

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

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PHARMACOLOGY REVIEW(S)

**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: **204353**

Supporting document/s: NDA Resubmission, SDN #20, eCTD # 0019

Applicant's letter date: Feb 10, 2014

CDER stamp date: Feb 10, 2014

Product: **Canagliflozin + metformin IR (CanaMet, INVOKAMET®)**

Indication: Type 2 diabetes

Applicant: Janssen Pharmaceuticals Inc.

Review Division: DMEP

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1 Executive Summary

1.1 Recommendations

1.1.1 Approvability: Nonclinical data supports approval of NDA 204353

1.1.2 Additional Nonclinical Recommendations: No new nonclinical studies are recommended.

1.1.3 Labeling Recommendations (there is no change in the PT section of the label from the first submission)

8.1 Pregnancy

Teratogenic Effects

Pregnancy category C

There are no adequate and well-controlled studies in pregnant women with INVOKAMET or its individual components. During pregnancy, consider appropriate alternative therapies, especially during the second and third trimesters. INVOKAMET should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus

Canagliflozin

There are no adequate and well-controlled studies of canagliflozin in pregnant women. Based on results from rat studies, canagliflozin may affect renal development and maturation. In a juvenile rat study, increased kidney weights and renal pelvic and tubular dilatation were evident at greater than or equal to 0.5 times clinical exposure from a 300 mg dose [see Nonclinical Toxicology (13.2)]. These outcomes occurred with drug exposure during periods of animal development that correspond to the late second and third trimester of human development.

Metformin hydrochloride

There are no adequate and well-controlled studies in pregnant women with metformin. Metformin was not teratogenic in rats and rabbits at doses up to 600 mg/kg/day. This represents an exposure of about 2 and 6 times the maximum recommended human daily dose of 2,000 mg based on body surface area comparisons for rats and rabbits, respectively. Determination of fetal concentrations demonstrated a partial placental barrier to metformin.

Canagliflozin and Metformin Combination

Co-administration of canagliflozin and metformin to pregnant rats during the period of organogenesis was neither embryolethal nor teratogenic when tested at doses yielding systemic exposures (AUC) up to 11 and 13 times the maximum recommended human dose (MRHD) (canagliflozin 300 mg and metformin 2000 mg), respectively.

8.3 Nursing Mothers

INVOKAMET

No studies in lactating animals have been conducted with the combined components of INVOKAMET. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from INVOKAMET, a decision should be made whether to discontinue nursing or to discontinue INVOKAMET, taking into account the importance of the drug to the mother [see Nonclinical Toxicology (13.2)].

Canagliflozin

It is not known if canagliflozin is excreted in human milk. Canagliflozin is secreted in the milk of lactating rats reaching levels 1.4 times higher than that in maternal plasma. Data in juvenile rats directly exposed to canagliflozin showed risk to the developing kidney (renal pelvic and tubular dilatations) during maturation. Since human kidney maturation occurs in utero and during the first 2 years of life when lactational exposure may occur, there may be risk to the developing human kidney.

Metformin

Studies in lactating rats show that metformin is excreted into milk and reaches levels comparable to those in plasma. It is not known whether metformin is secreted in human milk.

8.4 Pediatric Use

Safety and effectiveness of INVOKAMET in pediatric patients under 18 years of age have not been established.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility (draft)

INVOKAMET

No animal studies have been conducted with the combined products in INVOKAMET to evaluate carcinogenesis, mutagenesis, or impairment of fertility. The following data are based on the findings in studies with canagliflozin and metformin individually.

Canagliflozin

Carcinogenesis

Carcinogenicity was evaluated in 2-year studies conducted in CD1 mice and Sprague-Dawley rats. Canagliflozin did not increase the incidence of tumors in mice dosed 10, 30, or 100mg/kg (≤ 14 times exposure from a 300mg clinical dose).

Testicular Leydig cell tumors, considered secondary to increased luteinizing hormone (LH), increased significantly in male rats at all doses tested (10, 30, and 100mg/kg). In a 12-week clinical study, LH did not increase in males treated with canagliflozin.

Renal tubular adenoma and carcinoma increased significantly in male and female rats dosed 100mg/kg, or approximately 12-times exposure from a 300mg clinical dose. Also, adrenal pheochromocytoma increased significantly in males and numerically in females dosed 100 mg/kg. Carbohydrate malabsorption associated with high doses of canagliflozin was considered

a necessary proximal event in the emergence of renal and adrenal tumors in rats. Clinical studies have not demonstrated carbohydrate malabsorption in humans at canagliflozin doses of up to 2-times the recommended clinical dose of 300mg.

Mutagenesis

Canagliflozin was not mutagenic with or without metabolic activation in the Ames assay. Canagliflozin was mutagenic in the in vitro mouse lymphoma assay with but not without metabolic activation. Canagliflozin was not mutagenic or clastogenic in an in vivo oral micronucleus assay in rats and an in vivo oral Comet assay in rats.

Impairment of Fertility

Canagliflozin had no effects on the ability of rats to mate and sire or maintain a litter up to the high dose of 100 mg/kg (approximately 14x and 18x the 300 mg clinical dose in males and females, respectively), although there were minor alterations in a number of reproductive parameters (decreased sperm velocity, increased number of abnormal sperm, slightly fewer corpora lutea, fewer implantation sites, and smaller litter sizes) at the highest dosage administered.

Metformin hydrochloride

Carcinogenesis

Long-term carcinogenicity studies have been performed in rats (dosing duration of 104 weeks) and mice (dosing duration of 91 weeks) at doses up to and including 900 mg/kg/day and 1500 mg/kg/day, respectively. These doses are both approximately 4 times the maximum recommended human daily dose of 2000 mg based on body surface area comparisons. No evidence of carcinogenicity with metformin was found in either male or female mice. Similarly, there was no tumorigenic potential observed with metformin in male rats. There was, however, an increased incidence of benign stromal uterine polyps in female rats treated with 900 mg/kg/day.

Mutagenesis

There was no evidence of a mutagenic potential of metformin in the following in vitro tests: Ames test (*S. typhimurium*), gene mutation test (mouse lymphoma cells), or chromosomal aberrations test (human lymphocytes). Results in the in vivo mouse micronucleus test were also negative.

Impairment of fertility

Fertility of male or female rats was unaffected by metformin when administered at doses as high as 600 mg/kg/day, which is approximately 3 times the maximum recommended human daily dose based on body surface area comparisons.

13.2 Animal Toxicology and/or Pharmacology

Canagliflozin

In a juvenile toxicity study in which canagliflozin was dosed directly to young rats from postnatal day (PND) 21 until PND 90 at doses of 4, 20, 65, or 100 mg/kg, increased kidney

weights and a dose-related increase in the incidence and severity renal pelvic and renal tubular dilatation were reported at all dose levels. Exposure at the lowest dose tested was greater than or equal to 0.5 times the maximum clinical dose of 300 mg. The renal pelvic dilatations observed in juvenile animals did not fully reverse within the 1-month recovery period. Similar effects on the developing kidney were not seen when canagliflozin was administered to pregnant rats or rabbits during the period of organogenesis or during a study in which maternal rats were dosed from gestation day (GD) 6 through PND 21 and pups were indirectly exposed in utero and throughout lactation.

In embryo-fetal development studies in rats and rabbits, canagliflozin was administered for intervals coinciding with the first trimester period of non-renal organogenesis in humans. No developmental toxicities were observed at any dose tested other than a slight increase in the number of fetuses with reduced ossification at a dose that was associated with maternal toxicity and that is approximately 19 times the human exposure to canagliflozin at the 300 mg clinical dose.

1.2 Brief Discussion of Nonclinical Findings

This is a resubmission of the canagliflozin and metformin-IR fixed dose combination (CanaMet-IR FDC) NDA 204353. The division had issued a complete response (CR) letter following the review of the original FDC on Dec 12, 2013. The CR letter contained no nonclinical deficiency and the resubmission contains no new pivotal nonclinical studies. The resubmission, however, contains several amendments and minor pharmacokinetic studies previously submitted to canagliflozin (NDA 204042) and original CanaMet-IR FDC NDA 204353. The amendments and minor studies contain no new information pertinent to the label. The nonclinical sections (8.1, 8.3, 8.4, 13.1 and 13.2) of the resubmitted label remain unchanged and acceptable. The original CanaMet-IR application submitted on Dec 12, 2012 contained several nonclinical studies including pivotal combination dose studies (3-month toxicity and reproductive toxicology in rats) were reviewed with the original submission and support the approval of the CanaMet-IR FDC (NDA 204353). The nonclinical studies and amendments in the resubmission do not affect the label or the pharmtox recommendations to the label. Pharmtox reviewer recommends approval of CanaMet-IR FDC with no change to the pharmtox sections of the label.

2 Drug Information**2.1 Drug: INVOKAMET®****2.1.2 Generic Name:** Canagliflozin+metformin**2.1.3 Code Name for canagliflozin:** JNJ-28431754 hemihydrate**Code Name for metformin:** JNJ-1158196-AAC (metformin)

Janssen is purchasing metformin from (b) (4). A letter of authorization was provided by (b) (4) to reference DMF (b) (4)

2.1.4 Chemical Name

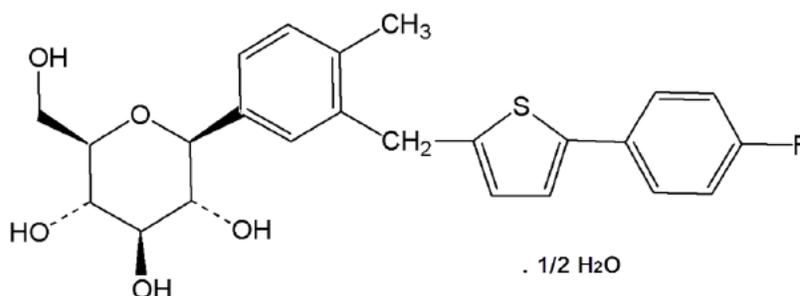
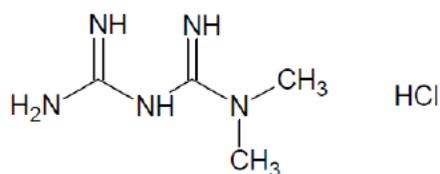
canagliflozin: (1S)-1,5-anhydro-1-C-[3-[[5-(4-fluorophenyl)-thienyl]methyl]-4-methylphenyl]-D-Glucitol

metformin: 1,1-Dimethylbiguanide hydrochloride, N,N-dimethyl-monochloride, imidodicarbonimidic diamide

2.1.5 Molecular Formula/Molecular Weight

Canagliflozin hemihydrate: $C_{24}H_{25}FO_5S \cdot H_2O$, MW 454.5

Metformin hydrochloride: $C_4H_{11}N_5 \cdot HCl$, MW 165.6

2.1.6 Structure of canagliflozin (JNJ-28431754-ZAE):**Structure of metformin (JNJ-1158496-AAC):**

2.1.7 Pharmacologic class:

Canagliflozin: Sodium glucose co-transporter 2 (SGLT2) inhibitor
 Metformin: Biguanide antihyperglycemic

2.2 Relevant IND/s, NDA/s, and DMF/s

Canagliflozin NDA 204042

Metformin MDF (b) (4) is approved and marketed in US (b) (4) (b) (4)
 (b) (4)

2.3 Clinical Formulation

2.3.1 Drug Formulation

Active ingredients: JNJ-28431754 hemihydrate and Metformin hydrochloride

50/500 mg CANA/MET IR FDC tablets are white, capsule-shaped, film-coated tablets with “CM” on one side and “155” on the other side (b) (4)

50/1000 mg CANA/MET IR FDC tablets are beige, capsule-shaped, film-coated tablets with “CM” on one side and “551” on the other side (b) (4)

150/500 mg CANA/MET IR FDC tablets are yellow, capsule-shaped, film-coated tablets with “CM” on one side and “215” on the other side (b) (4)

150/1000 mg CANA/MET IR FDC tablets are purple, capsule-shaped, film-coated tablets with “CM” on one side and “611” on the other side (b) (4)

Inactive ingredients: Microcrystalline Cellulose, (b) (4), Croscarmellose Sodium, hypromellose, Magnesium Stearate, and coating (b) (4) white, beige yellow and purple amounts are provided in table below).

Amounts of Coating Ingredient	Components per Tablet Quality reference	Amount per unit (mg/tablet)			
		85F18422 White Used in 50/500-mg tablets	85F97458 Beige Used in 50/1000-mg tablets	85F32547 Yellow Used in 150/500-mg tablets	85F90093 Purple Used in 150/1000- mg tablets
Polyvinyl alcohol-partially hydrolyzed	Ph. Eur./USP	(b) (4)			
Titanium dioxide	Ph. Eur./USP				
Macrogol (b) (4) PEG (b) (4)	Ph. Eur./NF				
Talc	Ph. Eur./USP				
Yellow iron oxide (b) (4)	EU Directive				
Red iron oxide (b) (4)	2008/128/EC/NF EU Directive				
Black iron oxide (b) (4)	2008/128/EC/NF EU Directive				
	2008/128/EC				

-- = Not present

2.3.3 Comments on Impurities/Degradants of Concern in canagliflozin

Canagliflozin Impurities

The purity of canagliflozin (JNJ-28431754) as hemihydrate and an anhydrous were 97.7% (w/w) and 99.8%, respectively. All the impurities were less than the specification (b) (4) requiring no characterization. Inorganic impurities such as heavy metals were below (b) (4) ppm. The residual solvents were below ICH Q3C (R3) limits.

Canagliflozin batch #30178750 (98.5% purity) synthesized by (b) (4) was used in GLP toxicology, early clinical studies trials and in the stability studies. Batch #30936066 (98.5% purity) was manufactured by (b) (4) is considered to be the representative drug substance to be used in new clinical studies. The impurities and residual solvents in both batches were qualified by HPLC and assessed in the toxicology studies. The genotoxic degradant (b) (4) identified during the stability studies was present in the drug product and drug substance up to (b) (4) ppm (b) (4) µg per 300 mg tablet). Further analysis found the genotoxic impurity to be a (b) (4). With significantly higher daily exposure to (b) (4) in the diet, the agency and the sponsor agreed to set the specifications to genotoxic (b) (4) impurity for 100 and 300 mg tablets are shown below. The specifications for the (b) (4) impurity was also applied to the FDC.

100mg drug product (tablet):	(b) (4)	ppm ((b) (4)	µg/day)
300mg drug product (tablet):	(b) (4)	ppm ((b) (4)	µg/day)

Metformin Impurities

The purity of metformin used in the study was 99.6% (w/w). All the impurities were less than the specification (b) (4), requiring no characterization. Inorganic impurities such as heavy metals were below (b) (4) ppm. The residual solvents were below ICH Q3C (R3) limits.

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2.4 Proposed Clinical Population and Dosing Regimen for canagliflozin+metformin IR

Type II diabetic patients, Canagliflozin-Metformin IR fixed dose combinations (FDC):
50/500, 50/1000, 150/500 and 150/1000 mg BID

2.5 Regulatory Background

Canagliflozin (JNJ-28431754) NDA 204042 application was approved by the FDA on March 29, 2013 for the treatment of type 2 diabetes. The NDA 204353 for canagliflozin and immediate release metformin (CanaMet-IR) fixed dose combination (FDC) was submitted on Dec 12, 2012. The canagliflozin/metformin FDC is for twice-daily oral doses at four dosage strengths (50/500, 150/500, 50/1000 and 150/1000 mg). For the approval, the sponsor relied on the approved canagliflozin application and existing metformin data for Glucophage NDA under 505(b)(2). For the approval of the original FDC, the sponsor had provided a 3-month toxicology and an embryofetal development combination study in rats. The agency issued a complete response (CR) letter on Dec 11, 2013. The CR letter requested clinical and PK /PD data supporting bridging of once daily (QD) to twice daily (BID) canagliflozin dosing regimen. There was no nonclinical deficiency in the CR letter.

In this resubmission (NDA 204353) on Feb 2014, the sponsor has provided complete response to the clinical deficiency (reanalysis and modeling of clinical PK / PD data and bone safety update) and a few minor nonclinical studies. The nonclinical studies in the resubmission (listed below) are reviewed in PharmTox (PT) review with no impact on the label. The PT sections of the canagliflozin/metformin IR FDC label in the resubmission remain unchanged.

3.1 Studies Reviewed

- Study # BA1625 - Validation report for method BTM-1241-R0: Determination of metformin in rat plasma by LC/MS/MS
- Study# **TOX10433**: A 2-week repeated dose oral bridging PK study of JNJ-28431754- ZAE in the female rabbit
- Study#**FK10427**: An in vitro study on the transport of ¹⁴C-JNJ-28431754 by SLCO1B3 (human OATP1B3) and ABCG2 (human BCRP) and on the inhibition of SLCO1B3 and ABCG2 by ¹⁴C-JNJ-28431754.
- Study# **FK10426**: In Vitro Evaluation of Canagliflozin (JNJ-28431754) as an Inhibitor of UDP Glucuronosyltransferase Enzymes in Human Liver Microsomes
- Study# **FK5352**: Study of the Potential Effects of JNJ-28431754 in the Induction of CYP1A2, CYP2C9, CYP2C19, and CYP3A4 in Cryo-Preserved Human Hepatocytes
- Study# **FK7341**: A Study of the Potential Effects of High Dose JNJ-28431754 in the Induction of CYP1A2, CYP2B6 and CYP3A4 in Cryopreserved Human Hepatocytes
- Study# **FK7434**: A Study of the Potential Effects of JNJ-41488525 (M7) and JNJ-41980874 (M5) in the Induction of CYP1A2, CYP2B6, and CYP3A4 in Human Hepatocytes
- Study#DD12313: Effects of JNJ-28431754 on C-Peptide Clearance in Rats

3.2 Studies Not Reviewed: none

3.3 Previous Reviews Referenced

Acute and subchronic toxicology studies with canagliflozin alone were reviewed under canagliflozin NDA 204042. One month and 3-month canagliflozin in combination with metformin were reviewed with the first NDA 204353 submission. Therefore, only the summary of the combination dose studies are provided in NDA resubmission review.

- Study # TOX9521: Developmental toxicity study with JNJ-28431754-ZAE Combined with Metformin in the Rat (non-GLP)
- Study # TOX9590: Developmental toxicity study with JNJ-28431754-ZAE Combined with Metformin in the Rat (GLP)
- Study #TOX9582: 1-Month repeated dose oral toxicity study of JNJ-28431754 combined with metformin in the rat (non-GLP)
- Study # TOX9667: 3-Month Repeated Dose Oral Toxicity Study of JNJ-28431754-ZAE Combined with Metformin in the Rat (GLP)

4 Pharmacology

4.1 Primary Pharmacology

Canagliflozin is a selective, reversible inhibitor of SGLT2 transporter. The IC₅₀s for Chinese Hamster Ovary cells expressing human SGLT2 and SGLT1 were 4.1 nM (~1.8 ng/ml) and 664 nM (~291 ng/ml), respectively. Although the IC₅₀ data suggests a 160 fold selectively to SGLT2, the intestinal exposure after oral dosing can reach sufficient concentration to inhibit SGLT1 in the small intestine in rats (evidenced by soft feces, bloating, gas) and possibly in humans (minor bloating), secondary to retention of excess glucose in the distal GI tract.

The IC₅₀ for rat SGLT2 and SGLT1 were 3.7 nM and 555 nM, respectively. In mice the IC₅₀s for the same transporters were 2 nM and >1000 nM, respectively.

Canagliflozin appeared to be even more selective in mice than in rats. Based on IC₅₀ data, canagliflozin potency at inhibiting SGLT2 is similar among species tested (humans, rats and mice). However, in the in vivo studies, the effective dose of canagliflozin was approximately 10 fold lower in nondiabetic SD rats than in nondiabetic mice, suggesting that canagliflozin is likely to produce a pharmacological activity and toxicity in rats at 10x lower dose than in mice. This might explain the absence of renal, adrenal or bone findings in mice. Canagliflozin had notable activity at other receptors/transporters in the Cerep receptor screen assay.

Overview of In Vitro Effects of Canagliflozin in Nonclinical Pharmacology Studies

<u>In Vitro Effects</u>	<u>IC₅₀ (nM)</u>
Human SGLT2	4.2
Rat SGLT2	3.7
Human SGLT1	663
Rat SGLT1	555
Human SGLT3	Not detected
Human SGLT4	> 10,000
Human SGLT6	3,100
Human SMIT1	> 10,000
HepG2 cells	> 50,000
Human primary adipocytes	6,800
Rat skeletal muscle myoblast	> 10,000
Mouse SGLT1	>1,000
Mouse SGLT2	2.0

In humans, canagliflozin is extensively metabolized to two prominent glucuronide metabolites (M5 and M7). The potential SGLT1 and SGLT2 inhibitory activities of the two metabolites have been tested using Chinese hamster ovary cells expressing SGLT1 or SGLT2. Neither metabolite is inhibitor of the SGLT1 or SGLT2 transporter. The C_{max} for M7 and M5 after single dose of 200 mg are 1159 and 703 ng/ml in normal subjects, respectively.

In vitro inhibitory activity of canagliflozin and its major human glucuronide metabolites, M5 and M7	Human SGLT2	Human SGLT1
	IC ₅₀	IC ₅₀
Canagliflozin (JNJ-28431754)	4.2 nM	663 nM
Metabolite M5 (JNJ-41980874)	1014 nM	> 5000 nM
Metabolite M7 (JNJ-41488525)	7600 nM	>10000 nM

The effect of canagliflozin on renal glucose threshold was evaluated in various nonclinical animal models. Canagliflozin (1mg/kg) significantly decreased glucose re-absorption rate and reduced the glucose threshold in anesthetized Obese Zucker fatty rats (ZDF) instrumented with catheters and infused with glucose that raised blood glucose from 150 to 400 mg/dl. Although blood glucose was lower, urinary glucose excretion was dramatically increased in ZDF rats. Urine volume gradually increased with increase of urinary glucose. The table lists the in vivo effective dose of canagliflozin in various species. Although IC₅₀ for mouse and SD rat was similar, the effective dose was significantly lower in SD rats. Blood and Urine Glucose and Urine Volume Excreted Over 120 min after Administration of Single Oral Dose of Canagliflozin (1 mg/kg Dose) in Normoglycemic Rats.

Study#DD12313: Effects of JNJ-28431754 on C-Peptide Clearance in Rats

This non-GLP study was designed to assess the effect of canagliflozin on clearance of C-peptide in male SD rats. As a renal SGLT2 inhibitor, canagliflozin targets kidney for its pharmacological effect and since C-peptide, marker of insulin secretion is cleared by the kidney, the potential indirect effect of canagliflozin on insulin secretion could be due to altered C-peptide clearance by the kidney.

SD rats were treated with single oral dose of 10 mg/kg canagliflozin and plasma concentrations of C-peptide were measured before and after canagliflozin treatment (July 2012).

Group	Treatment
Group 1	Vehicle (0.5% Methocel) 10 ml/kg, p.o.; then Saline: 1 ml/kg, i.v.
Group 2	Vehicle (0.5% Methocel) 10 ml/kg, p.o.; C-Peptide: 5 nmol/ml/kg, i.v.
Group 3	Canagliflozin, 10 mpk, 10 ml/kg, p.o.; then C-Peptide: 5 nmol/ml/kg, i.v.

Single dose of canagliflozin (10 mg/kg) significantly reduced blood glucose levels in male SD rats.

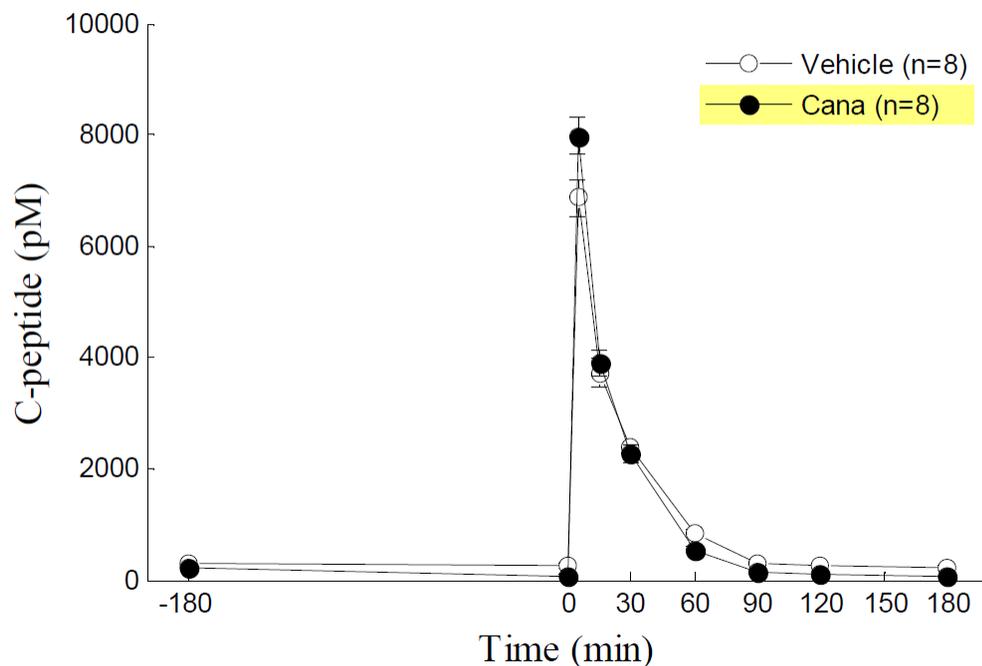
Effects of JNJ-28431754 on blood glucose levels (mg/dL)

Group	N	Time (min)							
		0	5	15	30	60	90	120	180
Vehicle	8	93.6 (5.9)	109.4 (4.6)	111.8 (5.2)	111.6 (4.0)	101.3 (2.9)	95.1 (4.6)	106.8 (8.6)	90.6 (6.4)
Cana	8	64.3* (8.3)	77.3* (6.1)	77.0* (4.3)	84.8* (4.9)	69.4* (3.9)	71.3* (4.9)	64.6* (3.9)	60.1* (4.7)

Data are mean (\pm s.e.m.). * $p < 0.05$ (t-test).

Serum C-peptide levels were similar in both canagliflozin and vehicle control. Canagliflozin had no effect on C-peptide clearance in male SD rats.

Serum C-peptide profiles for animals treated with vehicle or 10 mg/kg of canagliflozin.



Effects of JNJ-28431754 on C-peptide clearance values (ml/min/kg).

Group	N	Mean (SD)	95% CI for Mean
Vehicle	8	29.7 (3.1)	27.1-32.3
Cana	8	27.8 (4.5)	24.0-31.5

Based on this single dose study, canagliflozin (10 mg/kg) may not affect the serum C-peptide levels or its clearance by the kidneys in male SD rats.

4.2 Secondary Pharmacology

Canagliflozin-induced glucose excretion resulted in persistent polyuria in rats. Glucose loss also resulted in substantial weight loss, particularly in SD rats that were highly sensitive to the pharmacological effects of canagliflozin. It should be noted that in some instances animals had increased appetite, likely due to decrease in blood glucose. Since canagliflozin induced-glycosuria may increase urinary loss of electrolytes (e.g. Ca, Cl), the potential drug-related adverse activity may include changes in bone turnover rate and serum electrolyte imbalance.

4.3 Safety Pharmacology

Canagliflozin is pharmacologically active at renal SGLT2 and, at higher doses, at intestinal and renal SGLT1. Canagliflozin had no effects on CNS, cardiovascular, and respiratory systems.

Renal: Not evaluated

Gastrointestinal: Not examined

Abuse Liability: Not evaluated

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Canagliflozin

Canagliflozin is well absorbed in all the species tested. The bioavailability was highest in mice (88% to 137%) followed by 65% in dogs, 37% to 48% in monkeys and about 35% in rats. The $t^{1/2}$ after single dose ranged from 2.7 hrs in mice to 6.2 to 7.5 hrs in rats, dogs and monkeys. In the multiple dose studies, $t^{1/2}$ in rats ranged from 6.3 to 69 hrs and in dogs from 8 hr to 14 hrs. Plasma clearance ranged from low in mice, rats and monkeys to extremely low in dogs. The Vd was 1.4 to 2.3 L/kg in mice, monkeys and rats to 0.6 to 0.76 in dogs suggesting that in dogs. Repeated administration of canagliflozin increased exposure by 2.5 fold in dogs suggesting drug accumulation. Canagliflozin exposure tended to be higher in females in mice, rats and dogs. Canagliflozin had high plasma protein binding in all species including humans, ranging from 98.2 to 99.0%. The human serum albumin and human α -1 acid protein was 97.3 and 39.8%, respectively.

In humans, canagliflozin is metabolized primarily by the hepatic UDP-glucuronosyltransferase enzymes (UGT) to M7 and M5. Cytochrome P450 enzymes appeared to have minimal role in metabolism of canagliflozin except M9 metabolite (CYP3A4 and CYP2D6). The two prominent metabolites (M5 and M7) were not present in the *in-vitro* mouse, rat, dog and human hepatocyte metabolism studies. Qualitatively, the *In-vivo* metabolic profile of canagliflozin was similar to those in animal models. Quantitatively, the exposures to two prominent human M5 and M7 were significantly lower in animals than in humans. However, since both M5 and M7 are inactive and highly water soluble and readily excreted into urine and feces, there is no safety concern. Furthermore, analysis of bile in rats found far greater exposure to M7 (12%) and M5 (4%) than observed in plasma in the TK studies suggesting that low plasma levels might be due to rapid hydrolysis of M7 and M5 back to parent drug in rats. However, both M7 and

M5 were formed in nonpregnant rabbits but at much lower exposure than canagliflozin. The plasma canagliflozin exposure in non-pregnant rabbits was 16x the M7 and about 200x the M5. Metabolic induction studies found no notable in vitro CYP enzyme induction. PK parameters of canagliflozin appear to be gender dependent in rats and dogs. Canagliflozin exposure was moderately higher (up to 2-fold) in female mice, rats and dogs. Whether this was due to difference in CYP enzymes in animals is not clear. There is some in vitro human data suggesting that JNJ-28431754 may inhibit CYP3A4 and CYP2C9. Canagliflozin may act as substrate and inhibitor of MDR1 (IC₅₀ of 8.5 µg/mL) and MRP2 (IC₅₀ of 9.5 µg/mL,) transporters. Whether these transporters are contributing to the difference in drug exposure observed in animals is unknown.

Study#FK10427: An in vitro study on the transport of ¹⁴C-JNJ-28431754 by SLCO1B3 (human OATP1B3) and ABCG2 (human BCRP) and on the inhibition of SLCO1B3 and ABCG2 by ¹⁴C-JNJ-28431754.

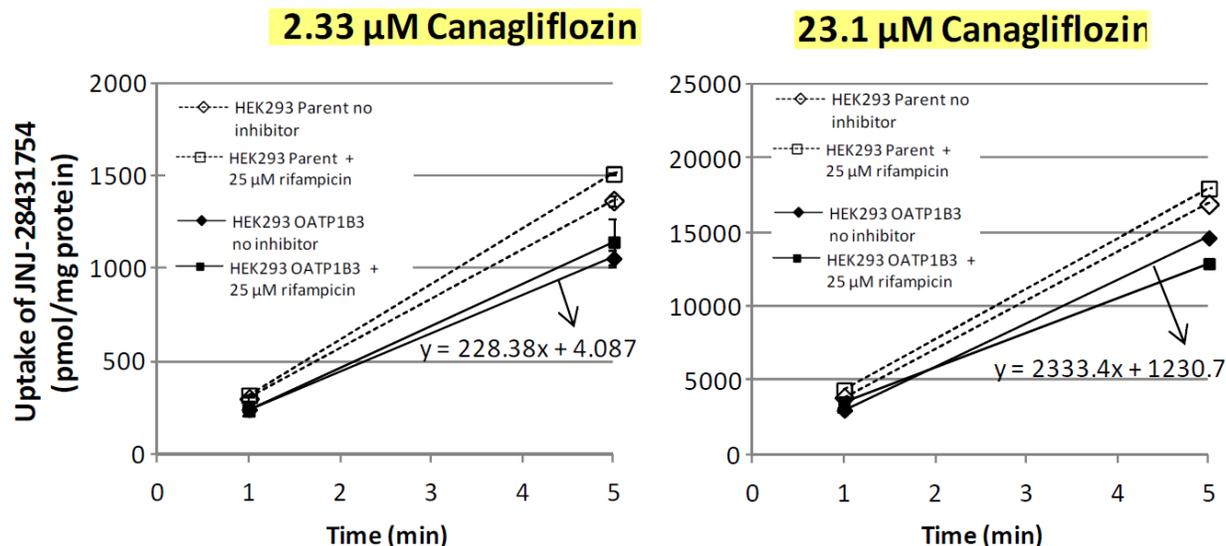
This in vitro study was designed to determine whether canagliflozin is substrate of or inhibitor of human PATP1B3 (SLCO1B3) or BCRP (ABCG2). The study was carried out at the sponsor's research facility in Beerse, Belgium. The effect of canagliflozin on the two transporters was tested using HEK-293 cells transfected with OATP1B3 (obtained from TNO, Netherlands) and MDCK-II cells transfected with BRCP (obtained from Solvo, Hungary). Rifampicin (0.64 to 25 µM) and cyclosporine (0.256 to 25 µM), inhibitors of OATP1B3 were used as positive control in the assay for the human transporter OATP1B3. KO143 an inhibitor of BCRP served as positive control for human BCRP transporter. Canagliflozin concentrations as an inhibitor of the transporter ranged from 1 to 100 µM (actual 0.529 to 66.7 µM). As substrate, canagliflozin was 2.33 or 23.1 µM. Substrates for both transporters are listed in the table below:

Test items and reference compounds

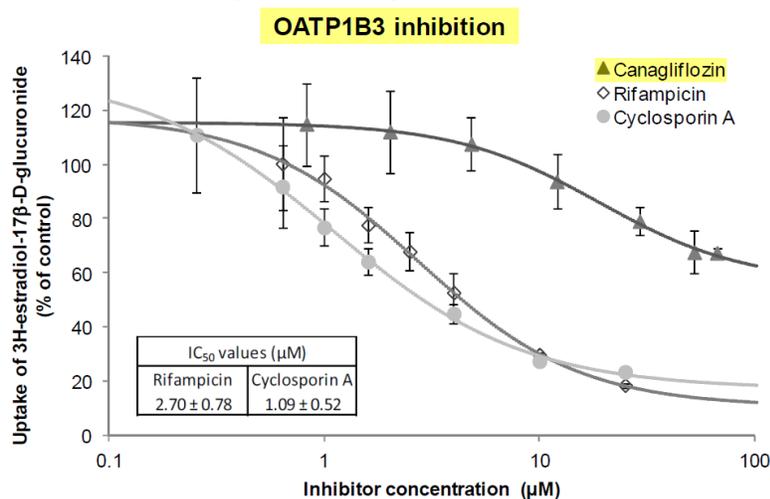
Compound Identification	JNJ-nr	vial	MW _{base} (Conv factor)	Compound Function	Batch Numbers	
					Unlabelled Compound	Labelled Compound
Test Compounds						
¹⁴ C-Canagliflozin	JNJ-28431754	5055	444.53 (1.02)	Test compound	ZR600348PFA271** A12IB3140	Batch 2611 S.A. 2.07 GBq/mmol Conc. 2.47 MBq/mL
³ H-Cyclosporin A*	JNJ-3132844	I151	1202.63 (1)	Inhibitor of OATP1B3	39195100-2	Batch 2689 S.A. 259 GBq/mmol Conc. 2.95 MBq/mL
Compounds for section 1 (substrate / inhibition OATP1B3)						
³ H-17β-estradiol-glucuronide	JNJ-5474729	I140	448.52 (1.05)	Substrate OATP1B3	39036872	Batch 2693 S.A. 1270 GBq/mmol Conc. 35.1 MBq/mL
Rifampicin	JNJ-304291	I003	822.96 (1)	Inhibitor of OATP1B3	EXTE_0201_210_1	-
Compounds for section 2 (substrate / inhibition BCRP transporter)						
KO143	/	-	469.57	BCRP inhibitor	031M4709V (Sigma)	-
³ H-Topotecan	JNJ-16913910	2960	421.46 (1.21)	Substrate BCRP	9604679	Batch 2681 S.A. 28.1 GBq/mmol Conc. 0.679 MBq/ml
PSC833	R118222	3028	1214.65 (1)	P-gp inhibitor	39287731	/
¹⁴ C-mannitol	-	-	182.17	Leakage marker	-	Batch 2665 S.A. 2.17 GBq/mmol Conc. 3.7 MBq/ml
³ H-mannitol	-	-	182.17	Leakage marker	D00129291 (Calbiochem)	Batch 2342 S.A. 448 GBq/mmol Conc. 28.4 MBq/ml

**Batch was used for non-specific binding experiment and first transwell experiment.

Uptake of ^{14}C -JNJ-28431754 (3 and 30 μM corrected for non-specific binding) in HEK-293 parent and OATP1B3 cell lines in presence and absence of the reference inhibitor Rifampicin (25 μM).



Effect of JNJ-28431754 and reference OATP1B3 inhibitors Rifampicin and Cyclosporin A on the uptake of ^3H -estradiol-17 β -glucuronide in OATP1B3 HEK-293 cells (1 μM ; 0.5 min; n=3). JNJ-28431754 concentrations are corrected for non specific binding.



Canagliflozin was not substrate of or inhibitor of human OATP1B3 transporter. However, Canagliflozin was substrate to BCRP but not an inhibitor of human BCRP.

Study# FK10426: In Vitro Evaluation of Canagliflozin (JNJ-28431754) as an Inhibitor of UDP Glucuronosyltransferase Enzymes in Human Liver Microsomes

Canagliflozin is metabolized in humans to **M7** and **M5** by hepatic UDP-glucuronosyltransferase (UGT) enzymes **UGT1A9** and **UGT2B4**, respectively. Glucuronyl transferase is a liver microsomal enzyme involved in phase 2 metabolic reactions, forms a conjugate between the glucuronic acid

and canagliflozin, leading to M5 and M7 glucuronide metabolites. Glucuronide conjugates are known by the attachment site of glucuronic acid (N-, O-, COO-, S- and C- Glucuronides). Potential inhibition of these enzymes can lead to accumulation or the parent or direct the metabolism to an alternate pathway. Furthermore, UGT enzyme inhibition can affect the metabolism of other drug co-administered with canagliflozin.

The objective of this study was therefore to determine whether canagliflozin (JNJ-28431754) is capable of inhibiting microsomal glucuronide enzymes (UGT1A1, UGT1A4, UGT1A6, UGT1A9 and UGT2B7). This study was carried out for the sponsor by (b) (4) (April 2014). Human microsomal enzymes were prepared from pooled human liver tissue collected from 16 male and female donated liver (non-transplantable). Microsomal preparation was incubated with marker substrates in the presence or absence of canagliflozin. Troglitazone and Probenecid were used as positive control. Marker substrates for the UGT enzymes are listed in the table. The listed marker substrates were selected based on use in published studies, solubility and affinity of the marker substrate for the enzyme.¹²³

Enzymes	Substrates	Troglitazone Concentrations	
		Enzyme	Concentration Studied
UGT1A1	17 β -Estradiol 3- β -D-glucuronidation	UGT1A1	12 μ M
UGT1A4	Trifluoperazine glucuronidation	UGT1A4	3 μ M
UGT1A6	1-Naphthol glucuronidation	UGT1A6	20 μ M
UGT1A9	Propofol glucuronidation	UGT1A9	20 μ M
UGT2B7	Morphine 3- β -D-glucuronidation	UGT2B7	100 μ M

Troglitazone and probenecid inhibited UGT1A1, UGT1A4, UGT1A6, UGT1A9 and UGT2B7 enzymes, suggesting that the study was performing as expected. Canagliflozin inhibited UGT1A1 and UGT1A6 with IC₅₀ of 91 and 50 μ M. The IC₅₀ for UGT1A4, UGT1A9 and UGT2B7 were greater than 100 μ M.

¹ Ito M, Yamamoto K, Sato H, Fujiyama Y, Bamba T (2001) Inhibitory effect of troglitazone on glucuronidation catalyzed by human uridine diphosphateglucuronosyltransferase 1A6. *Eur J Clin Pharmacol* 56(12):893-895.

² Takeda S, Kitajima Y, Ishii Y, Nishimura Y, Mackenzie PI, Oguri K, Yamada H. (2006) Inhibition of UDP-glucuronosyltransferase 2b7-catalyzed morphine glucuronidation by ketoconazole: dual mechanisms involving a novel noncompetitive mode. *Drug Metab Dispos* 34(8):1277-1282.

³ Walsky RL, Bauman JN, Bourcier K, Giddens G, Lapham K, Negahban A, Ryder TF, Obach RS, Hyland R, Goosen TC. (2012) Optimized assays for human UDP-glucuronosyltransferase (UGT) activities: altered alamethicin concentration and utility to screen for UGT inhibitors. *Drug Metab Dispos* 40(5):1051-1065.

Inhibition of Drug-Metabolizing Enzymes

Test Article: JNJ-28431754

Type of Study	<i>In Vitro</i> UGT Inhibition		Study No. XT125113
Incubation System	Human Liver Microsomes (HLM) (in Tris HCl (100 mM), MgCl ₂ (10 mM), EDTA (1 mM), D-saccharic acid 1,4 lactone (0.1 mM))		
Analytical	LC-MS/MS Assay (Sciex API 2000 or 3000™, ESI) Monitoring Probe Substrate Metabolite Formation		
DIRECT INHIBITION (IC ₅₀ Determination)			
Enzyme	Enzyme Reaction	Direct Inhibition	
		IC ₅₀ (μM) ^a	Inhibition Observed at 100 μM (%) ^b
UGT1A1	17β-Estradiol 3-β-D-glucuronidation	91	45
UGT1A4	Trifluoperazine glucuronidation	> 100	42
UGT1A6	1-Naphthol glucuronidation	50	78
UGT1A9	Propofol glucuronidation	> 100	40
UGT2B7	Morphine 3-β-D-glucuronidation	> 100	NA

^a Average data (i.e., percent of control activity) obtained from duplicate samples for each test article concentration were used to calculate IC₅₀ values.

^b Inhibition observed (%) is calculated with the following formula (results are rounded to two significant figures):

Inhibition observed (%) = 100% – Percent solvent control.

NA = Not applicable. Inhibition was not observed at the highest concentration of JNJ-28431754 evaluated (100 μM) since rates of metabolite formation in the presence of JNJ-28431754 were higher than the control rates.

Inhibition of Drug-Metabolizing Enzymes

Test Article: JNJ-28431754

Type of Study	<i>In Vitro</i> UGT Inhibition		Study No. XT125113
Incubation System	Human Liver Microsomes (HLM) (in Tris HCl (100 mM), MgCl ₂ (10 mM), EDTA (1 mM), D-saccharic acid 1,4 lactone (0.1 mM))		
Analytical	LC-MS/MS Assay (Sciex API 2000 or 3000™, ESI) Monitoring Probe Substrate Metabolite Formation		
DIRECT INHIBITION (IC ₅₀ Determination)			
Enzyme	Enzyme Reaction	Direct Inhibition	
		IC ₅₀ (μM) ^a	Inhibition Observed at 100 μM (%) ^b
UGT1A1	17β-Estradiol 3-β-D-glucuronidation	91	45
UGT1A4	Trifluoperazine glucuronidation	> 100	42
UGT1A6	1-Naphthol glucuronidation	50	78
UGT1A9	Propofol glucuronidation	> 100	40
UGT2B7	Morphine 3-β-D-glucuronidation	> 100	NA

^a Average data (i.e., percent of control activity) obtained from duplicate samples for each test article concentration were used to calculate IC₅₀ values.

^b Inhibition observed (%) is calculated with the following formula (results are rounded to two significant figures):

Inhibition observed (%) = 100% – Percent solvent control.

NA = Not applicable. Inhibition was not observed at the highest concentration of JNJ-28431754 evaluated (100 μM) since rates of metabolite formation in the presence of JNJ-28431754 were higher than the control rates.

Since human plasma canagliflozin levels are significantly less than the IC₅₀ for UGT enzymes (above), canagliflozin is not expected to inhibit its own metabolism or the metabolism of other UGT metabolized drugs.

Study# FK5352: Study of the Potential Effects of JNJ-28431754 in the Induction of CYP1A2, CYP2C9, CYP2C19, and CYP3A4 in Cryo-Preserved Human Hepatocytes (amendment)

This study was designed to assess the potential induction ability of JNJ-28431754 (0.4, 2 and 10 μM) of human cytochrome P450 enzymes, CYP1A2, CYP3A4, CYP2C9 and CYP2C19. Cryo-preserved human hepatocytes from three donors were used in the study. Rifampicin and β-naphthoflavone, the commonly used inducers of the P450 enzymes were also evaluated in the study (CYP1A2, CYP3A4, CYP2C9 and CYP2C19) conducted at the sponsor's facility (June 2006). This study was reviewed earlier but revised in the new amendment to include data for the negative controls (Dec 2012).

Canagliflozin did not induce CYP1A2, CYP2C19, CYP2C9 or CYP3A4 relative to positive control or in comparison to the negative control. Both positive controls induced the target CYP enzymes suggesting that the assay worked as expected.

Induction of Cytochrome P450 mRNA in Cryo-Preserved Human Hepatocytes (BD-44) after 48-Hour Treatment (FK5352)

Treatment	Mean ± SEM			
	1A2	2C19	2C9	3A4
0.1% DMSO	1.03 ± 0.07	1.00 ± 0.04	1.00 ± 0.05	1.12 ± 0.12
β-Naphthoflavone 50 μM	165.72 ± 24.26	1.24 ± 0.10	1.62 ± 0.12	0.24 ± 0.04
Rifampin 10 μM	1.29 ± 0.11	2.45 ± 0.18	2.30 ± 0.11	37.89 ± 3.00
JNJ-28431754 0.4 μM	0.90 ± 0.07	1.10 ± 0.12	0.95 ± 0.11	1.47 ± 0.21
JNJ-28431754 2 μM	0.83 ± 0.12	1.17 ± 0.14	0.83 ± 0.07	1.56 ± 0.30
JNJ-28431754 10 μM	1.46 ± 0.19	1.34 ± 0.13	1.07 ± 0.08	3.35 ± 0.42

mRNA (Donor BD-44) - Relative Percent Positive Control Induction (Negative Control=0, Positive Control=100) (FK5352)

Treatment	Mean ± SEM			
	1A2	2C19	2C9	3A4
β-Naphthoflavone 50 μM	100.00 ± 14.73	16.61 ± 6.56	47.52 ± 9.27	-2.38 ± 0.12
Rifampin 10 μM	0.16 ± 0.07	100.00 ± 12.38	100.00 ± 8.56	100.00 ± 8.16
JNJ-28431754 0.4 μM	-0.07 ± 0.04	6.84 ± 8.26	-4.36 ± 8.67	0.95 ± 0.56
JNJ-28431754 2 μM	-0.12 ± 0.07	11.73 ± 9.91	-13.07 ± 5.68	1.20 ± 0.81
JNJ-28431754 10 μM	0.26 ± 0.12	23.31 ± 8.84	5.33 ± 5.89	6.07 ± 1.14

Positive control for CYP1A2 = 50 μM β-naphthoflavone; positive control for CYP2C9, 2C19 and CYP3A4 = 10 μM rifampin. Data expressed as mean ± standard error.

Study# FK7341: A Study of the Potential Effects of High Dose JNJ-28431754 in the Induction of CYP1A2, CYP2B6 and CYP3A4 in Cryopreserved Human Hepatocytes (amendment)

This study was designed to determine CYP enzymes (CYP1A2, CYP2B6 and CYP3A4) induction by much higher dose of canagliflozin than previously tested in cultures of cryopreserved human hepatocytes from donors Hu4013, (b) (6).

Human Hepatocyte Characterization

Lot	Age	Sex	Race	Cause of Death	Tobacco Use	Alcohol Use	Substance Use	Medical History
Hu4013	19	M	C	Anoxia	Yes	Yes	No	Normal
(b) (6)	68	F	C	ICH	Yes	Yes	No	Normal
(b) (6)	38	F	C	CVA	Yes	Yes	No	Normal

List of Probe Substrates

CYP	Substrate	Vendor	Catalog Number
1A2	Phenacetin		(b) (4)
2B6	Bupropion		
3A4	Testosterone		

List of Positive Control Inducers

CYP	Inducer	Vendor	Catalog Number
1A2	β -Naphthoflavone		(b) (4)
2B6	Phenobarbital		
3A4	Rifampicin		

List of Metabolite Reference Standards

CYP	Metabolite/Internal Standard	Vendor	Catalog Number
1A2	Acetaminophen		(b) (4)
2B6	OH-Bupropion		
3A4	6 β -OH-Testosterone		
1A2 – I.S.	Acetaminophen-d4		
2B6 – I.S.	OH-Bupropion-d6		
3A4 – I.S.	6 β -OH-Testosterone-d3		

This study was originally reviewed with the Canagliflozin NDA application. In the amendment (Dec 2012), the sponsor has provided text and tabulated data describing the canagliflozin data relative to negative control in addition to the positive control. There is no change in the conclusion. Canagliflozin at 75 μ M produced a significant increase 24-hr post LDH release, suggesting that canagliflozin was cytotoxic at 75 μ M thus data collected at the highest dose of canagliflozin is not relevant. Canagliflozin at all doses resulted in significant decrease in mRNA following 48 hrs of incubation. Canagliflozin concentrations up to 75 μ M did not induce CYP1A2, CYP2B6 nor CYP3A4 activity relative to β -naphthoflavone or compared to the negative control.

Pharmacokinetics: Induction of Drug-Metabolizing Enzymes

Test Article: JNJ-28431754

Type of Study: Induction of CYP's 1A2, 2B6 and 3A4 relative to positive control in primary cryopreserved human hepatocytes

Study No. FK7341

Method: Primary cryopreserved human hepatocytes were incubated with JNJ-28431754 and positive controls for 48 hours. Induction of CYP's 1A2, 2B6 and 3A4 were assessed by measuring individual activities using CYP selective probes.

Tabulated Results

Treatments ^a	1A2	2B6	3A4
Rifampicin 10 μ M (fold increase)	ND	ND	7-14
Phenobarbital 1 mM (fold increase)	ND	10-25	ND
β -Naphthoflavone 50 μ M (fold increase)	16-52	ND	ND
JNJ-28431754 3 μ M (% of positive control)	0.06 \pm 0.52	-2.23 \pm 1.09	-3.35 \pm 1.02
JNJ-28431754 15 μ M (% of positive control)	3.87 \pm 3.16	0.22 \pm 3.42	-5.96 \pm 1.79
JNJ-28431754 75 μ M (% of positive control)	-3.22 \pm 2.26	-7.26 \pm 3.49	-11.1 \pm 4.13

^a Range of fold-increase over vehicle-treated samples in cells from three different donors, unless otherwise indicated

ND = not determined

Study# FK7434: A Study of the Potential Effects of JNJ-41488525 (M7) and JNJ-41980874 (M5) in the Induction of CYP1A2, CYP2B6, and CYP3A4 in Human Hepatocytes (amendment)

This study was designed to determine the induction potential of high dose of M7 (canagliflozin glucuronide metabolite) on CYP enzymes, CYP1A2, CYP2B6 and CYP3A4 in culture of cryopreserved human hepatocytes. In Dec 2012 amendment, the sponsor has provided additional data for mRNA for both donor (b) (6). Again, there is no change in the conclusion.

Hepatocytes from donors treated with M7 or M5, rifampicin, phenobarbital and β -naphthoflavone were tested for their ability to induce CYP1A2, CYP2B6 and CYP3A4 mRNA. Canagliflozin o-glucuronide conjugates were not inducers of human hepatocyte CYP1A2, CYP2B6, or CYP3A4 enzyme. In contrast, positive controls, induced CYP1A2 by 23-88 fold, CYP2B6 by 7 to 10 fold and CYP3A4 by 8 to 14 fold, suggesting that the assay works as expected.

Pharmacokinetics: Induction of Drug-Metabolizing Enzymes

Test Article: JNJ-41488525 (M7)

Type of Study: Induction of CYP's 1A2, 2B6 and 3A4 relative to positive control in primary cryopreserved human hepatocytes

Study No. FK7434

Method: Primary cryopreserved human hepatocytes were incubated with JNJ-41488525 (M7) and positive controls for 48 hours. Induction of CYP's 1A2, 2B6 and 3A4 were assessed by measuring individual activities using CYP selective probes.

Tabulated Results

Treatments ^a	1A2	2B6	3A4
Rifampicin 10 μ M (fold increase)	ND	ND	8-13
Phenobarbital 1 mM (fold increase)	ND	7-9	ND
β -Naphthoflavone 50 μ M (fold increase)	36-83	ND	ND
JNJ-41488525 (M7) 3 μ M (fold increase)	1.5-2.1	1.0-1.5	1.6-1.7
JNJ-41488525 (M7) 15 μ M (fold increase)	0.9-1.3	0.7-0.8	0.5-1.2
JNJ-41488525 (M7) 75 μ M (fold increase)	0.8-1.1	0.6-0.7	0.7-0.8
JNJ-41488525 (M7) 3 μ M (% of positive control)	1.9 \pm 1.7	4.6 \pm 4.2	7.2 \pm 2.6
JNJ-41488525 (M7) 15 μ M (% of positive control)	0.4 \pm 0.7	-4.0 \pm 1.2	-3.1 \pm 4.7
JNJ-41488525 (M7) 75 μ M (% of positive control)	0.1 \pm 0.5	-5.5 \pm 0.4	-3.0 \pm 1.1

^a Range of fold-increase over vehicle-treated samples in cells from three different donors, unless otherwise indicated
ND = not determined

Pharmacokinetics: Induction of Drug-Metabolizing Enzymes

Test Article: JNJ-41980874 (M5)

Type of Study: Induction of CYP's 1A2, 2B6 and 3A4 relative to positive control in primary cryopreserved human hepatocytes

Study No. FK7434

Method: Primary cryopreserved human hepatocytes were incubated with JNJ-41980874 (M5) and positive controls for 48 hours. Induction of CYP's 1A2, 2B6 and 3A4 were assessed by measuring individual activities using CYP selective probes.

Tabulated Results

Treatments ^a	1A2	2B6	3A4
Rifampicin 10 μ M (fold increase)	ND	ND	9-14
Phenobarbital 1 mM (fold increase)	ND	9-10	ND
β -Naphthoflavone 50 μ M (fold increase)	23-88	ND	ND
JNJ-41980874 (M5) 3 μ M (fold increase)	4.5-8.1	1.1-1.3	0.9-1.2
JNJ-41980874 (M5) 15 μ M (fold increase)	1.1-1.6	0.7-0.8	0.8
JNJ-41980874 (M5) 75 μ M (fold increase)	0.8	0.5-0.7	0.6-0.7
JNJ-41980874 (M5) 3 μ M (% of positive control)	12 \pm 5.7	2.3 \pm 1.3	0.4 \pm 1.4
JNJ-41980874 (M5) 15 μ M (% of positive control)	0.5 \pm 0.3	-3.3 \pm 1.0	-2.3 \pm 0.6
JNJ-41980874 (M5) 75 μ M (% of positive control)	-0.4 \pm 0.4	-4.6 \pm 1.1	-3.2 \pm 0.3

^a Range of fold-increase over vehicle-treated samples in cells from three different donors, unless otherwise indicated
ND = not determined

Study# TOX10433: A 2-week repeated dose oral bridging PK study of JNJ-28431754- ZAE in the female rabbit

This 2-week bridging study was designed to determine the toxicokinetics of canagliflozin and its prominent glucuronide metabolites, M5 (JNJ-41980874) and M7 (JNJ-41488525) in non-pregnant rabbits.

Four New-Zealand White non-pregnant female rabbits were give daily oral dose of 160 mg/kg canagliflozin (batch#ZR600348PFA271) for two weeks (non-GLP, Oct 2012). Canagliflozin was prepared in 0.5% methocel (hydroxyethylcellulose) for oral gavage delivery (5 ml/kg). Blood samples were collected on Day 7 (101F, 102F, 103F and 104F) and Day 13 (103F and 104 F) at 0.5, 1, 2, 4, 7 and 24 hrs post canagliflozin dose. The study was conducted at the sponsor's facility at Beerse, Belgium. The TK analysis was carried out at Janssen Research site in Raritan, NJ.

Two rabbits (101F and 102 F) on Day 7 were sacrificed due to poor health marked by substantial weight loss (583 and 703 g of weight loss), inappetence and few feces. Rabbit 103F displayed similar adverse profile (356 g of wt. loss) during second week while one female (104F) had no abnormal findings.

Canagliflozin absorption was slow based on Tmax of 5 to 14 hrs. AUC exposure to canagliflozin, M5 and M7 metabolites on Day 7 were similar to Day 13. Exposure to canagliflozin was greater than M7 and substantially greater than M5 (Canagliflozin>M7>>M5). Canagliflozin exposure in non-pregnant rabbits was about 16 and 200x the M7 and M5 exposure, respectively. The AUC exposure to M7 was nearly 12 to 14x greater than M5 exposure in nonpregnant rabbits.

Day	Analyte	C _{max} (ng/mL)	t _{max} (h)	AUC _(0-24 h) (h*ng/mL)
7	JNJ-28431754	48500	9.00	862000
		(16300)	(10.0)	(562000)
7	JNJ-41980874 (M5)	282	4.75	4320
		(119)	(1.50)	(2810)
7	JNJ-41488525 (M7)	4520	4.75	60600
		(1930)	(1.50)	(20000)
13	JNJ-28431754	55100	14.0	858000
		NA	NA	NA
13	JNJ-41980874 (M5)	292	14.0	4430
		NA	NA	NA
13	JNJ-41488525 (M7)	3190	14.0	52800
		NA	NA	NA

NA: not applicable N = 4 Day 7 N = 2 Day 13

Combination with Metformin

Toxicokinetics of the combination with metformin comes from the 3-month toxicology and embryofetal development studies in rats. Oral administration of 300 mg/kg metformin had no notable impact on canagliflozin exposure in rats. However, canagliflozin appeared to increase metformin exposure by 1.4 to 1.8 fold.

In humans, oral administration of CanaMet-IR had no impact on canagliflozin AUC. However, food appeared to reduce peak plasma exposure for metformin (16%) in humans. Bioavailability of metformin in humans under fasting condition is about 55%. Metformin exposure in humans was not dose-proportional, likely due to poor absorption and tendency to be delayed by the presence of food in the GI. Metformin has high volume of distribution (654 ± 358 L) and negligible protein binding in humans and is cleared primarily by renal elimination (90%). Since renal clearance of metformin is approximately 3.5 times greater than creatinine clearance (tubular secretion), any change in renal clearance is likely to impact metformin clearance. Single dose of furosemide (a tubular transporter inhibitor) increased metformin C_{max} by 22% and AUC by 15% without significantly changing metformin renal clearance.

Canagliflozin (100 mg) had no impact on single dose of 1000 mg metformin AUC but C_{max} decreased by 14%. However, at high dose of 300 mg, canagliflozin increased metformin exposure by 20% with no change in C_{max} . The canagliflozin induced increase in metformin exposure at high doses appeared to be similar to the rat observation.

Geometric Mean Ratios and Their Associated 90% Confidence Intervals for Metformin Following Single-Dose Administration of Metformin (1,000 mg) with or Without Multiple Doses of Canagliflozin (100 mg qd) (Study NAP1004)

Parameter	Geometric Mean		Geometric Mean Ratio, % (90% CI)
	Metformin + Canagliflozin (Test) N=16	Metformin (Reference) N=18	
C_{max} , ng/mL	946	1,104	85.6 (72.9; 100.7)
AUC_{∞} , ng.h/mL	8,071	8,362	96.5 (81.9; 113.7)

N = maximum number of subjects with data.

Source: Mod5.3.3.4\NAP1004\Table 6

Geometric Mean Ratios and Their Associated 90% Confidence Intervals for Metformin (Single Dose of 2,000 mg) and Canagliflozin (Multiple Doses of 300 mg qd) Administered Alone and Following Co-Administration (Study DIA1028)

Parameter	Geometric Mean		Geometric Mean Ratio, % (90% CI)
	Metformin + Canagliflozin (Test) N=16	Metformin or Canagliflozin (Reference) N=16	
Metformin			
C_{max} , ng/mL	1,647	1,557	105.80 (93.17; 120.15)
AUC_{∞} , ng.h/mL	13,385	11,159	119.95 (107.68; 133.62)
Canagliflozin			
C_{max} , ng/mL	2,699	2,566	105.17 (95.78; 115.78)
AUC_{24h} , ng.h/mL	24,087	21,946	109.76 (104.96; 114.78)

N = maximum number of subjects with data.

Source: Mod5.3.3.4\DIA1028\Table 6, Table 8

5.2 Toxicokinetics

Oral gavage toxicology studies were carried with canagliflozin in combination with metformin prepared in 0.5% Methocel (hydroxypropyl methylcellulose) solution. Metformin had no notable impact on canagliflozin AUC and C_{max}. However, canagliflozin increased metformin AUC by 1.4 to 1.8 fold.

Daily Dose (mg/kg)	<u>4/300</u>		<u>20/300</u>		<u>100/300</u>		<u>100/0</u>	
No. of Animals	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10
Toxicokinetics:								
No. of Animals	M: 3	F: 3	M: 2	F: 3	M: 3	F: 3	M: 3	F: 3
AUC ₀₋₂₄ (ng·h/mL)								
JNJ-28431754								
Day 0	15600	12600	97600	90600	827000	3940000	757000	602000
Day 91	13500	16000	73500	90800	347000	434000	398000	489000
Metformin								
Day 0	68800	66400	78600	83700	69300	87700	-	-
Day 91	73300	81200	140000	113000	115000	119000	-	-

Plasma canagliflozin and metformin exposures in pregnant SD rats (Day 6 to 17) used in the embryofetal development study.

The C_{max}, t_{max} and AUC mean (SD) values of **JNJ-28431754** after multiple oral dosing

Group	Compound Dosed	Dose (mg eq./kg/day)	C _{max} (ng/mL)	t _{max} (h)	AUC _(0-24 h) (h*ng/mL)
Low	JNJ-28431754/Metformin	10/300	2780 (361)	2.67 (1.15)	46800 (3100)
Medium	JNJ-28431754/Metformin	30/300	9030 (1570)	3.33 (3.21)	151000 (34300)
High1	JNJ-28431754/Metformin	60/300	18400 (4420)	3.00 (3.46)	300000 (42300)
High2	JNJ-28431754	60/0	19100 (1310)	5.00 (1.73)	284000 (43700)

The C_{max}, t_{max} and AUC mean (SD) values of **metformin** after multiple oral dosing

Group	Compound Dosed	Dose (mg eq./kg/day)	C _{max} (ng/mL)	t _{max} (h)	AUC _(0-24 h) (h*ng/mL)
Low	JNJ-28431754/Metformin	10/300	21400 (4010)	2.67 (1.15)	152000 (34000)
Medium	JNJ-28431754/Metformin	30/300	25000 (1050)	2.00 (0.00)	148000 (8950)
High1	JNJ-28431754/Metformin	60/300	23200 (3720)	1.33 (0.577)	151000 (22500)
S (Metformin Control)	Metformin	0/300	19100 (6910)	1.67 (0.577)	97200 (7530)

6 General Toxicology

6.1 Single-Dose Toxicity

There were no single dose combination studies.

6.2 Repeat-Dose Toxicity

The 3-month (GLP-1) combination dose toxicology study was reviewed with the initial CanaMet FDC. Therefore, the summary of the study is discussed here for reference. In the study, SD rats were dosed daily with canagliflozin (4, 20 and 100 mg/kg, LD, MD and HD) in combination with 300 mg/kg metformin (JNJ-1158196-AAC). Separate groups of rats also received either vehicle (C), 100 mg/kg canagliflozin alone (HDC) or 300 mg/kg metformin alone (MET). The drugs were prepared in aqueous suspension containing 0.5% hydroxypropyl methylcellulose (Methocel) for daily oral gavage delivery.

Study title: TOX9667: 3-Month Repeated Dose Oral Toxicity Study of canagliflozin (JNJ-28431754-ZAE) Combined with Metformin (JNJ-1158196-AAC) in the Rat

Key Study Findings

- Three deaths in males of unknown cause (1 Control, 1 LD Cana and 1 MET)
- Canagliflozin dose-dependently decreased BW (5 to 15%) and BW gain (10 to 26%) in males, independent of metformin.
- Food intake increased in both males and females at all doses, independent of metformin.
- Minor hematology changes were noted in HD canagliflozin (independent of metformin).
- Dose-related decrease in plasma glucose (all doses) and increase in BUN, Trig in both sexes at MD and HD were independent of metformin.
- No change in testosterone or LH was noted in males (increase in LH was stipulated to have increased the incidence of testicular tumors in the rat carcinogenicity study).
- Canagliflozin increased urine glucose (all doses), volume, Ca and P and decreased urine pH in some MD and HD dose canagliflozin rats (independent of metformin).
- Significant increase in renal (all doses) and adrenal weight in HD rats.
- Histopath findings included trabecular hyperostosis (all doses) in femur/tibia, renal tubule (all doses) and pelvic dilatation (HD) were consistent with other canagliflozin studies. Metformin did not contribute to histopath findings.
- HD canagliflozin appeared to increase renal cell proliferation in the outer strip of outer medulla (OSOM) but not in cortex. There was no significant increase in KIM-1 positivity in the cortex or OSOM in HD rats.

- Co-administration of canagliflozin with metformin did not significantly alter the kinetics of canagliflozin (variable). However, canagliflozin (20/300 and 100/300 mg/kg, CanaMet) increased metformin AUC by 1.4 to 1.8 fold but had no effect on Cmax.
- Canagliflozin exposure was higher after single dose in males but after multiples doses, canagliflozin exposure in females was slightly higher. There was no gender effect on metformin exposure.

Reviewer Comments:

Consistent with prior toxicology studies with canagliflozin alone, the combination study demonstrated dose-dependently increased urine glucose, and volume leading to lower body weight and BW gain and plasma glucose in rats. The glucose/calorie loss led to significant increase in food consumption but not enough to compensate for the glucosuria dependent weight loss. Canagliflozin-induced increases in kidney weight were associated with tubule and pelvic dilatation and pelvic calculi (100 mg/kg). Bladder and tubule dilatations and renal pelvic hyperplasia (2 HD females) were attributed to exaggerated pharmacological activity of canagliflozin and adaptive response to excessive diuresis. There was some evidence of cell proliferation in the outer medullary renal strip in the HD rats. There was no clear sign of cortex cell proliferation or KIM-1 positivity compared to control. Dose-related hyperostosis and calciuria attributed to canagliflozin was not altered by co-administration with metformin. Since canagliflozin-induced increase in testicular tumors in the two year bioassay were attribute to increase in LH, the plasma testosterone and LH levels were measured in HD and control males. Canagliflozin did not significantly change levels of either hormone. Overall, the known adverse canagliflozin signals (renal tubule, pelvic dilatations, glycosuria, polyuria, calciuria and trabecular bone hyperostosis) were unaffected by metformin. Metformin had no impact on canagliflozin exposure in rats; however, canagliflozin increased metformin AUC (1.4 to 1.8x) but not Cmax. Similar observations were also noted in humans. Since metformin is primarily excreted by the kidney (filtered and excreted), the increase in metformin AUC is likely due to canagliflozin-associated decrease in renal clearance.

In summary, co-administration of 300 mg/day metformin had no impact on canagliflozin-induced renal dilatation and bone hyperostosis in rats. Metformin alone was not associated with any adverse signal in the 3-month rat study. The canagliflozin metformin fixed dose combination does not appear to present risks beyond those already identified for its components. Based on renal and bone findings, the lowest dose of canagliflozin, 4 mg/kg, was selected as NOAEL (<1x the 300 mg clinical dose based on AUC). The NOAEL for metformin was greater than 300 mg/kg ($\geq 9x$ the 300 mg clinical dose based on AUC).

7 Genetic Toxicology

Genotoxicity of canagliflozin alone was reviewed with the original NDA for canagliflozin (204042). Canagliflozin was not mutagenic with or without metabolic activation in the Ames assay. Canagliflozin was mutagenic in the in vitro mouse lymphoma assay with but not without metabolic activation. Canagliflozin was not mutagenic or clastogenic in an in vivo oral micronucleus assay in rats and an in vivo oral Comet assay in rats.

8 Carcinogenicity

No animal studies have been conducted with the combined products in INVOKAMET to evaluate carcinogenesis, mutagenesis, or impairment of fertility. The following data are based on the findings in studies with canagliflozin and metformin individually.

Canagliflozin carcinogenicity was evaluated in 2-year studies conducted in CD1 mice and Sprague-Dawley rats. Canagliflozin did not increase the incidence of tumors in mice dosed 10, 30, or 100mg/kg (\leq 14 times exposure from a 300mg clinical dose).

Testicular Leydig cell tumors, considered secondary to increased luteinizing hormone (LH), increased significantly in male rats at all doses tested (10, 30, and 100mg/kg). In a 12-week clinical study, LH did not increase in males treated with canagliflozin.

Renal tubular adenoma and carcinoma increased significantly in male and female rats dosed 100mg/kg, or approximately 12-times exposure from a 300mg clinical dose. In addition, adrenal pheochromocytoma increased significantly in males and numerically in females dosed 100 mg/kg. Carbohydrate malabsorption associated with high doses of canagliflozin was considered a necessary proximal event in the emergence of renal and adrenal tumors in rats. Clinical studies have not demonstrated carbohydrate malabsorption in humans at canagliflozin doses of up to 2-times the recommended clinical dose of 300mg.

Metformin hydrochloride carcinogenicity of metformin was evaluated in rats (104 weeks) and mice (91 weeks) at doses up to and including 900 mg/kg/day and 1500 mg/kg/day, respectively. These doses are both approximately 4 times the maximum recommended human daily dose of 2000 mg based on body surface area comparisons. No evidence of carcinogenicity with metformin was found in either male or female mice. Similarly, there was no tumorigenic potential observed with metformin in male rats. There was, however, an increased incidence of benign stromal uterine polyps in female rats treated with 900 mg/kg/day.

9 Reproductive and Developmental Toxicology

The sponsor had tested the effects of combination of canagliflozin and metformin on the embryofetal development in SD rats (Study #TOX9590). The study was reviewed by Dr. Minck. Briefly, oral administration of canagliflozin/metformin of 0/0, 10/300, 30/300, 60/300, 60/0, 0/300 mg/kg resulted in dose related reductions in body weight gain in all groups. The combination appeared to cause greater decreases in BW gain. Food intake was reduced with MD and HD combination and both single treatment groups during the initial treatment period. There was no effect on cesarean section endpoints. There were no significant effects on fetal development. Slight increases in the incidences of skeletal variations were observed in the rats given the combination of the two drugs (relative to control or canagliflozin alone). It should be noted that skeletal variations were also present in the controls but at a lower frequency. The skeletal effect was likely transient due and due to initial maternal weight loss.

Observation	Select Skeletal Observations					
	Dosage in mg/kg/day (J&J drug/metformin)					
	0/1	10/300	30/300	60/300	60/0	0/300
number evaluated (fetuses/litters)	126/18	148/22	128/20	142/22	131/20	149/21
Skull						
interparietal incomplete ossification	7/3	19*/9	16*/9	44***/15**	5/3	18*/8
supraoccipital incomplete ossification	1/1	4/3	8*/5	9**/6*	1/1	5/4
Vertebral column						
cervical centrum unossified	24/12	50**/16	54***/17	69***/20*	35/15	47**/15
ventral tubercle unossified	20/8	45**/14	25/8	44**/14	20/11	24/9
thoracic centrum bipartite or dumbbell shaped ^a	3	6	11	16	6	7
lumbar rudimentary rib unilateral or bilateral ^a	12	18	22	28	28	15
Rib wavy	3/2	14**/7	7/5	1/1	15**/8*	5/5
Sternum						
extra ossification/fusion	5/5	5/4	8/4	5/3	4/2	17*/11
rudimentary 5th	2/2	5/5	2/2	10*/8*	2/2	9*/6
Metatarsal reduced ossification (>1)	3/3	3/1	1/1	16**/8	3/3	3/2

a: observations combined across categories so only fetal incidence shown – stats not calculated

* p<0.05; **p<0.01; ***p<0.001

Toxicity	Sex	NOAEL (Dose)	AUC ₀₋₂₄ (µg·hr/mL)	Clinical Safety Margin (rat AUC/human AUC ^a)
Rat Developmental Toxicity - Combination	maternal	10/300	46.8	2x / 11x
	developmental	60/300	300	11x / 11x

^aHuman AUC is considered 26.1 µg·hr/mL based on the average AUC at 300 mg QD from study DIA1023 and 300 mg bid from study DIA1007

In summary, co-administration of canagliflozin with metformin decreased maternal BW and increased alterations / delayed in fetal ossification. These findings were similar to rat embryofetal study with canagliflozin alone suggesting that metformin had no meaningful impact on maternal or embryofetal changes. Co-administration of metformin had no impact canagliflozin exposure; however, canagliflozin increased metformin exposure by 1.5 fold in rats. The maternal NAOEL based on weight loss was of 10/300 mg/kg, approximately 2x and 11x the clinical dose of 300/2000 mg QD FDC based on AUC. The NOAEL for fetal skeletal variations was 60/300 mg/kg, approximately 11 times the clinical dose for the FDC on AUC basis. The reviewer concludes that the addition of metformin to canagliflozin had no meaningful impact on canagliflozin induced maternal and embryofetal developmental changes in rats.

11 Integrated Summary and Safety Evaluation

This is a resubmission of the canagliflozin and metformin IR fixed dose combination NDA 204353 in response to the complete response (CR) letter issued by the Division on Dec 11, 2013. The focus of the CR letter was the lack of data bridging the efficacy of once daily dose of the approved canagliflozin dose (100 and 300 QD) to the proposed twice-daily dose of canagliflozin the sponsor is seeking in the CanaMet-IR FDC. The CanaMet-IR FDC will be administered twice daily at strengths of 50/500, 150/500, 50/1000 and 150/1000 mg canagliflozin/metformin. The CR letter contained no nonclinical deficiency and the resubmission contains no new pivotal nonclinical studies relevant to the label. The resubmission; however, contains several amendments to previously submitted metabolism studies and few minor nonclinical studies with canagliflozin. The nonclinical studies were also submitted to other canagliflozin applications and dealt with canagliflozin alone.

Overall, the nonclinical reports in the resubmission found the following: A) canagliflozin is not a substrate for or inhibitor of the OATP1B3 transporter, B) canagliflozin is a substrate for BCRO but not an inhibitor of BCRP, C) canagliflozin at maximum clinical exposure does not inhibit UDP glucuronyltransferase enzyme and is therefore not expected to affect its own metabolism, D) canagliflozin and its metabolites, M5 and M7, do not induce the prominent CYP450 enzymes such as CYP1A2, CYP2C19, CYP2C9, or CYP3A which are responsible for the metabolism of many co-administered drugs, E) canagliflozin has no effect on C-peptide release or clearance in rats, and finally, G) nonpregnant rabbits form both M5 and M7, the two prominent canagliflozin metabolites found in humans.

The studies submitted in this resubmission contain no new information pertinent to the label. The nonclinical sections (8.1, 8.3, 8.4, 13.1 and 13.2) of the resubmitted label remain unchanged and acceptable. The original CanaMet IR application submitted on Dec 12, 2012 contained several nonclinical studies including the pivotal 3-month combination toxicology and combination dose reproductive toxicology study in rats. The combination study findings were similar to studies with canagliflozin alone. Addition of metformin had no additive adverse effect on canagliflozin's effect in SD rats. These pivotal studies were reviewed with the original FDC submission and support the approval of the CanaMet-IR FDC (NDA 204353). The new nonclinical amendments in the resubmission do not affect the pharmtox recommendation to the label. Pharmtox reviewer recommends approval of CanaMet IR FDC resubmission with no change in the pharmtox sections of the label.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

FRED K ALAVI

06/09/2014

The nonclinical data supports the approval of CanaMet-IR FDC. There is no change in the pharmtox section of the label in the resubmission.

TODD M BOURCIER

06/10/2014

I concur

**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: 204353
Supporting document/s: 000
Applicant's letter date: Dec 12, 2012
CDER stamp date: Dec 12, 2012
Product: Canagliflozin + metformin (CanaMet, INVOKAMET®)
Indication: Type 2 diabetes
Applicant: Janssen Pharmaceuticals Inc.
Review Division: DMEP
Reviewer: Fred K. Alavi, PhD
Supervisor/Team Leader: Todd Bourcier, PhD
Division Director: Mary Parks, MD
Project Manager: Abolade Adeolu

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 204353 are owned by Janssen Pharmaceuticals or are data for which Janssen Pharmaceuticals has obtained a written right of reference. Any information or data necessary for approval of NDA 204353 that Janssen Pharmaceuticals does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that Janssen does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA 204353.

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1 Executive Summary

1.1 Recommendations

1.1.1 Approvability: Nonclinical data supports approval of NDA 204353

1.1.2 Additional Nonclinical Recommendations: No new nonclinical studies are recommended.

1.1.3 Labeling Recommendations



2 Pages Of Draft Labeling Have Been Withheld As b4 (CCI/TS) Immediately Following This Page

1.2 Brief Discussion of Nonclinical Findings

The sponsor is seeking the approval of canagliflozin as a fixed dose combination with metformin immediate release (INVOKAMET®). Canagliflozin is a selective sodium-glucose co-transporter 2 (SGLT2) inhibitor, approved by the FDA in March 29, 2013. Metformin Immediate Release is a biguanide class insulin sensitizer antihyperglycemic agent approved by the FDA in 1995. The sponsor cross-references the FDA's prior finding of safety/efficacy for Glucophage (NDA 20-357) in support of the metformin component of the FDC with canagliflozin. Janssen is purchasing the metformin drug substance from (b) (4) under DMF (b) (4). Metformin DMF (b) (4) is approved under (b) (4) and marketed in US since Jan of 2012. The safety of the canagliflozin-metformin fixed dose combination is supported by a 3-Month rat combination toxicology and a rat embryofetal development (EFD) study.

Canagliflozin pharmacological activity and nonclinical toxicology studies have been reviewed under NDA 20404; this review will summarize the relevant nonclinical information in support of the fixed dose combination with metformin. As a selective SGLT2 inhibitor, the antihyperglycemic effect of canagliflozin comes from its ability to reduce the renal threshold for glucose reabsorption, leading to spillage of glucose to the urine. This process is independent of insulin. Although canagliflozin is approximately 160 fold more selective to renal SGLT2 (IC₅₀ 4.1 nM) than SGLT1 (IC₅₀ 664 nM), at high concentrations, it is capable of inhibiting intestinal SGLT1. Canagliflozin (10 µM) had no remarkable inhibitory effect (<50% inhibition) on any the receptors in the Cerep receptor screen assay.

Toxicology studies conducted with canagliflozin in support of the monotherapy NDA identified two key target organs: the renal system and bone. Both renal and bone signals were considered related to canagliflozin-induced increases in urinary glucose, calcium, and associated diuresis in animals. In rats, canagliflozin resulted in dose-dependent increases in renal tubule, pelvic, and urinary tract dilatation. Dilatation of the renal and urinary tract appear to be an adaptive response to chronic glucose-induced osmotic diuresis. Changes in bone appeared to have two distinct features: an acute effect marked by hyperostosis of the trabecular (spongy) bone seen only in young rapidly growing rats, and a chronic effect marked by decrease in compact bone density

observed across species including rats and dogs. The decrease in compact bone density and strength appeared to be related to weight loss and Ca mobilization/excretion. Since Ca is fundamental to bone density and strength, any potential canagliflozin induced imbalance in Ca absorption and excretion may have long-term consequence on bone density and strength in humans.

The carcinogenicity of canagliflozin (10, 30 and 100 mg/kg) was tested in 2-year lifetime exposure studies in CD-1 mice and SD rats. Canagliflozin did not cause any tumor in mice at doses up to 100 mg/kg (7 to 14x the clinical dose of 300 mg QD BID, based on AUC). In rats, canagliflozin resulted in renal tubule and adrenal tumors at top dose of 100 mg/kg (12x and 21x the MRHD) in both sexes and testicular Leydig cell tumors at all doses in males with no safety margin. Incidences of bladder tumors noted in 2 HD males and 1 LD and 3 HD females were not significant. Renal and adrenal tumors are frequently observed with compounds known to inhibit carbohydrate absorption from the intestine, such as acarbose, and preventing carbohydrate malabsorption can prevent their emergence. Intestinal SGLT1 inhibition of absorption of carbohydrates by high doses of canagliflozin therefore is considered the most likely mode of action. Testicular Leydig cell tumors observed at all doses is considered secondary to increased luteinizing hormone (LH) and greater density of LH receptors in male rats. No such change in LH was observed in humans.

Metformin hydrochloride (JNJ-1158496) is a biguanide class drug that lowers both basal and postprandial glucose by decreasing hepatic glucose production and intestinal glucose absorption. Metformin also improves insulin sensitivity by increasing peripheral glucose uptake and utilization. Metformin was approved by the FDA in 1995. There is extensive clinical experience with use of metformin in type 2 diabetic adults and pediatric population. Although both metformin IR and XR are available for adults, only metformin IR is available to children in US. Chronic oral administration of metformin to rodents has not been associated with any type of tumor.

Canagliflozin-Metformin Combination

The safety of the canagliflozin-metformin fixed dose combination is supported by a 3-month toxicology (CanaMet doses of 4/300, 20/300 and 100/300 mg/kg/day) and an embryofetal development (EFD) study in rats (CanaMet doses of 10/300, 30/300, 60/300, 60/0 and 0/300 mg/kg/day). As previously observed with canagliflozin alone, the combination produced a dose dependent increase in urinary glucose excretion and urine volume and calcium and trabecular bone hyperostosis (at all doses) in SD rats. Furthermore, canagliflozin increased renal tubule and pelvic dilatation as a likely consequence of adaptation to polyuria. The addition of 300 mg/kg metformin had no impact on the toxicity profile of canagliflozin in rats. Furthermore, metformin had no impact on canagliflozin exposure; however, canagliflozin increased metformin AUC exposure by as much as 1.8 fold. Since metformin is primarily excreted by renal filtration, the increase in metformin AUC is likely due to canagliflozin's effect on renal hemodynamics in rats. Based on renal tubule dilatation, a dose of 4 mg/kg constituted the no adverse effect level (NOAEL). There was no safety margin for canagliflozin's effect on renal tubule dilatation, with an exposure multiple of less than unity relative to the clinical dose of 300 mg on an AUC basis. At a NOAEL greater than 300mg/kg, the safety margin for metformin was approximately 9x the maximum clinical dose of 2000mg/kg/day.

In the EFD study, similar to canagliflozin alone, co-administration of canagliflozin with metformin decreased maternal body weight (all doses), and increased the incidence of skeletal variations. The skeletal variations (incomplete, reduced or extra ossification) were likely due to maternal weight loss, and likely represent delayed development. The maternal NOAEL based on weight loss was 10/300 mg/kg while NOAEL for fetal skeletal variation was 60/300 mg/kg. As noted in the 3-month combination study, metformin had no effect on canagliflozin AUC exposure; however, canagliflozin increased metformin exposure by 1.5 fold.

Safety margin for canagliflozin (JNJ-28431754) and metformin

Species	Daily Cana/Met Dose, mg/kg	Canagliflozin AUC ₀₋₂₄ , µg.h/ml	Metformin AUC ₀₋₂₄ , µg.h/ml	AUC Safety margins, (Animal /Human)	
				Cana	Metformin
13-Week rat study Combination dose, NOAEL 4 mg/kg	4/300	M:12.6 F:21.3	M:73 F:81	<0.7	6
	20/300	M:74.4 F:94.6	M:140 F:113	4	9
	100/300**	M:365 F:470	M:115 F:119	16	9
Rat Embryofetal Developmental study- Combination dose	10/300#	F: 47.8	F:152	2	11
	30/300	F: 151	F:148	6	11
	60/300##	F: 300	F:151	11	11
	60/00	F: 284	--	11	
	0/300	--	F:97.2		7
Maximum Clinical Dose: Canagliflozin, 300 mg QD Metformin, 2000 mg QD		26.1	13.4		

* NOAEL for canagliflozin (4 mg/kg)

** NOAEL for metformin (300 mg/kg)

Maternal NOAEL

Developmental NOAEL in the embryofetal developmental study

In summary, consistent with prior studies with canagliflozin alone, the 3-month combination study with metformin identified dose-dependent increases in the incidence of trabecular bone hyperostosis, and increased urinary glucose and calcium excretion with resultant renal tubule and pelvic dilatation. Addition of metformin did not improve or worsen the canagliflozin toxicology profile in rats. In the EFD study, administration of canagliflozin with or without metformin was associated with dose-dependent decrease in maternal body weight and fetal skeletal variations such as reduced or incomplete ossification. The transient variations in fetal skeletal ossification were also seen with metformin alone but at lower incidence. Since these changes are transient,

they are unlikely to be clinically meaningful. The nonclinical combination dose studies support the safety and approval of the FDC tablets.

2 Drug Information

2.1 Drug: INVOKAMET®

2.1.2 **Generic Name:** Canagliflozin+metformin

2.1.3 **Code Name for canagliflozin: JNJ-28431754 hemihydrate**

Code Name for metformin: JNJ-1158196-AAC (metformin)

Janssen is purchasing metformin from (b) (4) India Limited. A letter of authorization was provided by (b) (4) to reference DMF (b) (4)

2.1.4 Chemical Name

canagliflozin: (1S)-1,5-anhydro-1-C-[3-[[5-(4-fluorophenyl)-thienyl]methyl]-4-methylphenyl]-D-Glucitol

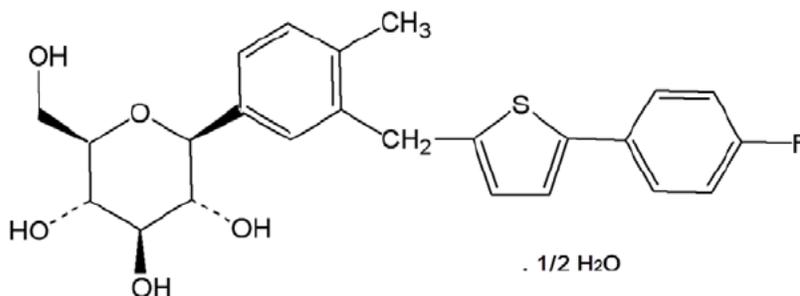
metformin: 1,1-Dimethylbiguanide hydrochloride, N,N-dimethylimidodicarbonimidic diamide monochloride,

2.1.5 Molecular Formula/Molecular Weight

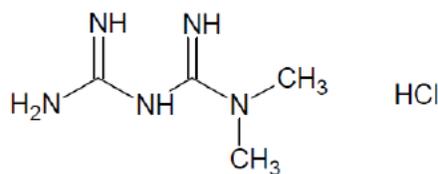
Canagliflozin hemihydrate: C₂₄H₂₅FO₅S. H₂O, MW 454.5

Metformin hydrochloride: C₄H₁₁N₅. HCl, MW 165.6

2.1.6 Structure of canagliflozin (JNJ-28431754-ZAE):



Structure of metformin (JNJ-1158496-AAC):



2.1.7 Pharmacologic class:

Canagliflozin: Sodium glucose co-transporter 2 (SGLT2) inhibitor
Metformin: Biguanide antihyperglycemic

2.2 Relevant IND/s, NDA/s, and DMF/s

Canagliflozin NDA 204042 (IND 76479, 11545, (b) (4))

Metformin MDF (b) (4) is approved and marketed in US (b) (4)

2.3 Clinical Formulation

2.3.1 Drug Formulation

Active ingredients: JNJ-28431754 hemihydrate and Metformin hydrochloride

50/500 mg CANA/MET IR FDC tablets are white, capsule-shaped, film-coated tablets with “CM” on one side and “155” on the other side (b) (4)

50/1000 mg CANA/MET IR FDC tablets are beige, capsule-shaped, film-coated tablets with “CM” on one side and “551” on the other side (b) (4)

150/500 mg CANA/MET IR FDC tablets are yellow, capsule-shaped, film-coated tablets with “CM” on one side and “215” on the other side (b) (4)

150/1000 mg CANA/MET IR FDC tablets are purple, capsule-shaped, film-coated tablets with “CM” on one side and “611” on the other side (b) (4)

Inactive ingredients: Microcrystalline Cellulose, (b) (4) (b) (4)
Croscarmellose Sodium, hypromellose, Magnesium Stearate, and coating
(b) (4) white, beige yellow and purple amounts are provided in table below).

Amounts of Coating Ingredient	(b)(4) Components per Tablet Quality reference	Amount per unit (mg/tablet)			
		85F18422 White Used in 50/500-mg tablets	85F97458 Beige Used in 50/1000-mg tablets	85F32547 Yellow Used in 150/500-mg tablets	85F90093 Purple Used in 150/1000-mg tablets
Polyvinyl alcohol-partially hydrolyzed	Ph. Eur./USP	(b)(4)			
Titanium dioxide	Ph. Eur./USP				
Macrogol (b)(4) PEG (b)(4)	Ph. Eur./NF				
Talc	Ph. Eur./USP				
Yellow iron oxide (b)(4)	EU Directive 2008/128/EC/NF				
Red iron oxide (b)(4)	EU Directive 2008/128/EC/NF				
Black iron oxide (b)(4)	EU Directive 2008/128/EC				
--	= Not present				

2.3.3 Comments on Impurities/Degradants of Concern in canagliflozin

Canagliflozin Impurities

The purity of canagliflozin (JNJ-28431754) as hemihydrate and an anhydrous were 97.7% (w/w) and 99.8%, respectively. All the impurities were less than the specification (b)(4), requiring no characterization. Inorganic impurities such as heavy metals were below (b)(4) ppm. The residual solvents were below ICH Q3C (R3) limits.

Canagliflozin batch #30178750 (98.5% purity) synthesized by (b)(4) was used in GLP toxicology, early clinical studies trials and in the stability studies. Batch #30936066 (98.5% purity) was manufactured by (b)(4) is considered to be the representative drug substance to be used in new clinical studies. The impurities and residual solvents in both batches were qualified by HPLC and assessed in the toxicology studies. The genotoxic degradant (b)(4) identified during the stability studies was present in the drug product and drug substance up to (b)(4) µg per 300 mg tablet). Further analysis found the genotoxic impurity to be a (b)(4). With significantly higher daily exposure to (b)(4) in the diet, the agency and the sponsor agreed to set the specifications to genotoxic (b)(4) impurity for 100 and 300 mg tablets are shown below. The specifications for the (b)(4) impurity was also applied to the FDC.

100mg drug product (tablet):	(b)(4) (b)(4) µg/day
300mg drug product (tablet):	(b)(4) (b)(4) µg/day

Metformin Impurities

The purity of metformin used in the study was 99.6% (w/w). All the impurities were less than the specification (b)(4), requiring no characterization. Inorganic impurities such as heavy metals were below (b)(4) ppm. The residual solvents were below ICH Q3C (R3) limits.

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2.4 Proposed Clinical Population and Dosing Regimen for canagliflozin +metformin

Type II diabetic patients, Canagliflozin-Metformin fixed dose combinations (FDC):
50/500, 50/1000, 150/500 and 150/1000 mg BID

2.5 Regulatory Background

Canagliflozin (JNJ-28431754) application was approved by the FDA on March 29, 2013. The sponsor is currently seeking regulatory approval of twice-daily orally administered fixed dose combination of canagliflozin with metformin hydrochloride immediate release (IR) at four dosage strengths (50/500, 150/500, 50/1000 and 150/1000 mg). The NDA application for the fixed dose combination submitted on Dec 20, 2012, relies primarily on the sponsors canagliflozin and existing metformin data, citing the Glucophage NDA under 505b2. The sponsor carried out additional nonclinical combination dose toxicology studies to support the FDC formulation. The additional toxicology studies consisted of a 3-month toxicology and embryofetal development study in rats administered the drug combination.

3.1 Studies Reviewed

- Study #TOX9582: 1-Month repeated dose oral toxicity study of JNJ-28431754 combined with metformin in the rat (non-GLP)
- Study # TOX9667: 3-Month Repeated Dose Oral Toxicity Study of JNJ-28431754-ZAE Combined with Metformin in the Rat (GLP)
- Study # BA1625 - Validation report for method BTM-1241-R0: Determination of metformin in rat plasma by LC/MS/MS

3.2 Studies Not Reviewed

none

3.3 Previous Reviews Referenced

Acute and subchronic toxicology studies with canagliflozin alone were reviewed under canagliflozin NDA 204042 / IND76479. Furthermore, the following combination dose developmental studies in rats were reviewed earlier under canagliflozin NDA thus only brief summary is provided for reference and labeling.

- Study # TOX9521: Developmental toxicity study with JNJ-28431754-ZAE Combined with Metformin in the Rat (non-GLP)
- Study # TOX9590: Developmental toxicity study with JNJ-28431754-ZAE Combined with Metformin in the Rat (GLP)

4 Pharmacology

4.1 Primary Pharmacology

Canagliflozin is a selective, reversible inhibitor of SGLT2 transporter. The IC₅₀s for Chinese Hamster Ovary cells expressing human SGLT2 and SGLT1 were 4.1 nM (~1.8 ng/ml) and 664 nM (~291 ng/ml), respectively. Although the IC₅₀ data suggests a 160 fold selectively to SGLT2, the intestinal exposure after oral dosing can reach sufficient concentration to inhibit SGLT1 in the small intestine in rats (evidenced by soft feces, bloating, gas) and possibly in humans (minor bloating), secondary to retention of excess glucose in the distal GI tract.

The IC₅₀ for rat SGLT2 and SGLT1 were 3.7 nM and 555 nM, respectively. In mice the IC₅₀s for the same transporters were 2 nM and >1000 nM, respectively. Canagliflozin appeared to be even more selective in mice than in rats. Based on IC₅₀ data, canagliflozin potency at inhibiting SGLT2 is similar among species tested (humans, rats and mice). However, in the in vivo studies, the effective dose of canagliflozin was approximately 10 fold lower in nondiabetic SD rats than in nondiabetic mice, suggesting that canagliflozin is likely to produce a pharmacological activity and toxicity in rats at 10x lower dose than in mice. This might explain the absence of renal, adrenal or bone findings in mice. Canagliflozin had notable activity at other receptors/transporters in the Cerep receptor screen assay.

Canagliflozin is extensively metabolized in humans to two prominent glucuronide metabolites (M5 and M7). The potential SGLT1 and SGLT2 inhibitory activities of the two metabolites were tested using Chinese hamster ovary cells expressing SGLT1 or SGLT2. Neither metabolite showed any meaningful inhibition of SGLT1 or SGLT2 transporters. The IC₅₀ for M5 and M7 were nearly 5000 times less than canagliflozin for SGLT2 and greater than 1000 fold for SGLT1 transporter. The C_{max} for M7 and M5 after single dose of 200 mg were 1159 and 703 ng/ml in normal subjects, respectively.

Overview of In Vitro Effects of Canagliflozin in Nonclinical Pharmacology Studies

In Vitro Effects	IC ₅₀ (nM)
Human SGLT2	4.2
Rat SGLT2	3.7
Human SGLT1	663
Rat SGLT1	555
Human SGLT3	Not detected
Human SGLT4	> 10,000
Human SGLT6	3,100
Human SMI1	> 10,000
HepG2 cells	> 50,000
Human primary adipocytes	6,800
Rat skeletal muscle myoblast	> 10,000
Mouse SGLT1	>1,000
Mouse SGLT2	2.0

In vitro inhibitory activity of canagliflozin and its major human glucuronide metabolites, M5 and M7	Human SGLT2	Human SGLT1
	IC ₅₀	IC ₅₀
Canagliflozin (JNJ-28431754)	4.2 nM	663 nM
Metabolite M5 (JNJ-41980874)	1014 nM	> 5000 nM
Metabolite M7 (JNJ-41488525)	7600 nM	>10000 nM

The effect of canagliflozin on renal glucose threshold was evaluated in various nonclinical animal models. Canagliflozin (1mg/kg) significantly decreased glucose re-absorption rate and reduced the glucose threshold in anesthetized Obese Zucker fatty rats (ZDF) instrumented with catheters and infused with glucose that raised blood glucose from 150 to 400 mg/dl. Although blood glucose was lower, urinary glucose excretion was dramatically increased in ZDF rats. Urine volume gradually increased with increase of urinary glucose. The table lists the in vivo effective dose of canagliflozin in various species. Although IC₅₀ for mouse and SD rat was similar, the effective dose was significantly lower in SD rats. Blood and Urine Glucose and Urine Volume Excreted Over 120 min after Administration of Single Oral Dose of Canagliflozin (1 mg/kg Dose) in Normoglycemic Rats.

4.2 Secondary Pharmacology

Canagliflozin-induced glucose excretion resulted in persistent polyuria in rats. Glucose loss also resulted in substantial weight loss, particularly in SD rats which were highly sensitive to the pharmacological effects of canagliflozin. It should be noted that in some instances animals had increased appetite, likely due to decrease in blood glucose. Since canagliflozin induced-glycosuria may increase urinary loss of electrolytes (Ca, Cl), the potential drug-related adverse activity may include changes in bone turnover rate and serum electrolyte imbalance.

4.3 Safety Pharmacology

Canagliflozin is pharmacologically active at renal SGLT2 and, at higher doses, at intestinal and renal SGLT1. Canagliflozin had no effects on CNS, cardiovascular, and respiratory systems.

Renal: Not evaluated

Gastrointestinal: Not examined

Abuse Liability: Not evaluated

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Canagliflozin

Canagliflozin is well absorbed in all the species tested. The bioavailability was highest in mice (88% to 137%) followed by 65% in dogs, 37% to 48% in monkeys and about 35% in rats. The t_{1/2} after single dose ranged from 2.7 hrs in mice to 6.2 to 7.5 hrs in rats, dogs and monkeys. In the multiple dose studies, t_{1/2} in rats ranged from 6.3 to 69 hrs and in dogs from 8 hr to 14 hrs.

Plasma clearance ranged from low in mice, rats and monkeys to extremely low in dogs. The Vd was 1.4 to 2.3 L/kg in mice, monkeys and rats to 0.6 to 0.76 in dogs suggesting that in dogs.

Repeated administration of canagliflozin increased exposure by 2.5 fold in dogs suggesting drug accumulation. Canagliflozin exposure tended to be higher in females in mice, rats and dogs. Canagliflozin had high plasma protein binding in all species

including humans, ranging from 98.2 to 99.0%. The human serum albumin and human α -1 acid protein was 97.3 and 39.8%, respectively.

Canagliflozin is metabolized primarily by the hepatic UDP-glucuronosyltransferase enzymes (UGT) to M7 and M5. Cytochrome P450 enzymes appeared to have minimal role in metabolism of canagliflozin except M9 (CYP3A4 and CYP2D6). The two prominent metabolites (M5 and M7) were not present in the *in-vitro* mouse, rat, dog and human hepatocyte metabolism studies. Qualitatively, the *In-vivo* metabolic profile of canagliflozin was similar to those in animal models. Quantitatively, the exposures to two prominent human M5 and M7, were significantly lower in animals than in humans. However, since both M5 and M7 are inactive and highly water soluble and readily excreted into urine and feces, there is no safety concern. Furthermore, analysis of bile in rats found far greater exposure to M7 (12%) and M5 (4%) than observed in plasma in the TK studies suggesting that low plasma levels might be due to rapid hydrolysis of M7 and M5 back to parent drug in rats.

Metabolic induction studies found no notable *in vitro* CYP enzyme induction. PK parameters of canagliflozin appear to be gender dependent in rats and dogs. Canagliflozin exposure was moderately higher (up to 2-fold) in female mice, rats and dogs. Whether this was due to difference in CYP enzymes in animals is not clear. There is some *in vitro* human data suggesting that JNJ-28431754 may inhibit CYP3A4 and CYP2C9. Canagliflozin may act as substrate and inhibitor of MDR1 (IC₅₀ of 8.5 μ g/mL) and MRP2 (IC₅₀ of 9.5 μ g/mL,) transporters. Whether these transporters are contributing to the difference in drug exposure observed in animals is unknown.

Combination with Metformin

Toxicokinetics of the combination with metformin comes from the 3-month toxicology and embryofetal development studies in rats. Oral administration of 300 mg/kg metformin had no notable impact on canagliflozin exposure in rats. However, canagliflozin appeared to increase metformin exposure by 1.4 to 1.8 fold.

In humans, oral administration of CanaMet had no impact on canagliflozin AUC. However, food appeared to reduce peak plasma exposure for metformin (16%) in humans. Bioavailability of metformin in humans under fasting condition is about 55%. Metformin exposure in humans was not dose-proportional, likely due to poor absorption and tendency to be delayed by the presence of food in the GI. Metformin has high volume of distribution (654 \pm 358 L) and negligible protein binding in humans and is cleared primarily by renal elimination (90%). Since renal clearance of metformin is approximately 3.5 times greater than creatinine clearance (tubular secretion), any change in renal clearance is likely to impact metformin clearance. Single dose of furosemide (a tubular transporter inhibitor) increased metformin C_{max} by 22% and AUC by 15% without significantly changing metformin renal clearance.

Canagliflozin (100 mg) had no impact on single dose of 1000 mg metformin AUC but C_{max} decreased by 14%. However, at high dose of 300 mg, canagliflozin increased metformin exposure by 20% with no change in C_{max}. The canagliflozin induced increase in metformin exposure at high doses appeared to be similar to the rat observation.

Geometric Mean Ratios and Their Associated 90% Confidence Intervals for Metformin Following Single-Dose Administration of Metformin (1,000 mg) with or Without Multiple Doses of Canagliflozin (100 mg qd) (Study NAP1004)

Parameter	Geometric Mean		Geometric Mean Ratio, % (90% CI)
	Metformin + Canagliflozin (Test) N=16	Metformin (Reference) N=18	
C _{max} , ng/mL	946	1,104	85.6 (72.9; 100.7)
AUC ₀₋₂₄ , ng.h/mL	8,071	8,362	96.5 (81.9; 113.7)

N – maximum number of subjects with data.

Source: Mod5.3.3.4\NAP1004\Table 6

Geometric Mean Ratios and Their Associated 90% Confidence Intervals for Metformin (Single Dose of 2,000 mg) and Canagliflozin (Multiple Doses of 300 mg qd) Administered Alone and Following Co-Administration (Study DIA1028)

Parameter	Geometric Mean		Geometric Mean Ratio, % (90% CI)
	Metformin + Canagliflozin (Test) N=16	Metformin or Canagliflozin (Reference) N=16	
Metformin			
C _{max} , ng/mL	1,647	1,557	105.80 (93.17; 120.15)
AUC ₀₋₂₄ , ng.h/mL	13,385	11,159	119.95 (107.68; 133.62)
Canagliflozin			
C _{max} , ng/mL	2,699	2,566	105.17 (95.78; 115.78)
AUC ₀₋₂₄ , ng.h/mL	24,087	21,946	109.76 (104.96; 114.78)

N – maximum number of subjects with data.

Source: Mod5.3.3.4\DIA1028\Table 6, Table 8

5.2 Toxicokinetics

Oral gavage toxicology studies were carried with canagliflozin in combination with metformin prepared in 0.5% Methocel (hydroxypropyl methylcellulose) solution. Metformin had no notable impact on canagliflozin AUC and C_{max}. However, canagliflozin increased metformin AUC by 1.4 to 1.8 fold.

Daily Dose (mg/kg)	4/300		20/300		100/300		100/0	
No. of Animals	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10
Toxicokinetics:								
No. of Animals	M: 3	F: 3	M: 2	F: 3	M: 3	F: 3	M: 3	F: 3
AUC ₀₋₂₄ (ng-h/mL)								
JNJ-28431754								
Day 0	15600	12600	97600	90600	827000	3940000	757000	602000
Day 91	13500	16000	73500	90800	347000	434000	398000	489000
Metformin								
Day 0	68800	66400	78600	83700	69300	87700	-	-
Day 91	73300	81200	140000	113000	115000	119000	-	-

Plasma canagliflozin and metformin exposures in pregnant SD rats (Day 6 to 17) used in the embryofeta development study.

The C_{max} , t_{max} and AUC mean (SD) values of JNJ-28431754 after multiple oral dosing

Group	Compound Dosed	Dose (mg eq./kg/day)	C_{max} (ng/mL)	t_{max} (h)	AUC _(0-24 h) (h*ng/mL)
Low	JNJ-28431754/Metformin	10/300	2780 (361)	2.67 (1.15)	46800 (3100)
Medium	JNJ-28431754/Metformin	30/300	9030 (1570)	3.33 (3.21)	151000 (34300)
High1	JNJ-28431754/Metformin	60/300	18400 (4420)	3.00 (3.46)	300000 (42300)
High2	JNJ-28431754	60/0	19100 (1310)	5.00 (1.73)	284000 (43700)

The C_{max} , t_{max} and AUC mean (SD) values of metformin after multiple oral dosing

Group	Compound Dosed	Dose (mg eq./kg/day)	C_{max} (ng/mL)	t_{max} (h)	AUC _(0-24 h) (h*ng/mL)
Low	JNJ-28431754/Metformin	10/300	21400 (4010)	2.67 (1.15)	152000 (34000)
Medium	JNJ-28431754/Metformin	30/300	25000 (1050)	2.00 (0.00)	148000 (8950)
High1	JNJ-28431754/Metformin	60/300	23200 (3720)	1.33 (0.577)	151000 (22500)
S (Metformin Control)	Metformin	0/300	19100 (6910)	1.67 (0.577)	97200 (7530)

6 General Toxicology

6.1 Single-Dose Toxicity

There were no single dose combination studies.

6.2 Repeat-Dose Toxicity

Study title: TOX9667: 3-Month Repeated Dose Oral Toxicity Study of canagliflozin (JNJ-28431754-ZAE) Combined with Metformin (JNJ-1158196-AAC) in the Rat

Study no.:	TOX9667
Study report location:	Janssen Research & Development, A division of Janssen Pharmaceuticals, Belgium
Conducting laboratory and location:	Drug Safety Sciences, Beerse site Turnhoutseweg 30, B-2340 Beerse, Belgium
Date of study initiation:	June 10, 2010
GLP compliance:	yes
QA statement:	Yes
Drug, lot #, and % purity:	Canagliflozin lot# ZR600348PFA091, 97.5% purity Metformin lot# 0910721, 99.6% purity was obtained from a commercial source used for clinical studies (b) (4)

Key Study Findings

- Three deaths in males of unknown cause (1 C, 1 LD Cana and 1 Met)
- Canagliflozin dose-dependently decreased BW (5 to 15%) and BW gain (10 to 26%) in males, independent of metformin (Met).
- Food intake increased in both males and females at all doses, independent of Met.
- Minor hematology changes were noted in HD canagliflozin, independent of Met.
- Dose-related decrease in plasma glucose (all doses) and increase in BUN, Trig in both sexes at MD and HD were independent of Met.
- No change in testosterone or LH was noted in males (increase in LH was stipulated to have increased the incidence of testicular tumors in the rat carcinogenicity study).
- Canagliflozin increased urine glucose (all doses), volume, Ca and P and decreased urine pH in some MD and HD dose canagliflozin rats (independent of metformin).
- Significant increase in renal (all doses) and adrenal weight in HD rats.
- Histopath findings included trabecular hyperostosis (all doses) in femur/tibia, renal tubule (all doses) and pelvic dilatation (HD) were consistent with other canagliflozin studies. Metformin did not contribute to histopath findings.

- HD canagliflozin appeared to increase renal cell proliferation in the outer strip of outer medulla (OSOM) but not in cortex. There was no significant increase in KIM-1 positivity in the cortex or OSOM in HD rats.
- Co-administration of canagliflozin with metformin did not significantly alter the kinetics of canagliflozin (variable). Canagliflozin (20/300 and 100/300 mg/kg, CanaMet) increased metformin AUC by 1.4 to 1.8 fold but had no effect on C_{max}.
- Canagliflozin exposure was higher after single dose in males but after multiples doses, canagliflozin exposure in females was slightly higher. There was no gender effect on metformin exposure.

Reviewer Comments:

Consistent with prior toxicology studies with canagliflozin alone, the combination study demonstrated dose-dependently increased urine glucose, and volume leading to lower body weight and BW gain and plasma glucose in rats. The glucose/calorie loss led to significant increase in food consumption but not enough to compensate for the glucosuria dependent weight loss. Canagliflozin-induced increases in kidney weight was associated with tubule and pelvic dilatation and pelvic calculi (100 mg/kg). Bladder and tubule dilatations and renal pelvic hyperplasia (2 HD females) were attributed to exaggerated pharmacological activity of canagliflozin and adaptive response to excessive diuresis. There was some evidence of cell proliferation in the outer medullary renal strip in the HD rats. There was no clear sign of cortex cell proliferation or KIM-1 positivity compared to control.

Dose-related hyperostosis and calciuria attributed to canagliflozin was not altered by co-administration with metformin. Since canagliflozin-induced increase in testicular tumors in the two year bioassay were attribute to increase in LH, the plasma testosterone and LH levels were measured in HD and control males. Canagliflozin did not significantly change levels of either hormone. Overall, the known adverse canagliflozin signals (renal tubule, pelvic dilatations, glycosuria, polyuria, calciuria and trabecular bone hyperostosis) were unaffected by metformin. Metformin had no impact on canagliflozin exposure in rats; however, canagliflozin increased metformin AUC (1.4 to 1.8x) but not C_{max}. Similar observations were also noted in humans. Since metformin is primarily excreted by the kidney (filtered and excreted), the increase in metformin AUC is likely due to canagliflozin-associated decrease in renal clearance.

In summary, co-administration of metformin 300 mg/day had no impact on canagliflozin-induced renal dilatation and bone hyperostosis in rats. Metformin alone was not associated with any adverse signal in the 3-month rat study. The canagliflozin metformin fixed dose combination does not appear to present risks beyond those already identified for its components. Based on renal and bone findings, the lowest dose of canagliflozin, 4 mg/kg, was selected as NOAEL (<1x the MRHD based on AUC). The NOAEL for metformin was greater than 300 mg/kg (≥ 9x the MRHD based on AUC).

Methods

Doses: **4/300, 20/300 and 100/300 mg/kg Canagliflozin/Metformin and 100 mg/kg Canagliflozin alone**

Frequency of dosing: Daily

Route of administration: Oral gavage

Dose volume: 5 ml/kg/day

Formulation/Vehicle: 0.5% w/v aqueous Methocel

Species/Strain: SD rats (CrI:CD® (SD) IGS), (b) (4)

Number/Sex/Group: 10/sex/dose (wire mesh housing, 5/cage/sex,)

Age: 5 weeks old arrival, 6 weeks old at the time of treatment

Weight: 136-217 g for female and male rats

Satellite groups: 3 /sex/group (housed 3/cage/sex)

Unique study design: Additional analysis included measurements of luteinizing hormone (LH) and testosterone. Renal cortex and outer stripe of the outer medulla (OSOM) cell proliferation (PCNA) was also evaluated. KIM-1 was measured to assess tubular injury in male rats at the end of 3 months.

Deviation from study protocol: Minor, not relevant.

<u>Dosage groups (colour code)</u>	<u>Identity number (computer number) of rats</u>	
	<u>Males</u>	<u>Females</u>
V: Vehicle (blue) Dosage: 0 mg eq./kg/day Concentration: 0 mg eq./ml Volume: 5 ml/kg/day	1 - 10	201 - 210
L : Low (red) Dosage: 4/300 mg eq./kg/day Concentration:0.8/60 mg eq./ml Volume: 5/5 ml/kg/day	21 - 30	221 - 230
M :Medium (yellow) Dosage:20/300 mg eq./kg/day Concentration:4/60 mg eq./ml Volume: 5/5ml/kg/day	41 - 50	241 - 250
H1 :High 1(green) Dosage:100/300 mg eq./kg/day Concentration:20/60 mg eq./ml Volume: 5/5 ml/kg/day	61 - 70	261 - 270
H2 :High 2(green + /) Dosage: 100/- mg eq./kg/day Concentration:20/- mg eq./ml Volume: 5/- ml/kg/day	81 - 90	281 - 290
ME: Metformin (white) Dosage: -/300 mg eq./kg/day Concentration:-/60 mg eq./ml Volume: -/5 ml/kg/day	101 - 110	301 - 310

Additional description of study protocol:

Dose selection was based on the 1-month non-GLP combination dose study (4/300, 20/300 and 100/300 mg/kg CanaMet in rats (appendix). There were no unexpected findings in the 1-month study. Metformin dose was based on published literature where 300 mg/kg has been shown to be well tolerable by rats while providing moderate safety multiples. In the study, canagliflozin doses produced previously identified renal and bone signals. Testosterone levels were decreased by 100 mg/kg canagliflozin by the end of the 4-week study. LH levels were however below the detection limits in both control and high dose canagliflozin group. Co-administration of metformin did not worsen or improve any of the canagliflozin induced adverse signals in rats. There were no notable adverse findings with metformin alone in rats.

Observational endpoints/timing

Clinical Findings	Once a day
Body weights	Weekly
Food consumption	Weekly
Ophthalmoscopy	Day -1, and at the end of the treatment.
EKG	Not done
Hematology	At the end of the study
Clinical chemistry	Under fasted conditions at the end of the study
Urinalysis	Under fasted conditions at the end of the study
Gross pathology	At the end of the study
Organ weights	At the end of the study
Histopathology	Adequate Battery: yes (x), no () Peer review: yes (x), no ()
Other	Additional analysis included measurements of LH and testosterone.

Observations and Results**Mortality**

- There were 3 moribund sacrifice, a LD male (#30, Day 49, brain infarct), control male (#1, Day 65, cause of death unknown) and a metformin alone male (#107, Day 67, cause of death unknown).
- Deaths were accidental since there was no dose-dependency or deaths at higher doses.

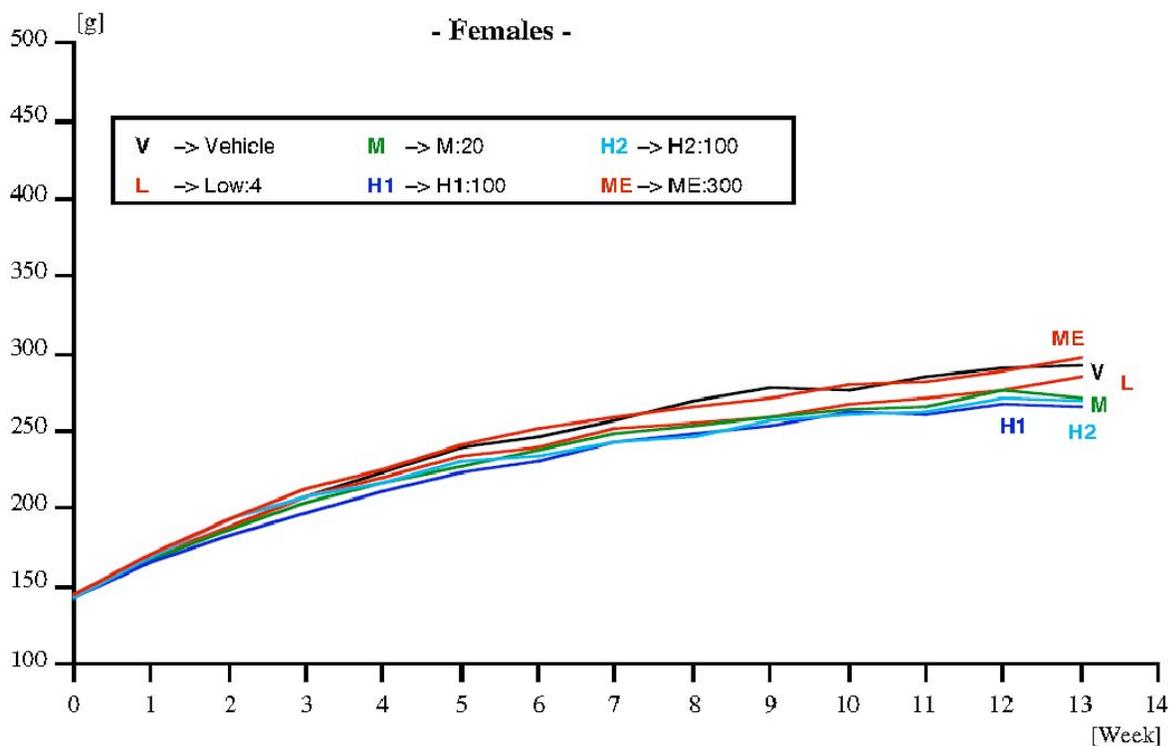
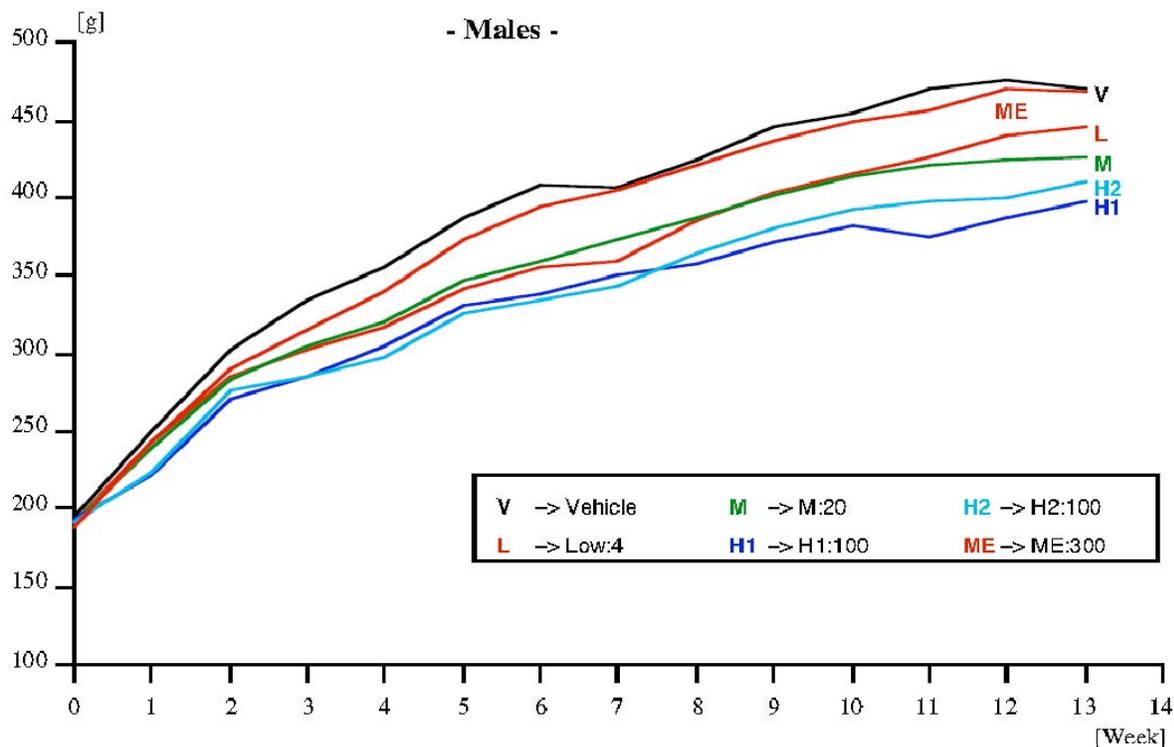
Clinical Signs

- All rats treated with canagliflozin and CanaMet resulted in soft feces in all male rats.

Body Weights

- Canagliflozin and CanaMet produced a dose-dependent decrease in BW (5 to 15%) and BW gain (10 to 26%) in males at all doses. The effect of HD canagliflozin and HD CanaMet were similar in these rats. Metformin alone had no notable effect on BW in males suggesting that the decrease in BW was solely due to canagliflozin-induced loss of urinary glucose.

- There was no meaningful change in BW or BW gain in female rats.



Feed Consumption

- There was a dose-related increase in food intake in all canagliflozin treated rats in males (up to 32%) and in females (up to 21%) to compensate for urinary loss of glucose/calories. Metformin alone did not affect food intake.

Ophthalmoscopy: Ophthalmic examination of conjunctiva, sclera, cornea, anterior chamber, iris, and fundus before and at the end of the treatment found no drug related ophthalmic changes in the study.

Hematology

- Changes in hematology parameters were generally minor.
- HD canagliflozin and CanaMet resulted in a small increase in mean red cell volume was in both sexes.
- HD canagliflozin slightly decreased thrombocytes and increased reticulocytes in males.
- HD canagliflozin and CanaMet caused a small decrease in APTT and prothrombine. Metformin alone had no such effect, suggesting that hematology and coagulatory changes may have been due to canagliflozin-induced diuresis.

Clinical Chemistry

- Per pharmacology, canagliflozin produced a dose-dependent decrease in plasma glucose, increase in BUN and triglyceride, similar to previous toxicology studies with canagliflozin alone.
- HD canagliflozin increased ALP in males
- Metformin alone had no notable effect.

Report Title: 3-Month Repeated Dose Oral Toxicity Study of JNJ-28431754-ZAE combined with Metformin in the Rat

Daily Dose (mg/kg)	0(Vehicle)		4/300		20/300		100/300		100/0		
No. of Animals	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10	
Serum Chemistry^a											
Inorg. phosphorus (mg/dl)	6.8	-	-	-	1.074 *	-	1.147 **	-	-	-	-
Total protein (g/dl)	-	6.5	-	-	-	-	-	0.923 **	-	-	0.938 *
Glucose (mg/dl)	118	109	0.695 ***	0.826 ***	0.559 ***	0.569***	0.517 ***	0.505 ***	0.483 ***	0.505***	
Triglycerides (mg/dl)	39	35	-	1.343 **	2.103 ***	1.457 **	3.128 ***	2.514 ***	2.308 ***	1.686 **	
Urea nitrogen (mg/dl)	14.5	16.4	1.179	-	1.607 ***	1.421 **	1.793 ***	1.463 ***	2.248 ***	1.848***	
Creatinine (mg/dl)	-	0.31	-	-	-	0.806 **	-	0.677 ***	-	0.871 **	
Total bilirubin (mg/dl)	-	0.11	-	-	-	0.636***	-	0.364 ***	-	0.636***	
Alk. phosphatase (U/l)	86	-	-	-	-	-	1.500 **	-	1.640 ***	-	

^a At end of dosing period. For vehicles, group means are shown. For treated groups, multiples of vehicles are shown. Statistical significance is based on actual data (not on the multiples of vehicles).

- No noteworthy findings

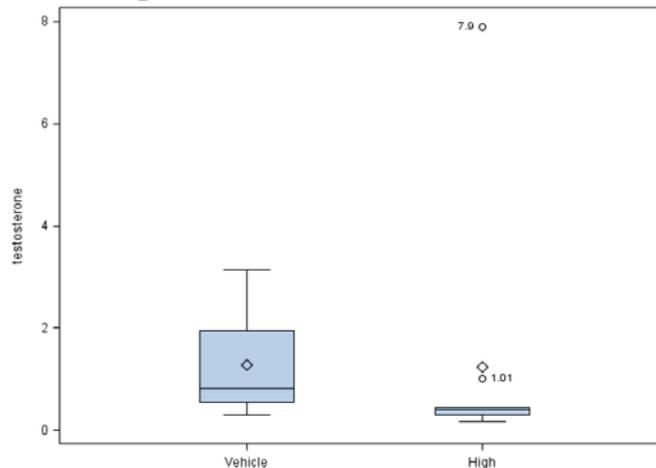
* - p<0.05, ** - p<0.01, ***-p<0.001

Serum Testosterone in male rats treated with canagliflozin

In addition to standard clinical chemistry parameters, drug effect on testosterone was analyzed (non-GLP) in nine control and HD canagliflozin rats (100 mg/kg) in-house and at (b) (4). The in-house analysis of terminal samples found no significant change in testosterone or LH in male rats. Metformin alone had no effect on testosterone in rats. In the 4-WK study with the combination, testosterone levels decreased in the canagliflozin treated rat; however, the LH levels were below detection limits and unaffected by the treatment. The investigators at the (b) (4) lab concluded that sample collection or prolonged storage may have played a role.

		Dosage Groups (mg eq / kg)	
		MALES	
Parameter	Unit	Vehicle	H2:100
Testosterone		2.98	3.09
	nmol/l	(0.96)	(2.33)
Luteinizing hormone		0.78	0.78
	ng/ml	(0.00)	(0.00)

Analysis of testosterone at (b) (4) found no meaningful change in testosterone (1.27 ng/ml in control vs. 1.2 ng/ml Canagliflozin). When the two outliers (7.9 and 1.01 ng/ml) based on box plot (below) were removed, the testosterone levels were significantly lower in HD canagliflozin males than control.



The normal range for serum testosterone in rats (n=10) measured with Testosterone Rat/mouse ELISA assay is shown below.

	Range (ng/ml)	Mean (ng/ml)
Male ♂	0.66 – 5.4	3.06
Female ♀	0.11 – 0.31	0.21

In male mice (n=10), the testosterone concentrations ranges between 1.7 and 14.4 ng/ml with mean value of 6.78 ng/ml (measured between 11 AM and 3 PM).

	Range (ng/ml)	Mean (ng/ml)
Male mice ♂	1.7 – 14.4	6.78

Serum LH analysis

There was no detectable serum LH in control or canagliflozin treated male rats at the end of the 3-month study, similar to the 1-month dose ranging study. It is not clear why LH levels were below detection limit since one would have expected an increase in LH in response to decrease in testosterone levels in the canagliflozin group in at least the 1-month study. Analytical methods used in LH analysis were unlikely to have been a factor since LH was undetectable whether measured by the sponsor or (b) (4). Whether poor sampling or storage had played a role is not clear. Since hormonal measurements were intentionally added to the 1- and 3-month study design, one would have expected greater care in handling of the serum samples. In summary, the low or undetectable levels of LH clearly did not support the hypothesis that canagliflozin-induced increase in testicular leydig cell tumors were caused by the elevation in serum LH in male rats.

Urinalysis

- As previously observed with canagliflozin alone, canagliflozin in combination with metformin dose-dependently increased urine glucose, volume, Ca, inorganic phosphate, magnesium and urinary proteins in male and female rats.
- Metformin alone had no effect on any of the parameters in either gender.

Parameter	Unit	Dosage Groups (mg eq / kg)					
		Vehicle	Low:4	M:20	H1:100	H2:100	ME:300
MALES							
Specific gravity		1.029 (0.005)	1.048 (0.001) *	1.048 (0.001) **	1.046 (0.002) *	1.044 (0.002)	1.030 (0.006)
pH		7.3 (0.2)	6.8 (0.2)	6.1 (0.1) ***	5.7 (0.2) ***	5.7 (0.2) ***	7.2 (0.2)
Volume	ml	15.3 (3.2)	17.4 (2.0)	25.1 (3.1) *	30.8 (2.3) ***	36.1 (3.1) ***	15.7 (3.9)
Proteins	Score	0.89 (0.26)	0.89 (0.20)	0.70 (0.40)	0.40 (0.16)	0.00 (0.00) *	1.00 (0.29)
Glucose	Score	0.00 (0.00)	3.33 (0.29) ***	3.50 (0.17) ***	3.40 (0.16) ***	3.40 (0.22) ***	0.00 (0.00)
Ketones	Score	1.11 (0.26)	1.56 (0.18)	3.90 (0.10) ***	4.00 (0.00) ***	4.00 (0.00) ***	1.11 (0.26)
Occult Blood	Score	1.22 (0.22)	1.22 (0.15)	1.40 (0.31)	1.10 (0.10)	1.00 (0.00)	1.22 (0.15)
Tripel phosphate crystals	Score	2.50 (0.22)	1.11 (0.42)	0.20 (0.20) ***	0.00 (0.00) ***	0.00 (0.00) ***	2.33 (0.21)
Sodium	mmol/g Crea	63.6 (8.6)	81.8 (5.4)	140.2 (18.4) *	234.3 (21.1) ***	197.6 (16.4) ***	41.9 (8.3)
Potassium	mmol/g Crea	129.7 (7.4)	175.3 (10.5) **	231.5 (25.8) **	317.3 (15.2) ***	267.9 (10.9) ***	114.2 (10.0)
Chloride	mmol/g Crea	55.5 (6.5)	104.7 (10.3) **	123.8 (7.7) ***	115.8 (11.8) ***	78.5 (9.0)	67.9 (10.7)
Calcium	mg/mg Crea	0.053 (0.010)	0.165 (0.026) ***	0.540 (0.080) ***	1.479 (0.190) ***	0.895 (0.105) ***	0.045 (0.007)
Phosphate	mg/mg Crea	0.84 (0.11)	1.68 (0.16) ***	3.37 (0.26) ***	4.49 (0.25) ***	4.23 (0.13) ***	0.93 (0.12)
Glucose	mg/mg Crea	0.13 (0.04)	148.93 (8.87) ***	216.67 (16.02) ***	272.28 (9.24) ***	264.04 (6.92) ***	0.15 (0.02)
Creatinine	mg	11.65 (0.78)	10.89 (0.61)	10.04 (0.43) *	9.08 (0.39) **	10.56 (0.38)	10.98 (0.95)
Protein	mg/mg Crea	1.0 (0.1)	1.2 (0.1)	1.8 (0.4) **	2.0 (0.2) ***	1.6 (0.1) **	0.9 (0.1)
Magnesium	mg/mg Crea	0.141 (0.022)	0.322 (0.037) *	0.597 (0.072) ***	0.911 (0.055) ***	0.842 (0.040) ***	0.173 (0.022)

Significance versus Vehicle computed by Mann-Whitney U test (two-tailed): * P<.05 ** P<.01 *** P<.001

Parameter	Unit	Dosage Groups (mg eq / kg)					
		Vehicle	Low:4	M:20	H1:100	H2:100	
FEMALES							
Specific gravity		1.025 (0.002)	1.045 (0.002) ***	1.047 (0.001) ***	1.045 (0.002) ***	1.047 (0.001) ***	1.033 (0.004)
pH		6.9 (0.1)	6.3 (0.1) **	5.9 (0.1) ***	5.3 (0.2) ***	5.4 (0.2) ***	6.6 (0.2)
Volume	ml	11.7 (1.5)	13.8 (1.4)	15.6 (1.9)	25.5 (2.0) ***	20.8 (1.2) ***	10.1 (2.4)
Proteins	Score	0.10 (0.10)	0.10 (0.10)	0.10 (0.10)	0.10 (0.10)	0.00 (0.00)	0.30 (0.15)
Glucose	Score	0.00 (0.00)	3.80 (0.13) ***	3.40 (0.22) ***	3.70 (0.15) ***	3.70 (0.15) ***	0.00 (0.00)
Ketones	Score	0.70 (0.15)	1.00 (0.00)	2.60 (0.27) ***	3.80 (0.20) ***	3.50 (0.22) ***	0.90 (0.23)
Occult Blood	Score	0.30 (0.15)	1.00 (0.00) **	1.00 (0.15) *	0.70 (0.21)	0.40 (0.16)	0.50 (0.22)
Tripel phosphate crystals	Score	2.25 (0.16)	0.50 (0.22) ***	0.00 (0.00) ***	0.00 (0.00) ***	0.20 (0.20) ***	2.25 (0.25)
Sodium	mmol/g Crea	92.3 (7.0)	138.0 (10.5) **	118.4 (13.4)	260.7 (22.1) ***	171.8 (13.7) ***	100.8 (11.5)
Potassium	mmol/g Crea	190.1 (10.4)	209.8 (15.5)	243.8 (23.7)	371.9 (18.3) ***	306.4 (18.1) ***	146.8 (5.7) **
Chloride	mmol/g Crea	79.3 (8.4)	144.5 (14.9) **	120.9 (13.1) *	150.3 (10.7) ***	75.4 (8.8)	109.9 (11.3) *
Calcium	mg/mg Crea	0.336 (0.050)	0.579 (0.075) *	1.113 (0.174) ***	2.766 (0.277) ***	1.747 (0.126) ***	0.243 (0.047)
Phosphate	mg/mg Crea	1.53 (0.15)	2.68 (0.14) ***	3.68 (0.16) ***	6.07 (0.30) ***	4.82 (0.14) ***	1.29 (0.08)
Glucose	mg/mg Crea	0.14 (0.01)	125.07 (7.68) ***	214.53 (11.85) ***	309.72 (16.06) ***	266.79 (5.63) ***	0.13 (0.01)
Creatinine	mg	6.80 (0.36)	6.77 (0.23)	6.59 (0.18)	5.82 (0.20)	6.58 (0.17)	6.45 (0.34)
Protein	mg/mg Crea	0.3 (0.0)	0.4 (0.0) *	0.4 (0.0) *	0.8 (0.1) ***	0.4 (0.0) **	0.3 (0.0)
Magnesium	mg/mg Crea	0.431 (0.064)	0.501 (0.032)	0.760 (0.076) **	1.317 (0.085) ***	1.052 (0.045) ***	0.415 (0.055)

Significance versus Vehicle computed by Mann-Whitney U test (two-tailed): * P<.05 ** P<.01 *** P<.001

Gross Pathology

- HD female rats had swollen adrenal glands corresponding to increase in adrenal weight. There was no notable histopath correlates in these animals.
- Kidney and liver in the HD females appeared pale at necropsy.

Report Title: 3-Month Repeated Dose Oral Toxicity Study of JNJ-28431754-ZAE combined with Metformin in the Rat

Daily Dose (mg/kg)	0(Vehicle)		4/300		20/300		100/300		100/0	
No. of Animals	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10
Gross Pathology										
Terminal sacrifice group										
Animals Examined	10	10	10	10	10	10	10	10	10	10
Adrenal glands										
- Swollen	-	-	-	-	-	-	-	4	-	-
Kidneys										
- Discoloration: pale	-	-	-	-	-	1	-	2	-	7
Liver										
- Discoloration: pale	-	-	-	-	-	-	-	2	-	5
- Prominent lobular pattern	-	-	-	-	-	-	-	3	-	1
- Swollen	-	1	-	-	-	-	-	3	-	-

^a At end of dosing period. For vehicles, group means are shown. For treated groups, multiples of vehicles are shown. Statistical significance is based on actual data (not on the multiples of vehicles).

^b Both absolute and relative weights differed from vehicles in the direction indicated. Number indicates multiple of vehicles for the absolute organ weights.

Organ Weights

- Canagliflozin increased adrenal (HD females only), kidney (17 to 33%) and liver weight (15 to 26%) in both sexes in rats.
- Metformin had no notable impact on organ weight.

Report Title: 3-Month Repeated Dose Oral Toxicity Study of JNJ-28431754-ZAE combined with Metformin in the Rat

Daily Dose (mg/kg)	0(Vehicle)		4/300		20/300		100/300		100/0	
No. of Animals	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10
Organ Weights ^{a,b}										
Terminal sacrifice group										
Mean weight										
Adrenal glands	-	0.07837	-	-	-	-	-	1.275 *	-	-
Kidneys	2.74	1.94	1.252 **	1.170 *	1.314 **	1.227 *	1.212 **	1.330 **	1.197 **	1.129
Liver	9.45	7.03	-	-	1.153 *	-	1.192 *	1.266 **	-	-
Mean % body weight										
Adrenal glands (%body)	-	0.02949	-	-	-	-	-	1.513 **	-	-
Kidneys (%body)	0.63029	0.73308	1.383 *	1.240 *	1.541 **	1.349 **	1.618 **	1.569 **	1.484 **	1.291 **
Liver (%body)	2.16	2.63	-	-	1.356 **	-	1.597 **	1.498 **	-	-
Mean % brain weight										
Adrenal glands (%brain)	-	3.87	-	-	-	-	-	1.349 **	-	-
Kidneys (%brain)	132.4	95.86	1.245 **	1.202 *	1.307 **	1.253 *	1.234 **	1.410 **	1.205 *	1.181 *
Liver (%brain)	456.4	347.1	-	-	1.152 *	-	1.213 *	1.341 **	-	-

^a At end of dosing period. For vehicles, group means are shown. For treated groups, multiples of vehicles are shown. Statistical significance is based on actual data (not on the multiples of vehicles).

^b Both absolute and relative weights differed from vehicles in the direction indicated. Number indicates multiple of vehicles for the absolute organ weights.

- No noteworthy findings

* - p<0.05, ** - p<0.01, ***-p<0.001

Histopathology

Adequate Battery: Yes

Peer Review: Not mentioned

Histological Findings

- All the histopath findings described below such as hyperostosis, renal tubule and pelvic dilatations were previously reported in rat toxicology studies with canagliflozin alone.

- Drug-related increase in incidence and severity (minimal to slight) of hyperostosis of the cartilage in the trabecular bone of the tibia and femur. Hyperostosis is characterized by the increase in the quantity of non-ossified cartilage and bone.
- Canagliflozin increased dilatation of tubules at all doses.
- Canagliflozin caused renal pelvic hyperplasia/inflammation in two HD females.
- Hypereosinophilia of the hepatocytes was noted in the MD and HD males and HD females. The two rats have single foci of subcapsular necrosis, which may have been due to increased liver weight.
- There was no notable histopath findings in rats with metformin alone, suggesting that histopath findings in CanaMet were solely due to canagliflozin.

Report Title: 3-Month Repeated Dose Oral Toxicity Study of JNJ-28431754-ZAE combined with Metformin in the Rat

Daily Dose (mg/kg) No. of Animals	0(Vehicle)		4/300		20/300		100/300		100/0	
	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10
Bone, stifle										
- No. Examined	10	10	10	10	10	10	10	10	10	10
- Hyperostosis			3	4 *	7 **	9 **	10 **	10 **	10 **	10 **
Grade 1			3	4	5	8		3	3	3
Grade 2					2	1	9	7	7	7
Grade 3							1			
Kidneys										
- No. Examined	10	10	10	10	10	10	10	10	10	10
- Tubular dilation	-	-	4 *	-	7 **	8 **	8 **	9 **	9 **	8 **
Grade 1			4		7	8	8	9	9	8
- Chronic pyelitis	-	-	-	-	-	-	-	2	-	-
Grade 1								1		
Grade 2								1		
- Pelvic hyperplasia	-	-	-	-	-	-	-	2	-	-
Grade 1								1		
Grade 2								1		
Liver										
- No. Examined	10	10	10	10	10	10	10	10	10	10
- Hypereosinophilia	-	-	-	-	3	-	9 **	6 **	-	-
Grade 1					3		4	2		
Grade 2							5	4		

* At end of dosing period. For vehicles, group means are shown. For treated groups, multiples of vehicles are shown. Statistical significance is based on actual data (not on the multiples of vehicles).

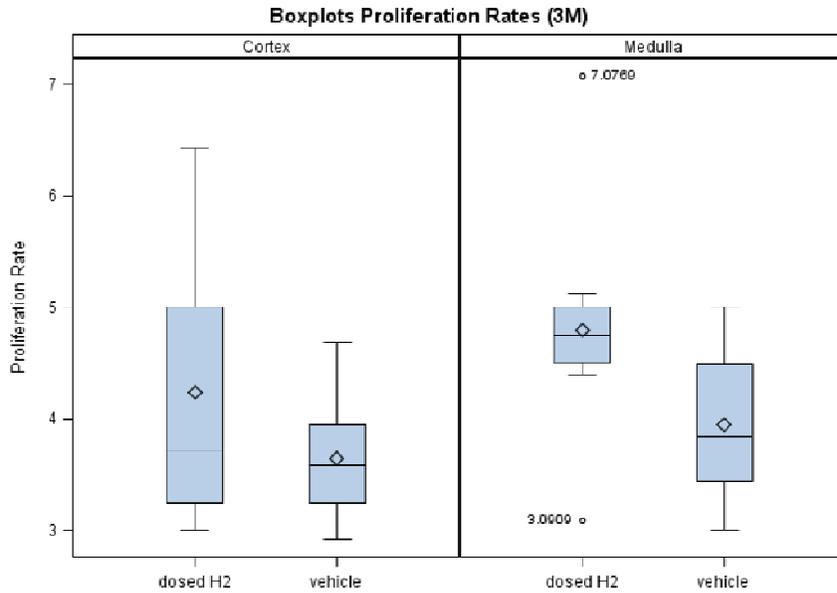
^b Both absolute and relative weights differed from vehicles in the direction indicated. Number indicates multiple of vehicles for the absolute organ weights.
- No noteworthy findings

* - p<0.05, ** - p<0.01, ***-p<0.001

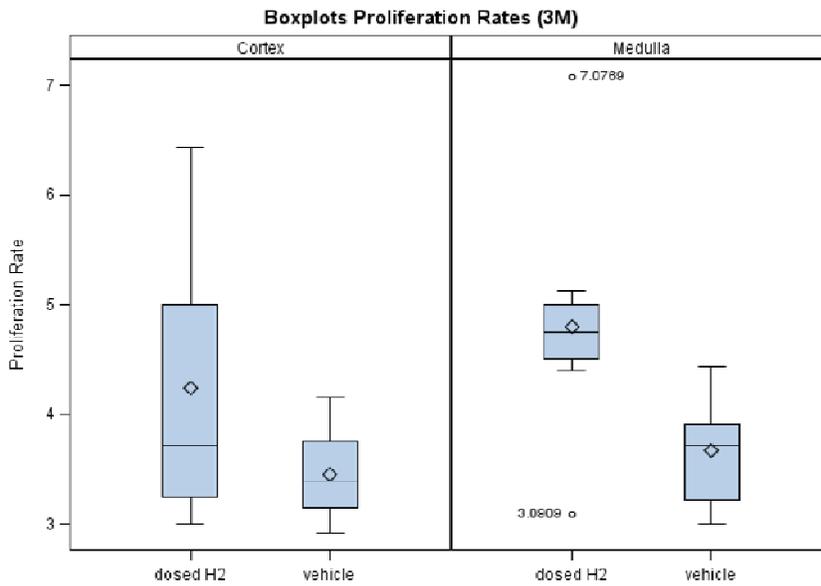
Analysis of Renal Cell Proliferation Biomarkers:

- High dose canagliflozin increased cell proliferation in the outer strip of the outer medulla (OSOM) when two animals (rat #7 and 8) were removed from analysis (p=0.0282). Including the two animals with high false positive background staining, the trend was positive but not statistically different (p=0.0658).
- The cortex cell (distal tubules) proliferation (PCNA positive counts) in the canagliflozin rats tended to be variable with or without the animal #7 and 8.
- There was no significant increase in KIM-1 positivity in the cortex or OSOM in HD canagliflozin rats treated compared to control, suggesting minimal renal tubule damage.

Box-Whisker plots of the proliferation rates: all examined animals.



Box-Whisker plots of the proliferation rates: rats Nos. 7 and 8 excluded.



Summary table on incidences: **KIM-1**

	Vehicle (n=8)		High dose (n= 9)	
	Focal	multifocal	Focal	multifocal
KIM-1 positivity cortex	6	0		7
Grade 1	6			7
Grade 2			(1)*	
KIM-1 positivity OSOM	2	4	1	8
Grade 1	2	4		7
Grade 2			1	1

*this rat has also multifocal positivity

Toxicokinetics:

- Canagliflozin exposure in females tended to be higher than males after multiple dosing.
- Canagliflozin exposure at 100 mg/kg was at slightly increased (15%) when co-administered with metformin. Metformin had no impact on canagliflozin exposure at lower doses (4 and 20 mg/kg).
- Metformin exposure was similar between males and females.
- Metformin exposure was somewhat higher when given in combination with canagliflozin doses \geq 20 mg/kg.
- Based on presence of hyperostosis at all doses, the NOAEL for canagliflozin was \leq 4 mg/ (AUC of 13.5 and 16.0 μ g.h/ml in male and female rats, respectively). The NOAEL exposure multiples was less than clinical exposure (300 mg QD, AUC₀₋₂₄ of 26.1 μ g.h/ml). The NOAEL for metformin was $>$ 300 mg/kg (AUC 39.8 and 48.9 ng.h/ml in males and females, respectively).

Dosing Formulation Analysis: The purity of canagliflozin was 97.6%. The canagliflozin solution concentrations were within the specified target (\pm 10%). Canagliflozin solution was stable for up to 22 days when stored in refrigerator and at least one day at room temperature. Canagliflozin in vehicle was below detection limit.

7 Genetic Toxicology

Genotoxicity of canagliflozin alone was reviewed with the original NDA for canagliflozin (204042). *Canagliflozin was not mutagenic with or without metabolic activation in the Ames assay. Canagliflozin was mutagenic in the in vitro mouse lymphoma assay with but not without metabolic activation. Canagliflozin was not mutagenic or clastogenic in an in vivo oral micronucleus assay in rats and an in vivo oral Comet assay in rats.*

8 Carcinogenicity

No animal studies have been conducted with the combined products in INVOKAMET to evaluate carcinogenesis, mutagenesis, or impairment of fertility. The following data are based on the findings in studies with canagliflozin and metformin individually.

Canagliflozin carcinogenicity was evaluated in 2-year studies conducted in CD1 mice and Sprague-Dawley rats. Canagliflozin did not increase the incidence of tumors in mice dosed 10, 30, or 100mg/kg (\leq 14 times exposure from a 300mg clinical dose). Testicular Leydig cell tumors, considered secondary to increased luteinizing hormone (LH), increased significantly in male rats at all doses tested (10, 30, and 100mg/kg). In a 12-week clinical study, LH did not increase in males treated with canagliflozin. Renal tubular adenoma and carcinoma increased significantly in male and female rats dosed 100mg/kg, or approximately 12-times exposure from a 300mg clinical dose. Also, adrenal pheochromocytoma increased significantly in males and numerically in females dosed 100 mg/kg. Carbohydrate malabsorption associated with high doses of

canagliflozin was considered a necessary proximal event in the emergence of renal and adrenal tumors in rats. Clinical studies have not demonstrated carbohydrate malabsorption in humans at canagliflozin doses of up to 2-times the recommended clinical dose of 300mg.

Metformin hydrochloride carcinogenicity of metformin was evaluated in rats (104 weeks) and mice (91 weeks) at doses up to and including 900 mg/kg/day and 1500 mg/kg/day, respectively. These doses are both approximately 4 times the maximum recommended human daily dose of 2000 mg based on body surface area comparisons. No evidence of carcinogenicity with metformin was found in either male or female mice. Similarly, there was no tumorigenic potential observed with metformin in male rats. There was, however, an increased incidence of benign stromal uterine polyps in female rats treated with 900 mg/kg/day.

9 Reproductive and Developmental Toxicology

The sponsor had tested the effects of combination of canagliflozin and metformin on the embryofetal development in SD rats (Study #TOX9590). The study was reviewed by Dr. Minck. Briefly, oral administration of canagliflozin/metformin of 0/0, 10/300, 30/300, 60/300, 60/0, 0/300 mg/kg resulted in dose related reductions in body weight gain in all groups. The combination appeared to cause greater decreases in BW gain. Feed intake was reduced with MD and HD combination and both single treatment groups during the initial treatment period. There was no effect on cesarean section endpoints. There were no significant effects on fetal development. Slight increases in the incidences of skeletal variations were observed in the rats given the combination of the two drugs (relative to control or canagliflozin alone). It should be noted that skeletal variations were also present in the controls but at a lower frequency. The skeletal effect was likely transient due and due to initial maternal weight loss.

Observation	Select Skeletal Observations					
	Dosage in mg/kg/day (J&J drug/metformin)					
	0/1	10/300	30/300	60/300	60/0	0/300
number evaluated (fetuses/litters)	126/18	148/22	128/20	142/22	131/20	149/21
Skull						
interparietal incomplete ossification	7/3	19*/9	16*/9	44***/15***	5/3	18*/8
supraoccipital incomplete ossification	1/1	4/3	8*/5	9**/6*	1/1	5/4
Vertebral column						
cervical centrum unossified	24/12	50**/16	54***/17	69***/20*	35/15	47***/15
ventral tubercle unossified	20/8	45**/14	25/8	44**/14	20/11	24/9
thoracic centrum bipartite or dumbbell shaped ^a	3	6	11	16	6	7
lumbar rudimentary rib unilateral or bilateral ^a	12	18	22	28	28	15
Rib wavy	3/2	14**/7	7/5	1/1	15**/8*	5/5
Sternum						
extra ossification/fusion	5/5	5/4	8/4	5/3	4/2	17*/11
rudimentary 5th Metatarsal	2/2	5/5	2/2	10*/8*	2/2	9*/6
reduced ossification (>1)	3/3	3/1	1/1	16**/8	3/3	3/2

^a: observations combined across categories so only fetal incidence shown – stats not calculated

* p<0.05; **p<0.01; ***p<0.001

Toxicity	Sex	NOAEL (Dose)	AUC ₀₋₂₄ (µg·hr/mL)	Clinical Safety Margin (rat AUC/human AUC ^a)
Rat Developmental Toxicity - Combination	maternal	10/300	46.8	2x /11x
	developmental	60/300	300	11x / 11x

^aHuman AUC is considered 26.1 µg·hr/mL based on the average AUC at 300 mg qd from study DIA1023 and 300 mg bid from study DIA1007

In summary, co-administration of canagliflozin with metformin decreased maternal BW and increased alterations / delayed in fetal ossification. These findings were similar to rat embryofetal study with canagliflozin alone suggesting that metformin had no meaningful impact on maternal or embryofetal changes. Co-administration of metformin had no impact canagliflozin exposure; however, canagliflozin increased metformin exposure by 1.5 fold in rats. The maternal NAOEL based on weight loss was of 10/300 mg/kg, approximately 2x and 11x the MRHD of 300/2000 mg QD FDC based on AUC. The NOAEL for fetal skeletal variations was 60/300 mg/kg, approximately 11x MRHD for the FDC on AUC basis.

The reviewer concludes that the addition of metformin to canagliflozin had no meaningful impact on canagliflozin induced maternal and embryofetal developmental changes in rats.

11 Integrated Summary and Safety Evaluation

The sponsor is seeking the approval of canagliflozin-metformin (INVOKAMET®) fixed dose combination. Canagliflozin is a selective sodium-glucose co-transporter 2 (SGLT2) inhibitor, approved by the FDA in March 29, 2013. Metformin (Glucophage) is a biguanide class insulin sensitizer antihyperglycemic agent approved by the FDA in 1995. The sponsor submitted a 3-month general toxicology study and an embryofetal development study in rats administered the combination in support of the safety of the FDC drug product.

Canagliflozin in the 3-month combination study resulted in glucosuria, polyuria, weight loss, renal and pelvic dilatations and trabecular bone hyperostosis. These changes were not impacted by the metformin co-administration suggesting that metformin does not significantly alter the toxicity profile of canagliflozin. Metformin also had no impact on canagliflozin exposure. In contrast, canagliflozin increased metformin exposure by 1.4 to 1.8 fold. Since metformin is excreted unchanged by the kidneys and canagliflozin can alter renal hemodynamics, the increase in metformin AUC is likely due to canagliflozin's effect on renal hemodynamics. The increase in metformin AUC was also seen in humans with the 300 mg dose.

Analysis of cell proliferation biomarkers in the HD canagliflozin (100 mg/kg) group found increased renal cell proliferation in the OSOM relative to the control. However, there was no statistically significant increase in KIM-1 positivity to suggest renal tubule damage.

The 3-month study design also included analysis of serum testosterone and LH to support the hypothesis that canagliflozin-induced increase in testicular leydig cell tumors in rats were due to increased LH. Analysis of the two hormones found no statistically significant change in testosterone while LH stayed below the detection limit in both control and canagliflozin groups. A similar observation was also noted in the 1-month dose-ranging study in rats. Although in the 1-month study testosterone levels were slightly reduced in canagliflozin rats, the serum LH was below detection limit thus providing no evidence to support an acute canagliflozin induced increase in LH. Whether the undetectable LH levels at 1- or 3-months were due to poor sample collection or storage is not clear. One would have expected utmost care since the analysis of the two hormones were part of the study design and an increase in LH should have been detected at least after 4 weeks of treatment.

In the rat embryofetal development study, oral administration of canagliflozin/metformin resulted in dose related reduction in body weight gain in all groups whether they were given in combination or alone. The combination appeared to cause a greater decrease in BW gain. Feed intake was reduced with the MD and HD combinations and both single treatment groups during the initial treatment period. There was no effect on cesarean section endpoints. There were no significant effects on fetal development. However, a slight increase in the incidences of skeletal variations were observed in the rats given the combination of the two drugs (relative to control or canagliflozin alone). The skeletal effect was likely transient due to initial maternal weight loss. The maternal NOAEL based on weight loss was 10/300 mg/kg, approximately 2x and 11x the

maximum recommended FDC of 300/2000 mg QD based on AUC. The development toxicity due to skeletal variation was 60/300 mg/kg, approximately 11x the MRHD based on AUC.

Safety margin for canagliflozin (JNJ-28431754) and metformin

Species	Daily Cana/Met Dose, mg/kg	Canagliflozin AUC ₀₋₂₄ , µg.h/ml	Metformin AUC ₀₋₂₄ , µg.h/ml	AUC Safety margins, (Animal /Human)	
				Cana	Metformin
13-Week rat study Combination dose, NOAEL 4 mkd	* 4/300	M:12.6 F:21.3	M:73 F:81	<0.7	6
	20/300	M:74.4 F:94.6	M:140 F:113	4	9
	100/300**	M:365 F:470	M:115 F:119	16	9
Rat Embryofetal Developmental study- Combination dose	10/300#	F: 47.8	F:152	2	11
	30/300	F: 151	F:148	6	11
	60/300##	F: 300	F:151	11	11
	60/00	F: 284	--	11	
	0/300	--	F:97.2		7
Maximum Clinical Dose: Canagliflozin, 300 mg Metformin, 2000 mg		26.1	13.4		

* NOAEL for canagliflozin (4 mg/kg) ** NOAEL for metformin (300 mg/kg)

Maternal NOAEL ## Developmental NOAEL in the embryofetal developmental study

In summary, previous toxicological assessment of canagliflozin alone identified kidney and bone as the primary target organs. The dilatation of renal tubule and pelvis appeared to be an adaptive response to persistent diuresis caused by the osmotic effect of excreted glucose in rats. Both renal and bone findings were also seen when canagliflozin was co-administered with metformin suggesting that metformin played no additional role. The NOAEL for renal dilatation was about 4 mg/kg for canagliflozin and 300 mg/kg for metformin. In the rat EFD study, canagliflozin decreased maternal weight resulting in a NOAEL of 10 mg/kg, approximately 2x the MRHD based on AUC. Slight increase in skeletal variations such as reduced or incomplete ossification is likely due to decrease in the maternal weight and expected to be transient. The NOAEL for metformin in both toxicity and EFD study was 300 mg/kg, approximately 11x the clinical dose of 2000 mg QD, based on AUC. Co-administration of metformin had no significant impact on toxicity or embryofetal development effect of canagliflozin in rats. Metformin had no impact on canagliflozin exposure; however, canagliflozin increased metformin exposure by 1.4 to 1.8 fold. The increase in metformin exposure is likely caused by canagliflozin-induced changes in renal hemodynamics. Overall, canagliflozin fixed dose combination with metformin is considered safe and the studies support approval of canagliflozin/metformin fixed dose combination for use in humans.

Appendix

Study title: 1-Month Repeated Dose Oral Toxicity Study of JNJ-28431754-ZAE Combined with Metformin in the Rat (non-GLP).

Methods: In this 1-month non-GLP dose-ranging study in SD rats, canagliflozin (JNJ-28431754-ZAE) doses of 4, 20 and 100 mg/kg were combined with 300 mg/kg metformin (JNJ-1158196-AAC) for daily oral gavage delivery. Canagliflozin doses had been previously used toxicology studies with canagliflozin alone while metformin dose was based on published literature to provide maximal tolerable exposure. The dose and concentrations of each drug component are shown in the table below. The drugs were prepared in aqueous suspension containing 0.5% hydroxypropyl methylcellulose (Methocel).

Dosage groups JNJ-28431754-ZAE	V	L	M	H1	H2	ME
mg eq./kg b.w./day	00	4	20	100	100	-
mg eq./ml	0	0.8	4	20	20	-
ml/kg b.w./day	5	5	5	5	5	-
Dosage groups JNJ-1158196-AAC	V	L	M	H1	H2	ME
mg eq./kg b.w./day	00	300	300	300	-	300
mg eq./ml	0	60	60	60	-	60
ml/kg b.w./day	5	5	5	5	-	5

Findings:

- Canagliflozin dose-dependently reduced BW and BW gain in males and to small extend in females (MD and H1). The decrease in BW is secondary to canagliflozin induced urinary glucose excretion. The associated increase in food intake was unable to compensate for caloric loss of glucose in urine.
- Canagliflozin dose-dependently increased urinary glucose, ketonuria, specific gravity and urine volume (secondary to osmotic effect of glucose).
- In spite of decrease in BW, the adrenal and kidney weights were increased in both sexes at all doses of CanaMet (L, M, H1) and HD canagliflozin alone (H2) suggesting that these changes were due to canagliflozin alone.
- HD canagliflozin and CanaMet increased in liver weigh.
- Adrenal gland swelling was noted in the MD and HD CanaMet with no histological correlate.
- There was a dose-related increase in the incidence and severity of hyperostosis in the cartilage of tibia and femur trabecular bone in all canagliflozin groups.
- There were no histopath findings (i.e. hyperostosis) attributable to metformin. There was no difference between H1 (HD CanaMet) and H2 (high dose Cana alone).

- A small but significant increase in renal cell proliferation of the outer stipe of the outer medulla (PCNA-positivity) but not in the cortex was noted in the HD canagliflozin group.
- The slight increases KIM-1 positivity (marker of tubular injury) in the HD canagliflozin group was not statistically significant.
- Analysis of serum from control and high dose canagliflozin found a small decrease in serum testosterone relative to control; however, the LH levels were undetectable in both control and HD canagliflozin rats at the end of the 1-month study. The reviewer had expected an increase in LH to support the hypothesis that canagliflozin induced increase in leydig cells tumors were due to early increase in LH in rats.
- Co-administration of metformin had no impact on any of the canagliflozin-induced changes in rats. Furthermore, metformin had no effect on canagliflozin exposure. However, canagliflozin increased metformin AUC exposure by up to 1.6 fold.

In summary, co-administration of canagliflozin with metformin resulted in known canagliflozin-induced toxicity signals such as glycosuria, polyuria, calciuria, trabecular bone hyperostosis and renal pelvis dilatation in rats. Metformin did not improve or worsen any of the histopath changes attributed to canagliflozin in rats.

Notable findings in the 1-month rat canagliflozin-metformin combination dose-ranging study:

Experiment: TOX - 9582

1-Month RD Oral Tox Study in the Rat

JNJ-28431754-ZAE + METFORMIN - OR/GAV - RAT

BODY WEIGHT

Mean values per dosage group in g

Week/Day	Dosage Group (mg eq. / kg)											
	Males						Females					
	Vehicle	Low:4	Med.:20	H1:100	H2:100	Metformin	Vehicle	Low:4	Med.:20	H1:100	H2:100	Metformin
0 / 0	213 (3.0)	311 (3.5)	312 (3.5)	314 (3.6)	314 (3.3)	311 (3.5)	198 (2.4)	197 (3.0)	197 (3.2)	192 (2.6)	196 (2.5)	196 (2.8)
1 / 7	362 (7.9)	346 (5.0)	346 (6.1)	312 *** (4.1)	327 ** (3.3)	348 (5.3)	218 (3.5)	215 (3.6)	213 (3.8)	206 * (3.5)	220 (2.4)	219 (3.6)
2 / 14	395 (10.7)	368 * (6.8)	363 * (5.6)	340 *** (4.6)	353 ** (4.2)	373 (6.9)	234 (4.9)	236 (3.1)	226 (4.4)	228 (4.2)	236 (3.3)	231 (3.2)
3 / 21	426 (13.2)	393 (8.7)	392 * (8.5)	361 *** (3.2)	379 ** (5.0)	397 (8.4)	249 (4.5)	243 (3.3)	237 (4.7)	237 (4.6)	241 (3.5)	245 (5.0)
4 / 28	446 (16.1)	402 * (8.3)	398 * (7.4)	366 *** (4.8)	390 ** (6.1)	410 (8.5)	259 (3.9)	254 (3.9)	245 (4.7)	246 (5.5)	255 (3.3)	253 (5.5)

Significance computed versus the Vehicle group by Mann-Whitney U test (two-tailed): * p <.05 ** p <.01 *** p <.001

Standard error is shown between brackets

L: 4/300 mg eq./kg/day

M: 20/300 mg eq./kg/day

H1: 100/300 mg eq./kg/day

H2: 100/- mg eq./kg/day

MF: -/300 mg eq./kg/day

Experiment TOX9582
 1-Month RD Oral Tox Study in the Rat
 JNJ-28431754-ZAE + METFORMIN - OR/GAV - RAT

Parameter	Unit	Dosage Groups (mg eq. /kg)	
		MALES	
		Vehicle	H2:100/0
Testosterone		5.31	1.25
	nmol/l	(1.83)	(0.51)
			**
Luteinizing hormone		< 0.78	< 0.78
	ng/ml	(0.00)	(0.00)

Standard Error is shown between brackets if more than 2 animals
 Mann-Whitney U test (two-tailed): * P<.05 ** P<.01 *** P<.001
 <x (below the lower limit of quantification)

Experiment TOX9582
 1-Month RD Oral Tox Study in the Rat
 JNJ-28431754-ZAE + METFORMIN - OR/GAV - RAT

Parameter	Unit	Dosage Groups (mg eq. / kg)					
		MALES					
		Vehicle	Low: 4/300	Med.: 20/300	H1: 100/300	H2: 100/0	Metf.: 0/300
Sodium	mmol/l	144 (0)	144 (0)	143 (1)	142 (1) *	143 (0)	143 (0)
Potassium	mmol/l	5.0 (0.1)	5.2 (0.1)	5.1 (0.2)	5.5 (0.1) *	5.1 (0.1)	5.4 (0.1)
Chloride	mmol/l	103 (0)	102 (0)	99 (0) ***	101 (1)	101 (1)	103 (0)
Calcium	mg/dl	10.0 (0.0)	9.7 (0.0) ***	9.6 (0.1) ***	9.8 (0.1) **	9.6 (0.1) ***	9.8 (0.1)
Inorg. phosphorus	mg/dl	7.9 (0.1)	7.5 (0.1)	7.9 (0.1)	7.8 (0.2)	7.2 (0.2) *	7.9 (0.2)
Total protein	g/dl	5.8 (0.1)	5.7 (0.0)	5.7 (0.1)	5.6 (0.1)	5.6 (0.1)	5.6 (0.1)
Albumin	g/dl	4.2 (0.1)	4.2 (0.1)	4.2 (0.0)	4.1 (0.1)	4.1 (0.1)	4.1 (0.0)
Glucose	mg/dl	105 (6)	84 (3) *	61 (2) ***	57 (1) ***	56 (3) ***	116 (5)
Cholesterol	mg/dl	47 (3)	40 (3)	41 (3)	55 (2)	60 (3) **	54 (3)
Triglycerides	mg/dl	50 (6)	44 (5)	61 (5)	129 (13) ***	94 (6) ***	37 (4)
Urea nitrogen	mg/dl	13.2 (0.5)	20.1 (1.1) ***	22.6 (0.9) ***	24.5 (1.3) ***	30.0 (1.0) ***	13.5 (0.6)
Creatinine	mg/dl	0.24 (0.01)	0.22 (0.01)	0.22 (0.01)	0.21 (0.01) *	0.22 (0.01)	0.25 (0.01)
Total bilirubin	mg/dl	0.14 (0.01)	0.14 (0.01)	0.12 (0.01) *	0.10 (0.01) ***	0.11 (0.01) *	0.14 (0.01)
Alk. phosphatase	U/l	119 (8)	128 (10)	130 (10)	142 (10)	156 (8) **	121 (5)
Aspartate aminotransferase	U/l	122 (6)	163 (8) ***	167 (7) ***	165 (9) ***	163 (9) **	123 (5)
Alanine aminotransferase	U/l	32 (1)	44 (1) ***	48 (2) ***	55 (3) ***	56 (2) ***	35 (2)
Gamma glutamyl transferase	U/l	0 (0)	0 (0)	0 (0)	1 (0)	1 (0)	0 (0)

Standard Error is shown between brackets if more than 2 animals

Significance versus Vehicle computed by Mann-Whitney U test (two-tailed): * P<.05 ** P<.01 *** P<.001

Experiment TOX9582
 1-Month RD Oral Tox Study in the Rat
 JNJ-28431754-ZAE + METFORMIN - OR/GAV - RAT

Parameter	Unit	Dosage Groups (mg eq. / kg)					Meff.: 0/300
		FEMALES					
		Vehicle	Low: 4/300	Med.: 20/300	H1: 100/300	H2: 100/0	
Sodium	mmol/l	143 (0)	142 (1)	142 (0)	141 (1)	142 (0)	141 (0) **
Potassium	mmol/l	5.1 (0.2)	4.7 (0.1)	4.6 (0.1) *	5.3 (0.2)	4.7 (0.1)	5.3 (0.1)
Chloride	mmol/l	102 (0)	100 (1) **	99 (1) **	98 (0) ***	102 (1)	102 (0)
Calcium	mg/dl	10.3 (0.1)	10.0 (0.1) *	9.8 (0.1) **	9.8 (0.1) **	9.8 (0.0) ***	10.2 (0.1)
Inorg. phosphorus	mg/dl	7.2 (0.2)	6.9 (0.1)	7.1 (0.1)	7.4 (0.1)	6.5 (0.2) *	7.3 (0.2)
Total protein	g/dl	6.3 (0.1)	6.1 (0.1)	6.1 (0.1)	5.8 (0.1) **	6.0 (0.1) *	6.1 (0.1)
Albumin	g/dl	4.6 (0.1)	4.4 (0.0) *	4.3 (0.1) **	4.2 (0.1) ***	4.3 (0.0) **	4.4 (0.1)
Glucose	mg/dl	101 (4)	79 (3) **	64 (2) ***	56 (2) ***	55 (2) ***	110 (4)
Cholesterol	mg/dl	74 (5)	68 (5)	52 (4) **	58 (3) *	64 (5)	74 (4)
Triglycerides	mg/dl	26 (2)	39 (1) ***	45 (6) **	88 (14) ***	54 (7) ***	30 (2)
Urea nitrogen	mg/dl	17.0 (0.7)	19.8 (0.6) **	22.9 (0.6) ***	28.8 (1.3) ***	30.6 (0.5) ***	15.2 (0.6)
Creatinine	mg/dl	0.32 (0.01)	0.27 (0.01) **	0.25 (0.01) ***	0.22 (0.01) ***	0.25 (0.01) ***	0.28 (0.01)
Total bilirubin	mg/dl	0.19 (0.01)	0.17 (0.01)	0.14 (0.00) ***	0.12 (0.01) ***	0.14 (0.01) ***	0.17 (0.01)
Alk. phosphatase	U/l	95 (6)	77 (5) *	72 (4) **	78 (5) *	83 (5)	86 (7)
Aspartate aminotransferase	U/l	121 (5)	140 (8)	143 (5) **	137 (7)	133 (4)	109 (5) *
Alanine aminotransferase	U/l	30 (1)	42 (3) ***	44 (2) ***	55 (3) ***	50 (3) ***	27 (1)
Gamma glutamyl transferase	U/l	1 (0)	1 (0)	2 (0) *	2 (0) *	1 (0)	1 (0)

Standard Error is shown between brackets if more than 2 animals
 Significance versus Vehicle computed by Mann-Whitney U test (two-tailed): * P<.05 ** P<.01 *** P<.001

URINALYSIS

Terminal, recorded in week 5

Parameter	Unit	Dosage Groups (mg eq. / kg)					Metf.:
		Vehicle	Low: 4/300	Med.: 20/300	H1: 100/300	H2: 100/0	
Specific gravity		1.019 (0.003)	1.044 (0.003) ***	1.043 (0.003) ***	1.046 (0.002) ***	1.046 (0.001) ***	1.018 (0.003)
pH		7.7 (0.2)	6.6 (0.1) ***	6.1 (0.1) ***	5.5 (0.2) ***	5.8 (0.1) ***	7.3 (0.2)
Volume	ml	22.5 (3.5)	21.7 (2.8)	29.2 (3.1)	34.4 (2.9) **	34.9 (1.1) **	26.4 (4.8)
Proteins	Score	0.50 (0.22)	0.60 (0.16)	0.40 (0.16)	0.50 (0.17)	0.10 (0.10)	0.40 (0.16)
Glucose	Score	0.00 (0.00)	4.00 (0.00) ***	4.00 (0.00) ***	4.00 (0.00) ***	4.00 (0.00) ***	0.00 (0.00)
Ketones	Score	1.10 (0.23)	1.70 (0.21)	3.20 (0.25) ***	4.00 (0.00) ***	3.90 (0.10) ***	0.60 (0.22)

URINALYSIS

Terminal, recorded in week 5

Parameter	Unit	Dosage Groups (mg eq. / kg)					Metf.:
		Vehicle	Low: 4/300	Med.: 20/300	H1: 100/300	H2: 100/0	
Specific gravity		1.022 (0.004)	1.047 (0.002) ***	1.047 (0.003) ***	1.048 (0.001) ***	1.045 (0.002) **	1.033 (0.004)
pH		7.5 (0.3)	6.2 (0.1) **	5.8 (0.1) ***	5.1 (0.1) ***	5.6 (0.2) ***	6.4 (0.1) *
Volume	ml	17.2 (2.7)	13.0 (1.7)	15.5 (2.6)	20.0 (1.7)	24.5 (1.4) *	10.0 (2.0)
Proteins	Score	0.20 (0.13)	0.10 (0.10)	0.20 (0.13)	0.00 (0.00)	0.00 (0.00)	0.50 (0.17)
Glucose	Score	0.00 (0.00)	4.00 (0.00) ***	3.70 (0.15) ***	3.90 (0.10) ***	4.00 (0.00) ***	0.00 (0.00)
Ketones	Score	0.30 (0.21)	0.80 (0.13)	2.10 (0.28) ***	3.70 (0.15) ***	2.90 (0.31) ***	0.70 (0.21)

Standard Error is shown between brackets if more than 2 animals

Significance versus Vehicle computed by Mann-Whitney U test (two-tailed): * P<.05 ** P<.01 *** P<.001

1-Month RD Oral Tox Study in the Rat
 JNJ-28431754-ZAE - METFORMIN - OR/GAV - RAT

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX Necropsy Status: TERMINAL SACRIFICE GROUP (K0)							
Sex	Males						
Dose Group No. Animals per Dose Group	V 10	L 10	M 10	H1 10	H2 10	ME 10	
BONE, STIFLE	No.Examined	10	10	10	10	10	10
- Hyperostosis		-	10 **	10 **	10 **	10 **	-
	Grade 1	-	10	10	-	4	-
	Grade 2	-	-	-	9	6	-
	Grade 3	-	-	-	1	-	-
KIDNEYS	No.Examined	10	-	3	10	2	-
- Basophilic tubules		1	-	-	-	-	-
	Grade 1	1	-	-	-	-	-
- Dilated pelvis		-	-	2 *	1	-	-
URETER(S)	No.Examined	10	-	3	10	1	-
URINARY BLADDER	No.Examined	10	1	-	10	3	-
- Seminal plug		1	-	-	-	2	-

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX Necropsy Status: TERMINAL SACRIFICE GROUP (K0)							
Sex	Females						
Dose Group No. Animals per Dose Group	V 10	L 10	M 10	H1 10	H2 10	ME 10	
ADRENAL GLANDS	No.Examined	10	-	2	10	2	-
- Vacuolation		-	-	-	-	1	-
	Grade 1	-	-	-	-	1	-
BONE, STIFLE	No.Examined	10	10	10	10	10	10
- Hyperostosis		1	7 **	8 **	10 **	10 **	-
	Grade 1	1	7	8	2	6	-
	Grade 2	-	-	-	8	4	-
KIDNEYS	No.Examined	10	1	1	10	3	3
- Dilated pelvis		-	-	-	1	-	-
- Pyelonephritis		-	-	-	-	-	1
	Grade 2	-	-	-	-	-	1
- Chronic inflammation		3	-	-	2	-	-
	Grade 1	3	-	-	2	-	-
- Cyst(s)		1	-	-	-	-	-
	Grade 1	1	-	-	-	-	-
- Mineral cortex		-	-	-	1	-	-
	Grade 1	-	-	-	1	-	-
- Acute inflammation		-	1	-	-	-	-
	Grade 4	-	1	-	-	-	-

One-Sided Exact Fisher Test: *) p<=0.05; **) p<=0.01; Control= V,
 Group V, Vehicle, females: JNJ-28431754-ZAE (0 mg eq./kg)
 Group L, Low (4/300 mg eq./kg), females: JNJ-28431754-ZAE (4 mg eq./kg)
 Group M, Medium (20/300 mg eq./kg), females: JNJ-28431754-ZAE (20 mg eq./kg)
 Group H1, High1 (100/300 mg eq./kg), females: JNJ-28431754-ZAE (100 mg eq./kg)
 Group H2, High2 (100/0 mg eq./kg), females: JNJ-28431754-ZAE (100 mg eq./kg)
 Group ME, Metformin (0/300 mg eq./kg), females: JNJ-28431754-ZAE (300 mg eq./kg)

GRADE 1 = Minimal / very few / very small
 GRADE 2 = Slight / few / small
 GRADE 3 = Moderate / moderate number / moderate size

Toxicokinetic profile of canagliflozin (4, 20 and 100 mg/kg) coadministered with or without 300 mg/kg metformin in rats.

JNJ-28431754-ZAE, Oral, Rats			TOX9582			Annex TK 6			
Analyte	Gender	Day	Compound Dosed	Dose (mg eq./kg)	Group	C _{max} (ng/mL)	t _{max} (h)	AUC _(0-∞) (h*ng/mL)	AUC _(0-24 h) (h*ng/mL)
JNJ 28431754	Male	0	JNJ-28431754/Metformin	4/300	L	900	7.00	14600	
			JNJ-28431754/Metformin	20/300	M	5070	7.00	99100	
			JNJ-28431754/Metformin	100/300	H1	23200	18.3	896000 ^a	
			JNJ-28431754	100	H2	38100	5.00	674000	
	Male	28	JNJ-28431754/Metformin	4/300	L	1050	3.67		14400
			JNJ-28431754/Metformin	20/300	M	4830	6.00		71800
			JNJ-28431754/Metformin	100/300	H1	20300	6.00		329000
			JNJ-28431754	100	H2	20800	6.00		318000
	Female	0	JNJ-28431754/Metformin	4/300	L	873	5.33	19700	
			JNJ-28431754/Metformin	20/300	M	4760	6.00	129000	
			JNJ-28431754/Metformin	100/300	H1	25200	3.67	2530000	
			JNJ-28431754	100	H2	38100	5.00	790000	
Female	28	JNJ-28431754/Metformin	4/300	L	1360	1.67		16200	
		JNJ-28431754/Metformin	20/300	M	5760	1.33		77500	
		JNJ-28431754/Metformin	100/300	H1	27100	1.33		428000	
		JNJ-28431754	100	H2	27000	3.33		392000	
Metformin	Male	0	JNJ-28431754/Metformin	4/300	L	13500	2.00	80400	
			JNJ-28431754/Metformin	20/300	M	13400	1.67	84700	
			JNJ-28431754/Metformin	100/300	H1	5860	2.67	112000	
			Metformin	300	ME	16700	2.00	83100	
	Male	28	JNJ-28431754/Metformin	4/300	L	17000	2.00		94200
			JNJ-28431754/Metformin	20/300	M	17600	1.67		124000
			JNJ-28431754/Metformin	100/300	H1	11800	1.33		90800
			Metformin	300	ME	13800	1.33		104000
	Female	0	JNJ-28431754/Metformin	4/300	L	13800	2.00	75800	
			JNJ-28431754/Metformin	20/300	M	14200	1.67	81300	
			JNJ-28431754/Metformin	100/300	H1	12800	2.00	78000	
			Metformin	300	ME	17800	1.67	72200	
Female	28	JNJ-28431754/Metformin	4/300	L	16300	1.00		72500	
		JNJ-28431754/Metformin	20/300	M	16900	1.00		93900	
		JNJ-28431754/Metformin	100/300	H1	11300	2.00		103000	
		Metformin	300	ME	16400	1.67		94200	

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/s/

FRED K ALAVI
08/21/2013
CanaMet FDC Approval recommended

TODD M BOURCIER
08/21/2013
p/t supports approval

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 204353

Applicant: Janssen
Pharmaceuticals Inc.

Stamp Date: Dec 12, 2012

Drug Name: Canagliflozin/metformin IR

NDA/BLA Type: 505(b)(2)

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	x		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	x		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	x		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	x		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	x		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?	x		
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	x		All the pivotal toxicology studies for canagliflozin and in combination with metformin (1-, 13-WK toxicology and embryofetal developmental studies in rats) were carried out according to GLP regulations.
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	x		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m ² or comparative serum/plasma levels) and in accordance with 201.57?	x		The proposed labeling sections with plasma level exposure multiples appear to be in accordance with 21 CFR 201.57.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	x		A genotoxic degradant (b)(4) - (b)(4) at levels in excess of (b)(4) µg/day threshold was identified during the stability studies. The genotoxicity was attributed to (b)(4) moiety also present (u)(4). Therefore the degradant was considered a very weak genotoxicant similar to (b)(4). Since it poses minimal risk to humans, the maximum concentrations up to (b)(4) µg/day µg/day per 100 mg tablet was considered acceptable. Other impurities in the product were less than the qualification threshold of (b)(4) thus requiring no qualification.
11	Has the applicant addressed any abuse potential issues in the submission?	x		Not applicable. Canagliflozin primarily targets kidney tubules (SGLT2) and to some degree SGLT1 in the intestinal. Canagliflozin has poor brain distribution with no known CNS effect based on safety pharmacology and toxicology studies.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?		x	Canagliflozin is a new molecular entity that will require prescription and will not be marketed as over the counter.

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE?

Yes

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Fred Alavi, PhD

Jan 24, 2013

Reviewing Pharmacologist

Date

Todd Bourcier

Jan 24, 2013

Team Leader/Supervisor

Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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/s/

FRED K ALAVI

02/01/2013

45-Day check list - No nonclinical filing issues

TODD M BOURCIER

02/01/2013