SYNOPSIS

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Name of Sponsor/Company	Janssen Research & Development
Name of Finished Product	To be determined
Name of Active Ingredient	JNJ-28431754 (Canagliflozin)

Protocol No.: 28431754DIA1023 (Amendment INT-2)

Title of Study: A Double-blind, Placebo-Controlled, Randomized, Parallel-Group, Multi-Center Study to Evaluate the Multiple-Dose Pharmacokinetic and Pharmacodynamic Characteristics of JNJ-28431754 (Canagliflozin) in Subjects With Type 2 Diabetes Mellitus

NCT No.: NCT01128985

Clinical Registry No.: CR017200

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Study Centers: Two sites at Fort Myers, FL and Miramar, FL in United States of America

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Phase of Development: 1

Objectives: The primary objective was to evaluate the pharmacokinetics (PK) of canagliflozin and its major metabolites M7 and M5 after multiple oral doses of canagliflozin in adult subjects
with type 2 diabetes mellitus (T2DM). The secondary objective was to evaluate the pharmacodynamics (PD) of canagliflozin in subjects with T2DM. Safety and tolerability were also assessed.

Methodology: This was a double-blind, randomized, placebo-controlled, multiple-dose (7 days), multicenter PK and PD study with 4 parallel groups. The study consisted of 5 phases: (1) a screening phase of approximately 3 weeks (Days -44 to -23), (2) a washout phase (including dietary counseling for all subjects, and washout of current antihyperglycemic agents [AHAs] for subjects currently on AHAs) of approximately 3 weeks (Days -22 to -3) (subjects whose fasting fingerstick glucose was >14.4 mM [260 mg/dL, confirmed by 2 consecutive measurements within 1 week] during the washout phase, were not eligible to enter the treatment phase), (3) a baseline phase of 2 days (Days -2 to -1); a blood sample was obtained on Day -2 to determine the subject's fasting plasma glucose [FPG] and all subjects received a single oral placebo dose in a single blind fashion on Day -1 followed by randomization to the treatment groups and stratification according to their FPG level measured on Day -2), (4) a double-blind treatment phase of 12 days (Days 1 to 12, with daily dosing on Days 1 to 7), and (5) a follow-up phase occurring 7 to 10 days after the last dose of study drug (Day 7) of the treatment phase. During the baseline phase, eligible subjects were randomized to 1 of 4 treatment groups and received either canagliflozin 50, 100, and 300 mg or matching placebo once daily for 7 consecutive days (Days 1 to 7).

Number of Subjects (planned and analyzed): Forty (40) subjects were enrolled to ensure at least 36 subjects completed the study. Thirty-six randomized subjects (placebo, N=9; 50-mg canagliflozin, N=9; 100-mg canagliflozin, N=8; and 300-mg canagliflozin N=10 subjects) completed the study.

Diagnosis and Main Criteria for Inclusion: Medically stable men and women diagnosed with T2DM for at least 1 year but not more than 12 years prior to Day -1, between 25 and 65 years of age, inclusive, with a body mass index (BMI) between 18 and 39.9 kg/m^2, inclusive at screening, and a body weight of not less than 50 kg were eligible for enrollment. Eligible subjects were to be on a generally stable approved AHA regimen for at least 2 months prior to

the first screening visit, including subjects who were (a) on a single AHA and had a HbA_{1c} value from 6.5% to 9.5% at screening, or (b) on low-dose dual oral AHA therapy (ie, <50% maximum labeled doses of both agents) and had a HbA_{1c} value from 6.5% to 9.5% at screening, or (c) not currently on AHA therapy and had a HbA_{1c} value from 7.0% to 10.0% at screening. Subjects with FPG concentrations between 7.8 mM (140 mg/dL) and 15 mM (270 mg/dL) on Day -2 were eligible for enrollment.

Test Product, Dose and Mode of Administration, Batch No.: Canagliflozin, 50 mg (as 2 x 25 mg tablets filled into single capsule) (Batch No.: PD2778), 100 mg (as 1 x 100 mg tablet) (Batch No.: PD3093), or 300 mg (as 1 x 300 mg tablet) (Batch No.: PD3158), for oral administration.

Reference Therapy, Dose and Mode of Administration, Batch No.: Matching placebo (Batch No.: PD3220), capsules, oral administration.

Duration of Treatment: Single doses of 50, 100, and 300 mg of canagliflozin or matching placebo were administered once daily for 7 consecutive days from Days 1 to 7 during the double-blind treatment phase.

Criteria for Evaluation:

Pharmacokinetic Evaluations: Pharmacokinetic parameters of canagliflozin and it metabolites (M7 and M5) were determined for each dose level using non-compartmental analysis:

- Day 1: C_{max}, t_{max}, and $AUC_{\tau,sd}$
- Day 7: $C_{max,ss}$ C_{trough}, $C_{min,ss}$, $t_{max,ss}$, $AUC_{\tau,ss}$, Acc Ratio, λ_z, $t_{1/2\lambda}$, CL_{ss}/F (only for parent), and Vd_{ss}/F (only for parent).

Urine samples were obtained at predetermined intervals up to 24 hours after dosing on Day 1 and up to 48 hours after dosing on Day 7 for determination of urine canagliflozin, and metabolites (M7 and M5) concentrations. The following urinary PK parameters were determined for canagliflozin and its metabolites (M7 and M5): $A_{e,\ t1\text{-}t2}$, A_e, $A_{e,\%dose}$ and CL_R.

The chiral conversion from canagliflozin (β-anomer) to the α-anomer was also evaluated.

Pharmacodynamics: Pharmacodynamic parameters were determined on Days -1, 1, and 7: $MPG_{0\text{-}24,}$ $MPG_{0\text{-}4,}$ IPG, UGE_{24}, $UGE_{24\text{-}48}$ (Day 7 only) and RT_G (renal threshold for glucose).

Safety: Safety and tolerability were monitored throughout the study by assessment of adverse events (AEs), clinical laboratory tests, 12-lead electrocardiograms (ECGs), vital signs, and physical examinations, conducted at screening, baseline, and end-of-study (or the time of early withdrawal).

Pharmacokinetic sampling times and bioanalytical methods:

Pharmacokinetics: Blood samples were to be collected on Day 1 and Day 7, and Day 2 to Day 12 and urine samples were to be collected on Day 1 and Day 7 for determination of canagliflozin and major metabolites (M5 and M7) plasma and urine concentrations. Concentrations of canagliflozin and its metabolites, M5 and M7, were determined using a validated liquid chromatography/tandem mass spectrometry; quantification range for canagliflozin was 5.0 to 10,000 ng/mL and for M7 and M5 metabolites was 100 to 100,000 ng/mL in plasma and urine. The LC-MS/MS method developed for quantification of the α-anomer had a calibration range of 5.00 to 100 ng/mL.

Pharmacodynamics: Blood samples for measurement of plasma glucose were collected at selected time points on Days -1 (baseline), 1, 2, 7, and 8. Urine samples were collected during predefined intervals on Day -1 (baseline) until dosing on Day 1, again during predefined intervals on Day 1 and Day 2, and on Days 7 to 9 for assessment of UGE.

Pharmacogenomics: A mandatory blood sample (10 mL) was collected from all subjects for pharmacogenomic research.

Statistical Methods:

The primary PD variable: Change from baseline (Day 1) in mean 24-hour plasma glucose.

PD population: All subjects with both pretreatment (Day -1) and posttreatment (Day 7) PD data and with no protocol deviations. Subjects were analyzed as treated.

Primary PD analysis: An analysis of covariance (ANCOVA) model was fitted with treatment as a factor and FPG at baseline as fixed effects for each time point (Day 1 and Day 7) separately. The least-squares (LS) means and intersubject variance were estimated from the model. Using the estimated LS mean and intersubject variance, the point estimate and 90% confidence intervals (CI) for the difference in mean MPG_{0-24} change from baseline between each canagliflozin dose group and placebo was constructed.

Additional analyses were performed on the change from baseline for RT_G, UGE_{0-24}, MPG_{0-24}, and incremental PG. An ANCOVA was fitted to each of these variables with treatment as a factor, and the baseline value as a fixed effect. Point estimates and 90% CIs for the difference between each canagliflozin dose and placebo were estimated.

Safety analysis: All subjects who received at least 1 dose of study drug were included in the safety analysis. For each treatment group, baseline values for all laboratory and safety evaluations, vital signs (pulse, SBP and DBP), and 12-lead ECG measurements) were defined as the last evaluation done before the first dose on Day 1. Safety was evaluated by examining the incidence and type of AEs, and changes in clinical laboratory test values, 12-lead ECGs, vital signs measurements, and physical examination results beginning with the screening phase through the final follow-up visit. The incidence of hypoglycemic events was tabulated by treatment group. Self-monitored blood glucose levels (fasting and 30 minutes after lunch) were listed.

PK analysis: PK parameters were presented using descriptive statistics.

Pharmacogenomics: Allele and genotype frequencies for analyzed genes were tabulated.

RESULTS: A total of 40 subjects were enrolled in the study. Of these, 2 randomized subjects were withdrawn before study drug administration on Day 1 due to the following reasons: 1 subject (002007) was withdrawn prior to the treatment phase based on the investigator's decision (ie, baseline FPG exceeded the protocol-specified limit) and 1 subject (002019) was withdrawn after the wash-out phase but prior to beginning the treatment phase because the wash-out phase had been inadvertently extended to 4 weeks (rather than the protocol-specified 3 weeks). These 2 subjects were replaced by subjects who were allocated to the same treatment group as the withdrawn subjects. Two additional subjects were screened but not randomized due to the following reasons: 1 subject (001901) had a serious adverse event (ie, atrial fibrillation) that was observed during the screening evaluation and this subject was considered a screen failure, and 1 subject (002014) was not randomized based on the investigator's decision (ie, due to low hemoglobin). Thirty-six subjects randomized to treatment completed the study with 9, 9, 8, and 10 subjects in the placebo and 50-, 100-, and 300-mg canagliflozin groups, respectively.

The majority of subjects were white (94%) and Hispanic or Latino (97%) with a median age of 55.5 years (range 33 to 64 years of age). The mean (±SD) baseline body weight (kg) was 83.1 (16.65) and BMI (kg/m^2) was 30.9 (4.64). Seventy-five percent of the subjects had a baseline FPG ≤200 mg/dL. Twenty-seven subjects with FPG ≤200 mg/dL at Day -2 and 9 subjects with FPG >200 mg/dL at Day -2 were randomized, and the stratification by FPG was relatively balanced within treatment groups. Because block size was 4 and the number of subjects in each strata was not a multiple of 4, both strata ended with an unfilled block and therefore the randomization was not exactly equally balanced across the 4 treatment groups. All other demographic and baseline characteristics of the subjects were comparable across treatment groups and were consistent with the protocol specifications.

No protocol deviations were reported for this study.

Pharmacokinetic and Pharmacodynamic Results:

Pharmacokinetics: Mean C_{max} and AUC values for canagliflozin, M7 and M5 increased in a dose dependent manner. For canagliflozin, mean C_{max} values increased to the ratios of 1.0:2.3:8.7 and mean AUC_{∞} values increased to the ratios of 1.0:2.0:7.6 on Day 7. Mean apparent $t_{1/2}$ values ranged from approximately 14 to 17 hours for all 3 analytes on Day 7 and appeared to be independent of the dose. At steady-state on Day 7, less than 1% of the administered

dose was excreted into urine as canagliflozin, approximately 27% to 32% of the administered dose was recovered as M7, and approximately 10% of the administered dose was recovered as M5 in the urine in 24 hours. Mean (SD) plasma canagliflozin, M7 and M5 pharmacokinetic parameters on Day 7 are shown in the table below.

Minimal accumulation of canagliflozin was observed at steady-state across the 3 doses with accumulation ratios ranging from 1.29 to 1.36. Mean accumulation ratios of AUC values for M7 on Day 7 ranged from 1.23 to 1.28 across the 3 doses. Similarly, the mean AUC accumulation ratio for M5 was 1.25 for the 50-mg dose, 1.22 for the 100-mg dose and 1.43 for the 300 mg-dose of canagliflozin.

For M7 and M5, the molar corrected mean plasma metabolite-to-parent C_{max} ratios decreased with increasing dose. For M7, the molar corrected mean plasma metabolite-to-parent AUC_{τ} ratio was similar between 50-mg and 100-mg doses and was slightly decreased at the 300-mg dose. For M5, the molar corrected mean plasma metabolite-to-parent AUC_{τ} ratio was similar between 100-mg and 300-mg doses and was slightly higher at the 50-mg dose.

Mean (SD) Canagliflozin, M7 and M5 Pharmacokinetic Parameters on Day 7

Parameters	50 mg (n=9)	100 mg (n=8)	300 mg (n=10)
Canagliflozin			
C_{max} (ng/mL)	536 (174)	1227 (481)	4678 (1685)
t_{max} (h)[a]	2.00 (1.00-5.00)	1.50 (1.00-5.00)	1.5 (1.00-2.00)
AUC_{τ} (ng.h/mL)	4059 (1105)	8225 (1947)	30995 (11146)
$t_{1/2}$ (h)	16.3 (4.8)	13.7 (2.1)	14.9 (4.8)
Acc Ratio[c]	1.30 (0.108)	1.29 (0.109)	1.36 (0.123)
Ae_{24} (% Dose)	0.833 (0.287)	0.746 (0.230)	0.752 (0.323)
M7			
C_{max} (ng/mL)	608 (305)	1276 (588)	3122 (542)
C_{max} (Metabolite/parent Ratios)[b]	0.824 (0.184)	0.800 (0.328)	0.528 (0.154)
t_{max} (h)[a]	3.00 (2.00-5.05)	2.50 (2.00-5.00)	2.00 (1.5-3.00)
AUC_{τ} (ng.h/mL)	5765 (3989)	10819 (5216)	28110 (7655)
AUC_{τ} (Metabolite/parent Ratios)[b]	1.00 (0.435)	0.979 (0.468)	0.700 (0.178)
$t_{1/2}$ (h)	17.2 (5.0)	13.9 (2.4)	15.0 (4.7)
Acc Ratio[c]	1.23 (0.146)	1.25 (0.122)	1.28 (0.186)
Ae_{24} (% Dose)	30.7 (6.97)	31.9 (11.0)	27.0 (4.09)
M5			
C_{max} (ng/mL)	324 (132)	559 (191)	1900 (534)
C_{max} (Metabolite/parent Ratios)[b]	0.469 (0.187)	0.371 (0.199)	0.312 (0.0778)
t_{max} (h)[a]	4.00 (1.50-6.00)	3.00 (1.50-6.00)	1.75 (1.00-4.00)
AUC_{τ} (ng.h/mL)	3607 (2109)	6003 (1943)	21911 (7865)
AUC_{τ} (Metabolite/parent Ratios)[b]	0.641 (0.253)	0.535 (0.149)	0.537 (0.149)
$t_{1/2}$ (h)	14.8 (3.9)	14.2 (2.6)	13.8 (4.6)
Acc Ratio[c]	1.25 (0.283)	1.22 (0.242)	1.43 (0.337)
Ae_{24} (% Dose)	10.1 (2.62)	9.57 (2.38)	10.5 (2.03)

[a] Median (min-max)

[b] Metabolite/Parent Ratio = [Parameter(metabolite)/Molecular weight(metabolite)]/[Parameter(parent)/Molecular weight(parent)]; Molecular weights: canagliflozin (454 g/mole); M7 and M5 (620.6 g/mole)

[b] Acc. Ratio = AUC_{τ} (Day 7)/AUC_{τ} (Day 1)

The chiral conversion from canagliflozin (β-anomer) to the α-anomer was evaluated. Plasma canagliflozin C_{max} values following the 300-mg dose were at least 700 to 900 times that of the α-anomer, suggesting that there is negligible chiral inversion of canagliflozin to the α-anomer in vivo.

Pharmacodynamic Results:

Plasma Glucose (PG): Plasma glucose was persistently reduced over the entire 24 hours on Day 1 for the 50-, 100-, and 300-mg once daily dose levels, and further decreased after 7 days of treatment, compared with Day -1. For the placebo treatment group, PG concentrations were not reduced on Day 1 and Day 7 relative to Day -1. At baseline (Day -1), MPG_{0-24} was comparable among treatment groups, ranging from approximately 195 to 217 mg/dL. The reductions from baseline in MPG_{0-24} at 50-, 100-, and 300-mg once daily canagliflozin doses were statistically significantly different from that of the placebo on Day 1 and Day 7. The mean 24-hour PG concentrations (MPG_{0-24}) on Day -1 (baseline) and Day 7 at each dose level compared with that of placebo are summarized in the table below.

Mean (SD) and Statistical Analysis of Mean 24-Hour Plasma Glucose Concentrations (MPG_{0-24}) for Each Treatment

Study Day	Placebo (n=9)	50 mg (n=9)	100 mg (n=8)	300 mg (n=10)
Day -1, Baseline (mg/dL)	207.23 (35.108)	216.70 (39.867)	195.40 (23.122)	196.73 (35.075)
Day 7 (mg/dL)	215.16 (46.459)	184.94 (38.291)	157.63 (18.392)	150.81 (22.792)
Change From Baseline	7.93 (26.939)	-31.76 (36.583)	-37.77 (13.659)	-45.91 (23.613)
Diff of LS Means [SE)][a]	NA	-42.21 [12.852][c]	-48.72 [13.335][b]	-57.26 [12.752][b]
90% CI		-64.004; -20.423	-71.329; -26.108	-78.877: -35.636

KEY: LS=least squares; NA=not applicable; n=subsample size; SD=standard deviation; SE=standard error

[a] P-values and CIs are based on the pairwise comparison between a dose of canagliflozin and placebo using least squares (LS) means from an ANCOVA model including treatment as a factor and FPG at baseline (Day 1 predose) as a covariate. No adjustments for multiplicity have been made.

[b] P-value <0.001

[c] P-value 0.003

Renal Threshold: Mean RT_G values on Day -1 ranged from approximately 212 to 244 mg/dL and tended to be higher in subjects with higher plasma glucose on Day -1. RT_G was reduced in a dose-dependent manner on both Day 1 and Day 7. The 100-mg canagliflozin dose provided near-maximal lowering of RT_G during the middle portion of the day, with slightly less lowering later in the day and in the overnight period, whereas the 300-mg canagliflozin dose maintained near-maximal lowering of RT_G throughout all of the time intervals. The 24-hour mean RT_G was lowered by >100 mg/dL compared with the Day -1 values in all 3 dose groups, whereas almost no change was observed in the placebo group across the days; the mean percent change in the 24-hour mean RT_G (Day 7 values relative to Day -1 values) was 52% in the 50-mg group and 64% in the 100- and 300-mg canagliflozin groups.

Pairwise Comparison in 24-Hour Mean RT_G Change From Baseline Between Each Canagliflozin Dose and Placebo With Associated 90% Confidence Intervals

Study Day	Placebo (n=9)	50 mg (n=9)	100 mg (n=8)	300 mg (n=10)
Day -1, Baseline (mg/dL)	235.49 (26.688)	244.17 (34.756)	212.06 (21.668)	237.13 (33.135)
Day 7 (mg/dL)	234.53 (31.882)	119.32 (32.869)	76.75 (15.855)	85.06 (20.049)
Change From Baseline (mg/dL)	-0.95 (31.238)	-124.8 (21.014)	-135.3 (19.826)	-152.1 (27.620)
Diff of LS Means [SE][a]	NA	-119.7 [10.224][b]	-145.6 [10.894][b]	-150.3 [9.908][b]
90% CI		-137.07; -102.40	-164.05; -127.11	-167.13; -133.53

KEY: LS=least squares; NA=not applicable; n=subsample size; SE=standard error

[a] P-values and CIs are based on the pairwise comparison between a dose of canagliflozin and placebo using least squares (LS) means from an ANCOVA model including treatment as a factor and baseline (Day -1) RT_G as a covariate. No adjustments for multiplicity have been made.

[b] P-value <0.001

Urinary Glucose Excretion (UGE): At baseline on Day -1, 24-hour UGE varied slightly between the treatment groups. Mean 24-hour UGE increased from Day -1 to Day 1 with mean (SD) increases of 66.2 (12.9), 101.7 (18.0) and 102.5 (23.9) g, for the 50-, 100-, and 300-mg dose groups, respectively. This increase in 24-hour UGE was maintained over the 7-day dosing period, ranging from approximately 85 to 103 g by Day 7. The 24-hour UGE increased statistically significantly compared to placebo for all canagliflozin doses after single dose as well as after multiple dose administration.

Pharmacogenomic Results: No subject withdrew consent for pharmacogenomic research. One UGT1A9*3 allele carrier had the lowest M7/canagliflozin ratios for AUC_τ and C_{max} in the 100-mg dose group suggesting that UGT1A9 enzyme may be involved in the metabolism of canagliflozin to the M7 metabolite.

Safety Results: No deaths, SAEs, or hypoglycemic-related AEs were reported. Overall, the incidence of treatment-emergent adverse events (TEAE) was similar in placebo (2 of 9 subjects, 22%) and canagliflozin groups (8 of 27 subjects, 30% of all canagliflozin-treated subjects). Among canagliflozin groups, the incidence of TEAEs was slightly higher in the 50-mg group (4 of 9 subjects, 44%) compared to the 100-mg (2 of 8 subjects, 25%) and 300-mg (2 of 10 subjects, 20%) groups. The only TEAE reported in the placebo group was back pain (2 of 9 subjects). The most common TEAEs in canagliflozin groups (all doses) included constipation (3 of 27 subjects), and headache (2 of 27 subjects). The most common TEAEs in the canagliflozin (all doses) groups belonged to the gastrointestinal disorders system organ class and the occurrence was higher in 50-mg canagliflozin group (3 of

9 subjects) compared to the 100-mg (1 of 8 subjects) and 300-mg (no subjects) groups. All TEAEs were considered mild in severity, and were considered as resolved by the investigator. None of the TEAEs were considered to be very likely or probably related to the study drug. One occurrence of pollakiuria in the canagliflozin 50-mg group, and 1 occurrence of headache in the canagliflozin 100-mg group were considered to be possibly related to the study drug by the investigator. One subject (001901) was withdrawn from the study during the screening phase due to a SAE of atrial fibrillation. The atrial fibrillation resolved within 2 days following treatment with acetylsalicylic acid, warfarin sodium, enoxaparin, and metoprolol tartrate.

There were no treatment-related, or canagliflozin dose-related trends for changes from baseline (Day 1, predose) in any of the routine clinical laboratory safety test results that were considered to be clinically significant. However, small changes for some clinical test analytes were observed.

There were no treatment-related, or canagliflozin dose-related changes from baseline in pulse, DBP, or SBP that were clinically significant. No clinically meaningful changes in body weight and no physical examination abnormalities or trends were observed during the study. No clinically meaningful mean changes from baseline in ECG parameters, including corrected QT intervals, were noted during the study.

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