose disposal is attributable to glucose disposal to UGE.

Mixed Meal Tolerance Test Assessments

Plasma Glucose, Insulin, and C-peptide:

No notable changes in plasma glucose, insulin, or C-peptide during the MMTT were seen in the placebo group. In the canagliflozin group, fasting and postprandial plasma glucose and insulin were decreased at Week 25 compared to baseline (both the fasted values and the AUCs following the meal), whereas plasma C-peptide concentrations were not different between visits; results were consistent with previous canagliflozin studies.

Plasma Glucagon, FFA, and β-hydroxybutyrate:

In the placebo group, mean plasma glucagon concentrations (both pre-meal and postprandial values) were modestly decreased from baseline to Week 24, whereas no change in glucagon during the MMTT was seen in the canagliflozin group.

Plasma FFA showed a small increase from baseline to Week 24 in the canagliflozin group, whereas overnight-fasted concentrations of β-hydroxybutyrate were more than doubled with canagliflozin treatment; no notable changes in either FFA or β-hydroxybutyrate were seen in the placebo group. Although the overnight-fasted β-hydroxybutyrate concentrations were increased with canagliflozin treatment, the postprandial concentrations of β-hydroxybutyrate were suppressed to a similar level at Week 24 as at baseline.

Beta-cell Function:

The relationship between the ISR and plasma glucose concentration was shifted upwards with canagliflozin treatment, indicating increased insulin secretion at any given plasma glucose concentration. The associated parameters, ISR at 9 mmol/L glucose and β-cell glucose sensitivity, were increased with canagliflozin treatment compared to placebo; results were consistent with previous canagliflozin studies.

Insulin Clearance During the MMTT:

Insulin clearance for endogenously secreted insulin during the MMTT was approximately 50% higher than the clearance of intravenously infused insulin calculated from the clamps, as expected based on the high first-pass hepatic extraction of insulin. Insulin clearance during the MMTT was increased by approximately 25% with canagliflozin treatment compared to placebo.

Calorimetry Results During MMTT:

Energy Production Rate: No between-group differences in EPR were observed in the pre-meal or the postprandial time intervals.

Respiratory Quotient and Carbohydrate and Fat Oxidation Rates: Compared to placebo, canagliflozin treatment reduced the respiratory quotient, decreased carbohydrate oxidation, and increased fat oxidation.

Urinary Glucose Excretion and Renal Threshold for Glucose Excretion:

Canagliflozin treatment increased UGE and decreased the RT_G; results were consistent with previous canagliflozin studies.

Disposition Index

The mean disposition index, calculated as the product of the IS index from the clamp ($S_{IP,clamp}$) and the beta-cell glucose-sensitivity from the MMTT, was increased by approximately 65% with canagliflozin treatment compared to an approximate 14% increase in the mean value in the placebo group.

SAFETY RESULTS:

Overall, the incidence of TEAEs in subjects was similar across both treatment groups (80.8% [21/26 subjects] of subjects in the canagliflozin group and 73.3% [22/30 subjects] of subjects in the placebo group). The most frequently reported (≥10% of subjects) TEAEs by preferred term in the canagliflozin group were nasopharyngitis (19.2% [5/26 subjects]), urinary tract infection (15.4% [4/26 subjects]), and diarrhea (11.5% [3/26 subjects]) compared with 3.3% (1/30 subjects) of subjects for each of the events in the placebo group. Most of the reported TEAEs were assessed by the investigator as mild or moderate in severity. Two of 26 subjects in the canagliflozin group (n=1 with contusion, foot fracture, ligament sprain, and limb injury; and n=1 with psychotic disorder) and 3 of 30 subjects in the placebo

group (n=1 with vomiting and back pain; n=1 with comminuted fracture; and n=1 with nausea and vomiting) reported with severe TEAEs.

No deaths were reported in the study. One subject in the canagliflozin group had treatment-emergent serious adverse event of psychotic disorder on Day 174. The study agent was withdrawn due to this event. The event was considered recovered on the next day (Day 175). This SAE was assessed as doubtfully related to the study agent by the investigator.

The incidence of TEAEs leading to study agent discontinuation in subjects who received treatment during the double-blind treatment phase was low in both the treatment groups, with 1 subject each in the canagliflozin group (3.8%) and in the placebo group (3.3%). The subject in the canagliflozin group with treatment-emergent SAE of psychotic disorder and subject in the placebo group with TEAE of vomiting were withdrawn from treatment. In addition, 1 subject randomized to the canagliflozin group had a severe AE of urticaria, during the pre-treatment phase and the study agent was not administered to this subject in the double-blind treatment phase.

The incidence of persistent AEs observed was higher in the placebo group (8 subjects) compared with the canagliflozin group (2 subjects). All the persistent AEs were mild or moderate in severity.

Six subjects (5 subjects in the canagliflozin group and 1 subject in the placebo group) had hypoglycemic episodes during the study. Most hypoglycemic episodes were asymptomatic and resolved within 10 to 91 minutes. Of these 6 subjects, 3 subjects reported TEAE of hypoglycemia (2 subjects in the canagliflozin group during the double-blind treatment phase and 1 subject in the placebo group during the pre-treatment phase).

There was a mean increase of approximately 8.4% in serum magnesium levels from baseline to Week 24 (Day 168) in the canagliflozin group. There was a mean increase of approximately 2.4% in serum magnesium levels from the baseline to Week 24 in the placebo group. There was a mean decrease from baseline ranging from approximately 12% to 16.9% in serum urate levels from Week 3 to Week 25 (Day 178) in the canagliflozin group. There was a mean decrease from baseline ranging from 0.11% to 4.3% in serum urate levels from Week 3 to Week 25 (Day 178) in the placebo group. These changes were consistent with previously completed canagliflozin studies.

Vital sign measurements (pulse rate and blood pressure), above or below the normal reference ranges were observed during the study. However, none were considered to be clinically meaningful.

The mean values of ECG parameters were generally similar between canagliflozin and placebo groups. No subject had a QT corrected according to Fridericia's formula (QTcF) or QT corrected according to Bazett's formula (QTcB) value of >500 msec or a change from baseline of >60 msec at follow-up in both treatment groups.

STUDY LIMITATIONS:

No notable study limitations were identified by the Sponsor.

CONCLUSIONS:

- For most subjects, steady-state plasma drug exposure of canagliflozin were achieved and maintained during the double-blind treatment phase. The C_{trough} concentrations for the 300 mg dose of canagliflozin are consistent with the range of C_{trough} values reported in an earlier multiple dose canagliflozin study.
- Canagliflozin treatment reduced HbA1c, BW, and FPG compared to placebo and the magnitude of these changes were consistent with results from previous studies.

- During an MMTT, canagliflozin treatment increased UGE, reduced fasting and postprandial levels of plasma glucose and insulin, and improved measures of beta-cell function; these results were consistent with results from previous studies.
- During a 2-step hyperinsulinemic euglycemic clamp study, the following changes in glucose production were observed with canagliflozin compared to placebo:
  - A modest numerical increase in basal EGP.
  - An increase in the percentage of suppression of EGP during the low insulin infusion, indicating increased hepatic IS.
  - No decreases in R_d and Tissue R_d during the high insulin infusion, with associated increases in the IS indices Tissue R_d/(Glucose×Insulin) and $S_{IP,clamp}$, suggesting improved muscle IS.
  - Greater numerical reductions in hepatic fat content were seen with canagliflozin compared to placebo, both in the full set of subjects and the subset of subjects with elevated liver fat at baseline. However, the 95% CIs for the between-treatment differences included 0 in both cases. In both groups, there was a strong correlation between weight loss and reductions in liver fat.
- No meaningful changes in EPR were seen with canagliflozin treatment. During fasting and postprandial conditions, canagliflozin treatment decreased carbohydrate and oxidation and increased fat oxidation, whereas, during clamped conditions, no changes in substrate utilization were seen.
- The disposition index, calculated as the product of $S_{IP,clamp}$ and β-cell glucose sensitivity, was increased by approximately 65% with canagliflozin compared to approximately 14% with placebo.
- No change in plasma glucagon concentrations was seen with canagliflozin treatment, although a modest numerical reduction was seen in the placebo group.
- Canagliflozin treatment modestly increased mean fasting plasma FFA and approximately doubled mean fasting β-hydroxybutyrate concentrations.
- Overall, treatment with canagliflozin 300 mg once daily was generally well tolerated in subjects with T2DM. The TEAEs and the safety laboratory test results observed in this study were consistent with the findings in completed canagliflozin studies in subjects with T2DM.

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