CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

204042Orig1s000

<u>PHARMACOLOGY REVIEW(S)</u>

Tertiary Pharmacology/Toxicology Review

From: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and
Toxicology, OND IO
NDA: 204042
Agency receipt date: May 31, 2012
Drug: canagliflozin
Sponsor: Janssen Pharmaceuticals Inc.
Indication: Type 2 Diabetes Mellitus treatment

Reviewing Division: Division of Metabolism and Endocrinology Products

Introductory Comments: The pharm/tox reviewers and supervisor concluded that the nonclinical data support approval of canagliflozin for the indication listed above.

The recommended pharmacologic class for canagliflozin is sodium-glucose cotransporter 2 (SGLT2) inhibitor.

Canagliflozin did not cause malformations in rats or rabbits at doses providing relatively large margins of exposure compared to humans. Some decrease in pup body weight and other parameters were noted in a rat pre- post-natal toxicity study at exposures only about twice the human exposure. Renal tubular dilatation and pelvic dilatation were noted in rats treated from postnatal day 21 to 90. These renal effects occurred at the lowest dose tested in these animals, 4 mg/kg, which produced an AUC in the animals about one half that in humans at the maximum recommended human dose. The pharm/tox reviewer and supervisor recommend against using canagliflozin in pregnant women during the 2nd and 3rd trimester and in nursing women.

Carcinogenicity studies of canagliflozin were conducted in rats and mice. The Executive Carcinogenicity Assessment Committee concurred that both of these studies were adequate. No drug-related neoplasms occurred in the mouse. The Committee concurred that renal tubular neoplasms at 100 mg/kg/d in males and females and adrenal pheochromocytomas at 100 mg/kg/d in males, as well as testicular Leydig cell tumors at all doses were clearly drug related. The Committee also noted that there was a numerical increase in pheochromocytomas in high dose females. The Committee noted that the increase in serum LH in rats was likely the causative event for the Leydig cell tumors. The Committee noted that malabsorption of dietary carbohydrate secondary to inhibition of intestinal SGLT1 was a likely key event in the development of the renal and adrenal neoplasms, but that a complete mode of carcinogenic action was not established. The pharm/tox supervisor noted that carbohydrate malabsorption did not apparently occur in clinical trials. Therefore, the risk of such tumors in humans may be low.

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Conclusions:

I agree with the division pharm/tox conclusion that canagliflozin can be approved from the pharm/tox perspective. I agree that canagliflozin may carry some risk to developing fetuses and newborns and that it is appropriate to include language in labeling warning of such risk.

Pharmacology/Toxicology
Center for Drug Evaluation and Research
Division of Metabolic & Endocrine Products

NDA SECONDARY REVIEW

Date:	05 February 2013
NDA #	204042
Sponsor:	Janssen Research & Development
Drug:	Canagliflozin (SGLT2 inhibitor) Type 2 Diabetes
Primary Reviewers:	Fred Alavi, Ph.D. Daniel Minck, Ph.D. (DART review)
Secondary Reviewer:	Todd Bourcier, Ph.D.

Janssen R&D seeks market approval for canagliflozin, proposed trade name Inkovana, as a treatment option for Type 2 diabetes. Canagliflozin is a small molecule inhibitor of the sodium glucose co-transporter 2 (SGLT2), a protein predominately expressed by the renal proximal tubule epithelium that serves as the primary mechanism by which the kidneys reabsorb filtered glucose. Inhibition of its function by canagliflozin results in the loss of most filtered glucose to the urine in an amount proportional to the glomerular filtration rate (GFR) and the predominating plasma glucose level. The urinary loss of filtered glucose by inhibition of SGLT2 is sufficient to reduce elevated plasma glucose levels present in type 2 diabetics.

As canagliflozin would be a first-in-class SGLT2 inhibitor for the treatment of diabetes, certain aspects of its pharmacology merit comment. SGLT2 actively transports filtered glucose into the proximal epithelium driven by the co-transport of sodium; the absorbed glucose leaves the basolateral side of the epithelium via GLUT2, a facilitative transporter unrelated to SGLT proteins. By interfering with the transport function of SGLT2, canagliflozin results in the loss of filtered glucose. This manifests clinically and in animals as glucosuria/polyuria, volume contraction, a reduction in plasma glucose, and caloric loss commensurate with modest loss of body weight. Because efficacy with canagliflozin is tied to GFR, conditions associated with reduced GFR are expected to reduce efficacy for lowering plasma glucose by this mechanism. Changes in the release of or tissue sensitivity to insulin with canagliflozin originate from the urinary loss of glucose and the resultant lowering of plasma glucose but not from a direct effect of the drug on the pancreas, adipose tissue, or other tissues other than the kidney. Any contribution of a possible effect on incretins secondary to marginal inhibition of intestinal SGLT1 is currently speculative. The renal site of drug action differentiates canagliflozin and the SGLT2 inhibitor drug class from other marketed oral anti-diabetic drugs.

Drs. Fred Alavi and Daniel Minck, the primary nonclinical reviewers, conclude that the pharmacology and toxicology data support approval of canagliflozin (100 & 300mg qd). *I concur with their assessment.*

The following summarizes key issues that arose during review of the nonclinical program with canagliflozin. The bone and carcinogenicity issues were discussed in greater detail in an FDA background document for an advisory committee held 10 Jan 2013.

Bone Health

A recurring finding with SGLT2 inhibitors, canagliflozin included, is disruption of calcium homeostasis in rodents and, to a much lesser degree, non-rodents. The calcium disruption manifests in rats as trabecular bone accretion, calcification of soft tissues, hypercalciuria, and complex changes in bone biomarkers. Consistently, serum (1,25)-dihydroxy vitamin D and parathyroid hormone drastically decline. In non-rodents, less severe changes in biomarkers are reported and bone histology does not change appreciably. Canagliflozin produced these effects in animals at clinically relevant exposure which prompted incorporation of robust bone monitoring in phase 3 clinical trials. Carbohydrate malabsorption secondary to inhibition of intestinal SGLT1 (see Dr. Alavi's review, pg 5) was the hypothesized underlying pathway, and Janssen conducted investigational studies that indeed support this pathway as leading to calcium disruption and trabecular accretion in rats. This is of interest, as this pathway also appears to be operable in human subjects[1,2]. However, at the therapeutic doses of 100 and 300mg, canagliflozin did not result in carbohydrate malabsorption (e.g., negative hydrogen breath tests), calcium disruption (e.g., vit D, PTH levels), or bone accretion in clinical trials (though bone loss secondary to body weight loss was noted). Dr. Alavi states that while the clinical risk is minimal, potentially small calcium imbalances caused by 'slight' carbohydrate malabsorption that presumably escaped clinical detection may adversely affect bone health, long-term. The confounding effect of weight loss on bone would make detection of additional drug effects difficult, but this possibility, however slight, would require dedicated monitoring in post-market trials lasting multiple years.

Carcinogenicity

The carcinogenic profile of SGLT2 inhibitors as a new therapeutic drug class is beginning to emerge. Among five investigational SGLT2 inhibitors that have filed final or interim findings from rodent carcinogenicity studies with the Division, four have reported neoplasms of the renal tubules, adrenal gland, or testicular Leydig cells (Table 1). The single agent that did not observe tumors in these tissues nonetheless reported an increased incidence of atypical hyperplasia of the renal tubules. The tumor response is now observed in two species, mice and rats. A common observation among the compounds listed in Table 1 is evidence of carbohydrate malabsorption and disrupted calcium balance, albeit to a varying extent depending on the pharmacology and kinetics specific to each compound.

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[1] Cashman KD (2006) A prebiotic substance persistently enhances intestinal calcium absorption and increases bone mineralization in young adolescents. Nutri Reviews 64(4), 189-196

[2] Scholz-Ahrens KE et al. (2007) Prebiotics, probiotics, and synbiotics affect mineral absorption, bone mineral content, and bone structure. J Nutri 137, 838s-846s.

Table 1: Neoplasms associated with SGLT2 inhibitors in rodents*			
	Tumor sites		
Drug	**Renal Tubules**	**Adrenal gland**	**Testicular Leydig**
Canagliflozin			
Drug A	(mice)		
Drug B	(mice)		(mice & rats)
Drug C			
Dapagliflozin*	none (atypical hyperplasia)	none	none

* From FDA briefing document, Advisory Committee for NDA 202293 (July 2011)

Canagliflozin increased neoplasms of the renal tubules, adrenals, and testicular Leydig cells of Sprague-Dawley rats. Canagliflozin is not genotoxic and therefore the positive tumor response in rats is postulated to occur via a non-genotoxic mode of action. No increase in neoplasms were seen in CD-1 mice at doses up to 14-times the 300mg maximal clinical dose, based on plasma drug exposure.

The Leydig cell tumors occurred at all doses tested with no safety margin to clinical exposure. The tumors were considered secondary to increased leutinizing hormone and decreased testosterone, as shown by changes in these hormones in the chronic and 2yr rat toxicology studies. The greater sensitivity of rats to this mode of action coupled with a lack of change in LH in the clinical trials minimizes the risk of this tumor type to human subjects.

Renal tubule adenoma/carcinoma and adrenal pheochromocytoma increased robustly and significantly in both male and female rats at 100mg/kg, or approximately 12-times clinical exposure from a 300mg maximal clinical dose. A small numerical increase in renal tumors (2 of 65 males) was seen at the next lower dose. Arguments that the mid-dose tumors were spontaneous are tenuous; rather, the two tumors are likely related to drug, but the signal was not robust and did not achieve statistical significance.

Carbohydrate malabsorption and possibly calcium imbalance appear to be key proximal events leading to the renal and adrenal tumors. Evidence for this is found in the extensive literature connecting poorly absorbed sugars with testicular and adrenal tumors in rodents, in studies connecting carbohydrate malabsorption with renal tumors induced by acarbose in rodents, and finally from Sponsor-conducted studies connecting carbohydrate consumption to renal/adrenal pre-neoplastic endpoints altered by canagliflozin.

This supports an approach whereby the clinical relevance of adrenal and renal malignancies in rodents given SGLT2 inhibitors is assessed on a case-by-case basis, with the existence of a safety margin and the presence or absence of these key events in clinical trial participants being primary considerations. For canagliflozin, significant carbohydrate malabsorption or calcium imbalance did not occur in clinical trials, and a 5- to 7-fold safety margin exists for the renal tubular and adrenal malignancies observed in rats. This suggests a low risk that chronic exposure to canagliflozin would result in similar human cancers.

It should be emphasized, however, that the carcinogenic mode of action for canagliflozin has only been cursorily defined. Key events subsequent to carbohydrate malabsorption necessary for

the emergence of renal and adrenal tumors remain uncharacterized. As canagliflozin would be the first marketed compound in this therapeutic class whose primary target organ is the kidney, it would be prudent to follow the incidence of renal/adrenal malignancies with post-market surveillance and in any long term post-approval clinical studies.

<u>Pregnancy and Lactation</u>
Dr. Minck and I recommend that canagliflozin not be used during the second and third trimesters of pregnancy or during nursing. This recommendation is based primarily on adverse findings in a study conducted in juvenile rats. Clinically relevant doses of canagliflozin given to rats from 3 to 13 weeks of age results in dilatation of the renal pelvis and tubules and a lower rate of body growth. The same duration of drug exposure (~10 weeks) but initiated in older rats (e.g., 6wks old) does not alter renal structures. This suggests a particularly susceptible window for renal toxicity if exposed to canagliflozin during post-natal weeks 3-6. This susceptible window in the young rats is characterized by active morphological and functional development of the kidneys. A similar period covering morphological and functional renal development in humans would be during the second/third trimesters of pregnancy, with functional renal development continuing in newborns until ~2yrs of age[3,4]. Canagliflozin is also present in the milk of lactating rats at a ~1:1 ratio to maternal plasma and is transferred to weaning pups in sufficient quantities to slow body weight gain.

Dr. Minck and I agree that canagliflozin presents a (renal) developmental risk to the fetus if exposed during the 2nd/3rd trimesters of pregnancy and to the newborn if exposed during nursing.

Labeling considerations for Pregnancy and Lactation
The preclinical data support pregnancy category C with recommendations to discontinue use of the drug during the 2nd/3rd trimesters of pregnancy and during nursing. These recommendations would ideally be included in Section 5, Warnings & Precautions, and also in the Highlights section of the label.

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[3] Suzuki, M (2009) J Toxicol Sci 34;SP267-271

[4] Zoetis T and Hurtt ME (2003) Birth Defects Res 68;111-120

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/s/

TODD M BOURCIER

02/07/2013

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:	204042
Supporting document/s:	000
Applicant's letter date:	June 30, 2012
CDER stamp date:	June 30, 2012
Product:	Canagliflozin (INVOKANA®)
Indication:	Type 2 diabetes
Applicant:	Janssen Pharmaceuticals Inc.
Review Division:	DMEP
Reviewer:	Daniel Minck, PhD
Supervisor/Team Leader:	Todd Bourcier, PhD
Division Director:	Mary Parks, MD
Project Manager:	Jena Weber

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1   Executive Summary

1.1   Introduction

This document provides a critical review of the non-clinical fertility, developmental toxicity, pre- and postnatal toxicity, and juvenile toxicity studies conducted with JNJ-28431754 (Canagliflozin).

1.2   Brief Discussion of Nonclinical Findings

The pivotal nonclinical developmental and reproductive studies were conducted in accordance with GLP regulations. Safety margins were based on the human exposure (AUC of 26.1 μg·hr/mL) at the proposed maximum recommended human dose (MRHD) of 300 mg using the average of the AUCs at 300 mg qd from study DIA1023 and 300 mg bid from study DIA1007.

In the rat fertility study, no toxicologically meaningful effects were noted on male or female reproductive performance or on the ability of the females to maintain the pregnancy. As a result, the HD (100 mg/kg/day) was identified as the NOAEL for reproductive functioning. At this dosage, the exposures were 14x (males) and 18x (females) the exposure at the MRHD. However, it should be noted that at the NOAEL dosage there was minimal parental toxicity (10%

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or more decrease in weight gain) and effects on sperm velocity morphology as well as on corpora lutea counts (leading to lower numbers of implantations and smaller litter sizes). These alterations did not affect reproductive capability in the rat, and were not considered adverse based on the observed incidences being within the historical range.

In both the rat and rabbit developmental toxicity studies with JNJ-28431754 there were no effects on embryo/fetal viability or the ability to maintain the pregnancy at dosages associated with minimal maternal toxicity (reduced weight gain and altered food consumption). Based on the observed effects, the maternal NOAEL was the low dose (10 mg/kg/day) in the rat (~2x the exposure at the MRHD) and the mid dose (40 mg/kg/day) in the rabbit (~3x the exposure at the MRHD). Effects on fetal development were limited to increases in the incidence of alterations in the ossification of various structures (i.e., absent, misshapen, or atypical appearance of various skeletal structures). As variations in ossification typically disappear as the animal matures, the observed effects are not considered to be an indication of risk to fetal development. Renal structures were not affected at any dose. Consequently, the NOAELs for fetal development were the highest dosages administered to the rat (100 mg/kg/day) and rabbit (160 mg/kg/day) resulting in exposure multiple of ~19x the MRHD in both species.

In the rat pre- and postnatal toxicity study, the LD (10 mg/kg/day) was considered the NOAEL for maternal animals based on body weight and post-mortem observations at the higher dosages. For the offspring, lower pup body weights were observed at the MD/HD throughout the pre-weaning period with some recovery observed during the post-weaning period. At the HD delays in development of the righting reflex and the attainment of sexual maturity indices were noted which are considered secondary to the lower pup body weights. Although there were no effects on the ability of the pups to reproduce, there were fewer corpora lutea and a decreased number of implantations at the HD. Renal structures, including the renal pelvis, were not affected at any dose. The Sponsor identified the LD (10 mg/kg) as the NOAEL for effects on F_1 growth and the HD (100 mg/kg) as the NOAEL for effects on F_1 reproductive performance, based on their interpretation that the effects on the righting reflex, age of sexual maturation, and reproductive effects observed at the HD all being secondary to the reduced body weights. Regardless of the cause of the various effects noted on one or more parameters in the F_1 animals at the MD and HD, their occurrence leads to the identification of the LD (10 mg/kg/day; ~2x the exposure at the MRHD) as the NOAEL for offspring development by FDA reviewers.

A study was also performed in juvenile animals in which the effects observed were consistent with those occurring in adult animals. The primary in-life observations were related to the pharmacology of the compound and included increased urine volume and glucosuria leading to increased feed consumption, reduced weight gain, alterations in multiple clinical pathology endpoints, etc, observed at all dosages. The primary post-mortem observations were also consistent with those reported in adult animals in bone (hyperostosis) and kidney. Importantly, renal pelvic and/or tubule dilatation was observed at an increased incidence at all dosages and appeared after a shorter treatment period than observed in adult animals. Although renal tubule dilatation did reverse, pelvic dilatation was still evident at an increased incidence at dosages $\geq$ 2.5x the MRHD.   Based on the reversal, the Sponsor considered the LD as the NOAEL. However, since treatment-related observations occurred at all dosages (4 mg/kg/day and above; 0.5x the MRHD and above), a NOAEL was not identified by FDA reviewers.

When JNJ-28431754 and metformin were co-administered to rats in a developmental toxicity study, effects on maternal animals similar to those reported above when JNJ-28431754 was administered by itself were noted. As before, there were no effects on pregnancy parameters. However, alterations in ossification occurred at a higher incidence in the combination groups

compared to that occurring with JNJ-28431754 alone. There was no difference in the exposure to JNJ-28431754 (other than to slightly reduce the time to t_{max}), but the exposure to metformin was 1.2 (C_{max}) to 1.6 (AUC) fold higher in the combination groups compared to that seen in the metformin alone group. The data suggest there may be a slight enhancement of maternal perturbations when JNJ-28431754 and metformin are co-administered. The maternal NOAEL was the 10/300 mg/kg/day combination dosage. At this combination dosage, the maternal exposure to JNJ-28431754 was ~0.5x the exposure at the MRHD. Because ossification delays were only noted at the HD, effects generally considered reversible and that may be related to the maternal toxicity, the NOAEL for fetal development was the HD combination (60/300 mg/kg/day; ~12x the exposure at the MRHD).

In summary, there were no effects on fertility or embryo/fetal development following exposure during gestation at exposure multiples of $\geq$ 14x the MRHD. There also were no effects when exposure occurred during late gestation and through nursing at 19x the MRHD (based on developmental toxicity exposure data). However, when exposure was to juvenile animals, effects on renal development were evident at $\geq$ 0.5x the MRHD after a shorter dosing interval than for adults (eg, seen after 10 weeks in juvenile animals and at 6 months in adults) and/or at lower dosages.

1.3   Recommendations

There were no effects on fertility or offspring development that indicates this drug should be withheld from marketing. However, JNJ-28431754 does induce renal tubule and pelvic dilatation in juvenile animals, occurring at an increased incidence relative to controls and appearing after a much shorter dosing period in the juvenile animals as compared to adult animals. The period of dosing in the juvenile animal study is characterized by active morphological and functional development of the kidney and is analogous to events occurring during the last half of gestation and continuing until approximately 2 years of age in humans. As a result, appropriate statements will need to be incorporated into the label to discontinue treatment/nursing during late gestation and lactation.

1.3.1   Additional Non-Clinical Recommendations

None related to DART

1.3.2   Comments on Labeling

The results of the juvenile toxicity study reveal an effect on the maturation of the kidney, events that start during gestation and continue through the first couple of years postnatally. As such, appropriate wording regarding the risk to the fetus and nursing infant will need to be incorporated into the label.

Reviewer's recommended labeling

HIGHLIGHTS OF PRESCRIBING INFORMATION

------------------------WARNINGS AND PRECAUTIONS-----------------------

- INVOKANA must not be used in the second and third trimesters of pregnancy. Animal studies demonstrate the developing kidney can be harmed by canagliflozin. When pregnancy is detected, INVOKANA should be discontinued. There are no adequate and well-controlled studies in pregnant women. (5.5, 8.1)
- INVOKANA must not be used by a nursing woman. Studies in pregnant rats have shown excretion of INVOKANA in milk, and direct dosing of young animals results in adverse changes to the developing kidney. It is not known whether TRADENAME and/or its metabolite are excreted in human milk. (5.6, 8.3)

-----------------------USE IN SPECIFIC POPULATIONS-----------------------
- Pregnancy: There are no adequate and well-controlled studies in pregnant women. INVOKANA™ should be used during pregnancy only if clearly needed (8.1)
- Nursing mothers: Discontinue drug or nursing, taking into consideration importance of drug to the mother (8.3)

5 WARNINGS AND PRECAUTIONS
5.5 Pregnancy
Based on animal data, INVOKANA may cause fetal harm. In rat studies, exposure of juvenile animals to canagliflozin was associated with an increased incidence and/or severity of renal pelvic and tubular dilatation. These outcomes occurred with drug exposures during periods of animal development that correlate with the second and third trimesters of human pregnancy. During pregnancy, consider appropriate alternative therapies, especially during the second and third trimesters. INVOKANA should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus [see Use in Specific Populations (8.1)].

5.6 Nursing Mothers
It is not known if INVOKANA is excreted in human milk. INVOKANA is excreted in rat milk and data from a study with direct exposure to juvenile rats showed risk to the developing kidney (renal pelvic and tubular dilatations) during maturation. Human kidney maturation occurs in utero and during the first 2 years of life when lactational exposure may occur. Because many drugs are excreted in human milk, and because of the potential for serious adverse reactions in nursing infants from INVOKANA, a decision should be made whether to discontinue nursing or to discontinue INVOKANA, taking into account the importance of the drug to the mother [see *Use in Specific Populations (8.3)*].

8 USE IN SPECIFIC POPULATIONS
8.1 Pregnancy
Pregnancy Category C
Risk Summary
There are no adequate and well-controlled studies of INVOKANA 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 ≥ 0.5x clinical exposure from a 300mg dose. This outcome occurred from exposure to drug during functional maturation of the kidney and corresponding to the late second and third trimester of human development. During pregnancy, consider appropriate alternative therapies, especially during the second and third trimesters. INVOKANA should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

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Clinical Considerations
To be addressed by clinical review team

Animal Data
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 ≥0.5 times the maximum clinical dose of 300mg. 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 maximum recommended human dose.

8.3 Nursing Mothers
It is not known if INVOKANA is excreted in human milk. INVOKANA 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 INVOKANA showed risk to the kidney (renal pelvic and tubular dilatation) 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. Because many drugs are excreted in human milk, and because of the potential for serious adverse reactions in nursing infants from INVOKAN, a decision should be made whether to discontinue nursing or to discontinue INVOKANA, taking into account the importance of the drug to the mother.

2   Drug Information

2.1   Drug

Generic Name: Canagliflozin

Code Name: JNJ-28431754

Chemical Name: (1S)-1,5-anhydro-1-C-[3-[[5-(4-fluorophenyl)-2-thienyl]methyl]-4-methylphenyl]-D-Glucitol

Molecular Formula/Molecular Weight: $C_{24}H_{25}FO_5S$, MW 444.5

Structure or Biochemical Description:
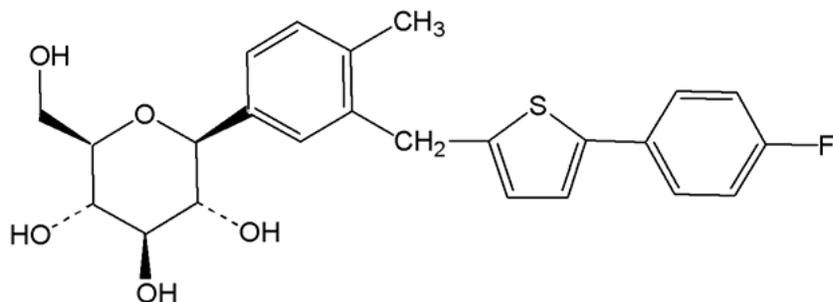
Pharmacologic Class: SGLT2 inhibition

Planned Clinical Route of Administration: Oral

3   Reproductive and Developmental Toxicology

3.1   Fertility and Early Embryonic Development

3.1.1   Oral Fertility Study of JNJ-28431754-ZAE in the Male and Female Rat

Study #	TOX8562
Study report location	seq 0034
CRO/Laboratory name and location	J&J Global Preclinical Development, Beerse Belgium
Date of study initiation	9 Oct 07
GLP compliance statement	yes
GLP issues identified	no
QA statement	yes
Drug, lot #, and % purity	Lot ZR600348PFA021; purity 97.6% (HPLC)

Key Study Findings
- Dosages of 4, 20, or 100 mg/kg/day administered to male and female rats.
- Weight gain was lower than controls in all treated male groups, although the effects resulted in absolute body weights that were greater than 10% lower than control only at the HD. In females, weight gain was ~15% lower during gestation at the HD only.
- Food consumption was increased in all treated groups of both males and females.
- There were no effects on mating performance or time to mating. Estrous cycles were not affected in females. There were reductions in sperm motility and velocity as well as a slight increase in the number of abnormal sperm at the HD, but no effects on overall sperm counts, male reproductive organ weights, or histopathology. Although effect on sperm parameters cannot be excluded as treatment-related, these alterations had no effects on the ability of the animals to sire a litter. In HD females, fewer corpora lutea were observed. Also noted was a slight reduction in the number of implants.

Reviewer Comments: The NOAEL for paternal toxicity was considered the MD. Although a specific basis for this determination was not provided, it is likely based on the > 10% reduction in absolute body weight at the HD. The NOAEL for females was the HD as there were no adverse effects noted. Although subtle effects were noted on various sperm and reproductive indices, the effects were not toxicologically significant and did not appear to effect reproductive performance, fertility, or the ability to maintain the pregnancy. As a result, the HD was identified as the NOAEL

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for reproductive performance. Based on exposure data from the 13 week study (in which the same dosages were administered), the exposure at the NOAEL for general toxicity in males is ~3x the MRHD; at the NOAEL for females and for fertility endpoints for both sexes, the exposures are ~14x for males and 18x for females relative to the exposure at the MRHD.

Methods

Doses	0, 4, 20, 100 mg/kg
Dosing Frequency	once daily; ♂ 4 wks precohabitation through determination of female fertility (~7 wks total); ♀ 2 wks precohabitation through GD7
Dose Volume	10 mL/kg
Route of Administration	oral gavage
Formulation/vehicle	aqueous suspension in demineralized water containing 0.5% w/v Methocel
Species/Strain	SPF Sprague-Dawley (Crl: CD) rat
Number/Sex/Group	24/sex/group
Study Design	standard fertility design; treated animals cohabited with each other
Protocol Deviations	none mentioned

Dosages used were the same as those utilized in the 13 week tox study. The NOAEL in that study was 4 mg/kg/day in males and 20 mg/kg/day in females based on hyperostosis. Other effects occurring at the mid and high dosage included reduced body weight gains in females (12% and 20% lower at 20 and 100 mkd, respectively), kidney mineralization, and alterations in multiple other clinical pathology parameters. At all dosages, increased urinary glucose, decreased serum glucose, increased feed consumption and other effects related to the pharmacodynamic activity of the test-article were noted.

Dosages used were the same as those utilized in the 13 week tox study. The NOAEL in that study was 4 mg/kg/day in males and 20 mg/kg/day in females based on hyperostosis. Other effects occurring at the mid and high dosage included reduced body weight gains in females (12% and 20% lower at 20 and 100 mkd, respectively), kidney mineralization, and alterations in multiple other clinical pathology parameters. At all dosages, increased urinary glucose, decreased serum glucose, increased feed consumption and other effects related to the pharmacodynamic activity of the test-article were noted.

<u>**Observations and Results**</u>
Mortality: No mortality occurred.

Clinical Signs: No toxicologically limiting adverse effects considered treatment-related were noted. Soft feces were noted for 1 cage of HD males and 2 cages of HD females during the first week of treatment. The group-housing precluded the ability to determine the actual number of animals affected.

Body Weight: In males, weight gain was lower in each treated group for each interval, with the cumulative reductions in weight gain from study start attaining statistical significance during the first week at the MD and HD and by week 3 at the LD. The reduced weight gains resulted in lower absolute body weights in all groups, with the results reaching statistical significance by the end of week 1 at the HD and by the end of week 3 at the MD. The absolute weight of the LD animals attained statistical significance only at week 6. Regardless of the reduced weight gains, only the HD group had an absolute weight more than 10% lower than controls by the end of the study. Data for selected days/intervals are summarized below:

Selected Body Weight Data - Males
Dosage - mg/kg/day (% of control value)

day/interval	0	4	20	100
0	356	355	356	354
28	467	447 (96)	447 (96)	424*** (91)
49	506	481 (95)	472* (93)	452*** (89)
0 - 28	111	92* (83)	91** (82)	80*** (72)
0 - 49	150	126* (84)	116** (77)	99*** (66)

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

For females, there were no clear dose-related effects on weight gain or body weights during the initial two week precohabitation period; animals at the LD and MD gained less weight than controls while HD animals gained more weight. During gestation, weight gain of the HD group was ~85% that of controls as a result of slightly lower weight gain during both GD 0-7 (88% of control, statistically significant) and GD 8-13 (82% of control).

> *Reviewer comment: Although the reduced weight gain at the HD during the first week of gestation was statistically significantly different from control, the magnitude was considered small and not biologically meaningful by the Sponsor. As absolute body weight data during gestation were not provided with the report, the actual difference in weights could not be evaluated to help put the weight change into perspective.*

Feed Consumption: Feed consumption was increased in all treated groups for both males and females. In males during the precohabitation period (across dose levels), the increase in mean food consumption per week ranged from 3% to 38% and generally followed a dose response pattern.   For females during precohabitation, the increases (dose related) ranged from 3% to 42% while during gestation the increases ranged from 7% to 29%. The increases in food consumption in females at all dosages during gestation were statistically significant.

Necropsy: There was a dose related increase in the incidence of distended colon (0, 4, 7, and 18 in C, L, M, and H dose groups, respectively) and cecum (0, 3, 6, and 21 in C, L, M, and H dose groups, respectively) in males. Dilation of the urinary bladed was also noted in males (1, 4, 9, and 12 in C, L, M, and H dose groups, respectively). These were attributed to the increased urine volume resulting from the pharmacologic activity of the compound. No treatment related effects were noted in females.

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.):
There were no toxicologically meaningful effects on fertility as the number of animals mating, the time to mate, and the resultant number of pregnancies were comparable between the groups. However, there were alterations in a number of parameters noted at the HD.

In females, there were no effects on estrous cyclicity. At the HD, gravid uterine weights were lower (12.6g compared to 14.3g in controls; p<0.01) than in controls, reflecting a slight decrease in litter size. The smaller litter size is likely a result from there being fewer corpora lutea that led to fewer implantations. Data are summarized in the Sponsor table below:

9

Treatment unit: mg eq./kg		Vehicle 0	Low 4	Medium 20	High 100
LITTER DATA					
Number of corpora lutea of pregancy/pregnant animal (3)		17.5	17.9	16.6	16.0 *
Number of implantations/pregnant animal	(3)	16.4	15.7	16.2	14.4 **
Pre-implantation loss (%)	(3)	5.08	6.55	4.15	8.84
Total number of resorptions / pregnant animal	(3)	0.57	0.70	1.21	0.71
Post-implantation loss (%)	(3)	3.45	8.26	7.46	5.07
Number of live fetuses/pregnant animal	(3)	15.8	15.0	15.0	13.7 ***
Number of dead fetuses/pregnant animal	(3)	0.00	0.00	0.00	0.00

Note: (3) refers to Mann-Whitney U-test; *p<0.05, **p<0.01, ***p<0.001

> *Reviewer comment: As the Sponsor indicated, the number of corpora lutea is within the range of publically available historical data, although a relationship to treatment cannot be completely ruled out. A possible male-related component may also have contributed to the fewer number of fetuses at the high dose (see below).*

In males, there were no effects on sperm count. At the HD, there was a statistically significant reduction in the percentage of progressively motile sperm, but no meaningful difference in the overall percentage of motile sperm. A more detailed analysis showed that sperm velocities (average, straight, curvilinear) were slower but the significance of these observations is unknown. There was also a slight increase in the percentage of abnormal appearing sperm, but the difference from control values was small and there was no dose response. These alterations had no effects on copulation or fertility rates, and these slight differences from control values are not considered toxicologically meaningful in the rat. Data are summarized in the Sponsor tables below:

	Vehicle 0	Low 4	Medium 20	High 100
Sperm Motility				
Animals: Examined/Total	24 / 24	23 / 24	24 / 24	24 / 24
Motile sperm (%)	79.2 (20.1)	76.0 (17.0)	77.6 (17.1)	71.0 (23.6)
	-	0.1759	0.2920	0.0692
Progressively motile sperm (%)	49.6 (20.3)	43.3 (18.1)	48.0 (17.7)	36.3 (20.2)
	-	0.1868	0.6059	0.0220*
Sperm Concentration: Cauda epididymidis				
Animals: Examined/Total	24 / 24	23 / 24	24 / 24	24 / 24
Weight (g)	0.3 (0.0)	0.3 (0.0)	0.3 (0.0)	0.3 (0.0)
	-	0.3070	0.7415	0.3325
Sperm count (millions/g)	623.0 (91.1)	605.1 (87.4)	619.9 (91.9)	633.5 (84.7)
	-	0.4959	0.8366	0.5227
Total (million)	203.4 (27.9)	201.5 (23.9)	204.3 (28.2)	213.2 (31.1)
	-	0.8149	0.5847	0.2610
Sperm Concentration: Testis				
Animals: Examined/Total	24 / 24	23 / 24	24 / 24	24 / 24
Weight (g)	1.6 (0.2)	1.6 (0.2)	1.7 (0.1)	1.7 (0.1)
	-	0.6704	0.5919	0.3865
Sperm count (millions/g)	77.4 (14.1)	85.0 (14.3)	86.6 (13.6)	83.9 (14.6)
	-	0.0773	0.0412*	0.1432
Total (million)	126.3 (22.9)	136.7 (27.2)	144.2 (24.9)	140.2 (22.6)
	-	0.1152	0.0192*	0.0634

Treatment unit: mg eq./kg
Significances computed versus Vehicle by Mann-Whitney U Test: Two Tailed
$*p<0.05, **p<0.01, ***p<0.001$

	Vehicle 0	Low 4	Medium 20	High 100
Sperm Morphology				
Animals: Examined/Total	24 / 24	23 / 24	24 / 24	23 / 24
Mean number of sperm cells examined per animal	195.2	194.6	200.0	197.3
p-val Sign.	-	0.9515	0.3173	1.0000
Normal number %	90.4	89.4	91.1	87.8
p-val Sign.	-	0.5722	0.3065	0.0440*
Abnormal number %	9.6	10.6	8.9	12.2
p-val Sign.	-	0.5722	0.3065	0.0440*

$*p<0.05$

Reviewer comment: This reviewer agrees with the Sponsor interpretation that there is no overall effect on fertility. However, it cannot be completely ruled out that the slight increase in the number of abnormal sperm in conjunction with the slight decrease in sperm with progressive motility led to the fewer implantations. The relevance of these observations to humans is unclear.

Dosing Formulation Analysis: Concentration, homogeneity, and stability of dosing formulations were acceptable.

Additional Notes:
1. The largest single unspecified impurity was [   ] (b) (4) , occurring at [   ] (b) (4) The qualification status of this impurity should be looked at.
2. Toxicokinetic data from the 13 week toxicity study were used for the estimation of exposure multiples. Data from that study (Study TOX8150) are shown below in the Sponsor table:

Table I: Mean (n=4) Plasma Toxicokinetic Parameters of JNJ-28431754 in Male and Female Rats Following Daily Oral Administration of JNJ-28431754-AAA (TOX8150)

Day	Dose (mg/kg/day)	Gender	C_{max} (ng/mL)	t_{max} (h)	AUC[a] (ng·h/mL)	$t_{1/2}$ (h)	CL/F (mL/h/kg)
0	4	Female	1210	2.75	19800	9.00	204
		Male	1000	3.75	15000	6.64	269
	20	Female	5400	3.75	104000	9.73	194
		Male	5220	2.75	84500	7.34	238
	100	Female	28300	6.00	682000[b]	12.5[b]	153[b]
		Male	31400	4.00	602000	10.3	168
90	4	Female	1500	1.75	21300	9.18	160
		Male	935	2.00	12600	15.2	214
	20	Female	6020	4.00	94600	8.33	185
		Male	6460	1.25	74400	7.45	241
	100	Female	33200	1.25	470000	9.51	182
		Male	25200	1.25	365000	8.78	234

[a] $AUC_{0-\infty}$ for Day 0 and AUC_{0-24h} for Day 90; [b] mean of n=3 values

3.2   Embryonic and Fetal Development

3.2.1   Oral Developmental Toxicity Study of JNJ-28431754-AAA in the Rat

Study #	TOX8327
Study report location	seq 0034
CRO/Laboratory name and location	J&J Global Preclinical Development, Beerse Belgium
Date of study initiation	3 May 07
GLP compliance statement	yes
GLP issues identified	no
QA statement	yes
Drug, lot #, and % purity	Lot ZR491348PFA021; purity 98.4% (HPLC)

Key Study Findings
- Dosages of 10, 30, or 100 mg/kg/day administered to rats to evaluate potential developmental toxicity.
- Transient reductions in maternal weight gain observed at the LD and MD, with the effect at the MD leading to a slightly lower weight gain (corrected) over the course of the study. At the HD, loss of weight followed by reduced weight gain was observed throughout the dosing period before a recovery was observed during the post-dosing period.
- Effects on feed consumption at the HD paralleled those on body weight.
- No effects on Cesarean section endpoints. Fetal development was not adversely impacted although there were increased skeletal variations (delays in ossification) observed. The effects on skeletal endpoints are considered transient and associated with the observed maternal toxicity.
- The bioanalytical data and subsequent toxicokinetic analysis conducted as part of this study were not considered acceptable by the Sponsor (specific reasons for this determination were

not identified). As a result, a separate bridging study was performed. Based on the data from the bridging study, exposures (AUC_{0-24}) at the end of dosing were approximately 42.5, 154.9, and 507.9 µg·hr/mL at 10, 30, and 100 mg/kg/day, respectively.

Reviewer Comments: The maternal NOAEL was identified as the LD based on the decreased weight gain over the course of the study at $\geq$ MD. At the LD, the exposure was ~2x the exposure at the MRHD. The NOAEL for developmental toxicity was the HD. Although there were minor skeletal anomalies noted at this dosage, the observed effects either did not follow a dose-response, are considered to be reflections of transient delays in the progression of normal development, or are those commonly observed in the presence of maternal toxicity. The exposure at the HD is approximately 19x the exposure at the MRHD.

Methods

Doses	0, 10, 30, 100 mg/kg
Frequency of dosing	once daily GD 6 – 17
Dose volume	10 mL/kg
Route of administration	oral gavage
Formulation/Vehicle	aqueous suspension in demineralized water containing 0.5% w/v Methocel
Species/Strain	SPF Sprague-Dawley (Crl: CD) rat
Number/Sex/Group	24 presumed gravid females/group
Satellite groups	3 (C) or 6 (L, M, H) females for tk sampling
Study design	standard

Prior to the conduct of this study, a dose-ranging study was performed (TOX8035) in which presumed gravid SD rats (8/group) were administered 0, 10, 50, or 250 mg/kg/day of JNJ-28431754-AAA on GD 6 thru 17. An additional group was also administered test article on GD 16 thru 20. Data for LD and MD animals were comparable to that of controls, with the exception of lower weight gain and feed consumption from GD 6 - 9. In contrast, treatment at the HD (both subgroups) was associated with multiple clinical signs (soft and decreased feces, wet urogenital region, dehydration, piloerection), mortality/morbidity, weight loss during the first few days of dosing and reduced weight gain over the entire dosing period, and a transient reduction in feed consumption. At Cesarean section, there were increased pre- and post-implantation losses (including total litter resorptions) resulting in decreased numbers of offspring, reduced fetal weights, and increased incidence of ossification delays of multiple structures (primarily of the sternum, ribs, vertebrae, and metatarsals) in the HD groups.

Observations and Results

Mortality: No treatment-related mortality. A single LD animal was euthanized on GD 14 following marked weight loss (100 g over 4 days) and showing red vaginal discharge. Postmortem examination revealed distention of the stomach and both the small and large intestines. In addition, all implants were resorbed. Although a specific cause of death could not be ascertained upon necropsy, the effects in this single animal were not considered treatment related in the absence of mortality or other similar signs at higher dosages (Note that mortality did occur in the drf study at 250 mg/kg; embryo/fetal loss and stomach/intestinal distention was noted in some of the early decedents as well as those surviving to study completion).

Clinical Signs: Alopecia seen sporadically in MD and HD animals, but at an incidence only slightly higher than in controls and is not considered drug-related.

Body Weight: Reduced weight gain occurred from GD 10 – 13 in the LD/MD groups resulting in a lower weight gain (adjusted for gravid uterine weight) over the study at the MD (18% lower).

Weight gain was decreased throughout the dosing period at the HD before a recovery was seen during the post-dosing period. Regardless, even with the increased weight gain at the end of the study, overall weight gain (adjusted for gravid uterine weight) was still decreased ~25% at the HD. There were no effects on gravid uterine weights. Note that absolute weight data were not provided. Data for selected intervals are summarized in the table below:

Body Weight Gain (g)

Interval[a]	Dosage (mg/kg/day)			
	0	10	30	100
GD 6 - 9	21	19	18	5***
GD 10 - 13	20	16*	11***	14**
GD 14-17	50	49	53	39***
GD 6 – 17[b]	91	84	82	58
gravid uterus	104	102	104	102
Corrected weight gain	39	35	32*	29**

a) GD = gestation day
b) reviewer calculated, no stats
*p<0.05, **p<0.01, ***p<0.001

Feed Consumption: Feed consumption was increased (6% - 8%) over controls at the LD/MD during much of the dosing period; food consumption continued to be increased (14%) during the post-dosing phase at the MD. At the HD, feed consumption was reduced (-24%) during the early treatment period (GD 6 – 9), comparable to controls through the remainder of the treatment period, and then increased (33%) over controls during the post-treatment phase.

Toxicokinetics: The bioanalytical and resultant toxicokinetic data obtained concurrent with the study were not considered acceptable leading to the conduct of a separate bridging study (FK7269) using test article lot ZR600348PFA021 (97.6% pure). The data from this subsequent study are summarized below.

Both C_{max} and AUC increased with dose in a slightly greater than proportional manner over the range evaluated. Plasma concentrations reached t_{max} between 2 and 6 hours after dose administration. There did not appear to be any accumulation with repeated dosing to pregnant animals.

Sex: Female rat

JNJ-28431754 (Plasma)										
PK Profile			Day 6				Day 16			
			C_{max}	t_{max}	t_{last}	AUC_∞	C_{max}	t_{max}	t_{last}	AUC_{last}
Treatment	Dose(mg/kg/day)	Type	ng/mL	h	h	h.ng/mL	ng/mL	h	h	h.ng/mL
Low	10	Mean	2173	5.00	24.00	32119	3337	4.00	24.00	42585
Medium	30	Mean	10883	4.00	24.00	150261	12000	4.00	24.00	154932
High	100	Mean	30633	2.00	24.00	584891	33533	6.00	24.00	507898

Necropsy: No treatment-related effects noted on maternal animals.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.): The numbers of corpora lutea, implantations, early and late resorptions, live and dead fetuses, and pre/post-implantation loss were comparable between drug-treated groups and the controls. There were a slightly higher number of early resorptions leading to an increased post-implantation loss rate at the LD, but the absence of effects on embryo/fetal viability at higher dosages support the interpretation that these effects were unrelated to treatment.

Offspring (Malformations, Variations, etc.): There were no effects on fetal weight or sex ratio. There were no treatment-related effects on fetal morphological or visceral development. Skeletal examination revealed a number of minor ossification-related anomalies in the treated groups that are considered to be transient alterations in the normal pattern of development.

There was a slight increase in the number of fetuses with reduced ossification of the metatarsal bones at the MD and HD, an effect generally regarded as an indication of delayed development resulting from maternal toxicity. There was a slight increase in the incidence of fetuses with wavy ribs at the LD and MD and of rudimentary 14th ribs in HD animals. The wavy ribs are not considered treatment-related based on the lack of a dose-response while the rudimentary rib at the HD is a common transient observation likely related to maternal toxicity. These observations are tabulated below:

Select Skeletal Observations

Observation	Dosage (mg/kg/day)			
	0	10	30	100
number evaluated (fetuses/litters)	164/23	154/23	163/23	159/24
Metatarsal				
reduced ossification (>1)	10/3	8/5	18/6	22*/8
Ribs				
wavy	2/2	7*/4	11**/4	4/4
Rudimentary rib				
bilateral	4/3	9/4	2/2	15**/10*
unilateral	1/1	11*/9*	4/4	24***/14***

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

> *Reviewer Comment: Reduced ossification of various structures is generally regarded as an indication of delayed development resulting from maternal toxicity, particularly when there is no overt teratogenic signal. The absence of a dose-response pattern to the wavy ribs support the interpretation that this observation is incidental and not related to treatment. The presence of rudimentary 14th ribs is considered to be a transient alteration as a rudimentary rib is typically incorporated into the transverse process of the vertebrae as ossification continues. The thoracic/lumbar region is also an area in which various skeletal anomalies are noted to occur when maternal toxicity is present. These minor variations in the pattern of skeletal development are not considered to be of toxicologic concern.*

Dosing Formulation Analysis: Dosing formulations were stable, within acceptable content range, and were homogenous.

3.2.2   Oral Developmental Toxicity Study of JNJ-28431754-AAA in the Rabbit

Study #	TOX8326
Study report location	seq 0034
CRO/Laboratory name and location	J&J Global Preclinical Development, Beerse Belgium
Date of study initiation	23 May 07
GLP compliance statement	yes
GLP issues identified	no
QA statement	yes
Drug, lot #, and % purity	Lot ZR491348PFA021; purity 98.4% (HPLC)

Key Study Findings

- Dosages of 10, 40, or 160 mg/kg/day administered to rabbits to evaluate potential developmental toxicity.
- A single HD animal was euthanized moribund on GD 25 as a result of persistent weight loss. Reduced to absent feces occurred in HD animals during both the treatment and post-treatment periods.
- There was a dose-related loss of body weight during the initial treatment period, but little effect on overall weight change over the entire study. Feed consumption was lower during the dosing period in the HD group, with the effects reaching statistical significance during the first half of the treatment period.
- There were no treatment-related postmortem effects in maternal animals nor were there any differences in Cesarean section parameters
- Fetal morphological development was not adversely impacted by drug treatment. There were increased incidences of variations noted in the drug-treated groups, but the observed effects are typical of those seen in rabbits or were alterations in the degree of ossification that are considered transient.
- Exposures (AUC_{0-24}) at the end of dosing were approximately 13.0, 84.9, and 486.6 µg·hr/mL at 10, 40, and 160 mg/kg/day, respectively.

Reviewer Comments: The maternal NOAEL for general toxicity was identified as the MD. Although the Sponsor did not specify a reason, it is likely based on the mortality and reduced feed consumption evident at the HD. At the NOAEL, the exposure was ~3x the exposure at the MRHD. The NOAEL for developmental toxicity was the HD even though there were minor visceral and skeletal anomalies noted in this group. However, the observed anomalies did not follow a dose-response, are commonly seen in the laboratory rabbit, and/or were considered to be reflections of transient delays in the progression of normal skeletal system development. The exposure at the HD is approximately 19x the exposure at the MRHD.

It should also be noted that there were fewer than the ICH recommended number of litters with viable fetuses for evaluation. This issue is discussed further in the Integrated Summary.

Methods

Doses	0, 10, 40, 160 mg/kg
Frequency of dosing	once daily GD 6 – 19
Dose volume	5 mL/kg
Route of administration	oral gavage
Formulation/Vehicle	aqueous suspension in demineralized water containing 0.5% w/v Methocel
Species/Strain	SPF New Zealand White rabbits
Number/Sex/Group	20 presumed gravid females/group
Satellite groups	NA
Study design	standard

Prior to initiating this study a dose-ranging study (TOX8147) was performed in which dosages of 0, 25, 100, 200, or 300 mg/kg were administered to presumed gravid rabbits (5/group) from GD 6 through 19. At 300 mg/kg, one animal was found dead and the remaining 4 animals were euthanized in moribund condition as a result of reduced to no fecal output. A dose-related loss of weight occurred during the initial treatment period in all drug-treated groups but weight gain was generally similar between groups thereafter, although inter- and intra-group variations were noted. Feed consumption was slightly lower during the dosing period at 200 mg/kg. Cesarean sections revealed increased pre- and post-implantation loss resulting in fewer live fetuses at 200 mg/kg. No external malformations were noted in the fetuses.

Observations and Results

Mortality: A single HD animal was euthanized on GD 25 following a period of prolonged weight loss and minimal food consumption. Postmortem examination revealed soft contents and distention in the cecum but little else; no implants were noted. A single C animal was found dead on day GD 20. Postmortem examination revealed hemorrhagic contents in the pleural cavity indicative of gavage trauma.

Clinical Signs: There was an increased incidence of reduced to no feces at the HD. Red vaginal discharge was noted in 3 HD animals, 1 of which subsequently delivered early.

Body Weight: A dose-related loss of weight was observed at the initiation of treatment between GD 6 to 8 in all drug-treated groups (-4, -17, -83g in the L, M, and H dose groups, respectively, as compared to a 19g gain in C), with the effects in the MD and HD reaching statistical significance. Weight gain for subsequent intervals during the treatment period in the drug-treated groups was variable, ranging from less than controls to greater than controls with no consistent pattern or relation to dose. During the post dose period, increased weight gain was seen in both the MD and HD groups relative to controls over the last few days of the study Days 25 to 27). The weight loss at the HD during the early treatment period was mostly recovered from by the end of the study, based on overall weight gain at the HD being within 5% of the overall weight gain seen in controls. Following adjustment for gravid uterine weights, all groups (including controls) lost weight in a non dose-related manner. Data are summarized for select intervals in the reviewer created table below:

Maternal Weight Gain
Dosage level (mg/kg/day)

interval	0	10	40	160
GD 6 – 19	155	172	164	52
GD 20 – 27	128	108	159	218
GD 6 – 27	283	280	323	270
gravid uterine wt	421	403	424	401
corrected wt gain	-138	-123	-101	-131

Interval weight gains reviewer calculated based on multiple weight gain interval data included with report. Slight differences in calculated figures result from rounding

Feed Consumption: Feed consumption was reduced throughout the dosing and post-dosing period at the HD, with the effects reaching statistical significance from GD 6 through 12. Feed consumption was also lower for various intervals at the LD and MD, but none of the effects were statistically significant.

Toxicokinetics: Exposure increased with dose in a greater than proportional manner. There appeared to be accumulation upon repeated dosing at the low and mid dosages, but not at the high dosage. Peak concentration were seen between 1 and 7 hours post-dosing, with t_{max} occurring later as the dosage increased [mean day 19 t_{max} (reviewer calculated) occurring at ~1.3, 2.3, and 5.3 hours post dosing in the L, M, and H dose groups, respectively].

	Female rabbit		
Dose (mg/kg/day)	10	40	160
	Day 6		
C_{max} (ng/ml)	1599	7280	48633
$AUC_{0\text{-inf}}$ (ng.h/ml)	5963	52736	517834
	Day 19		
C_{max} (ng/ml)	2390	10500	38067
$AUC_{0\text{-24 h}}$ (ng.h/ml)	13010	84858	486556

Necropsy: No treatment-related postmortem observations were noted.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, Litter Size, etc.): No effects on Cesarean sectioning parameters were observed.

Offspring (Malformations, Variations, etc.): There were no effects on fetal weight although the number of fetuses considered small was increased in the MD and HD groups (2, 2, 5, and 4 in the C, L, M, and H dose groups, respectively). There were no abnormalities noted that were considered treatment related, although a number of malformations were observed. Observed malformations included:

- 1 C fetus with domed head and hydrocephalus
- 1 MD fetus with acephalostomia, umbilical hernia, and absent digit
- 1 HD fetus with exencephaly

The MD and HD fetuses mentioned above were also small (each approximately 17g vs. average group weights of ~32g). The group distribution and dissimilar observations support the interpretation that these effects are not related to treatment.

Visceral examination revealed an increased incidence of common variations. There were 3 fetuses in both the MD and HD groups (from 2 and 1 litter, respectively) that had an absent accessory lobe of the lung, and an increased incidence of an additional artery arising from the aorta (note: likely the common carotid branching directly from the aorta rather than from the brachiocephalic trunk) in 11 fetuses (5 litters) of the HD group as compared to occurring in 2 fetuses (1 litter) from the control group. These observations are considered minor variations.

Skeletal examination revealed effects on the ossification of the hyoid, sternum, and lumbar vertebra as summarized in the table below:

Select Skeletal Observations

Observation	Dosage (mg/kg/day)			
	0	10	40	160
number evaluated (fetuses/litters)	145/15	128/15	173/19	117/14
Hyoid				
incomplete ossification	3/3	10*/5	2/2	10**/7
Sternum				
asymmetrical	-	3/3	5*/5*	3*/3*
unossified 5th	8/4	3/2	9/5	5/3
rudimentary 5th	7/4	17**/7	12/9	7/5
Lumbar vertebra				
rudimentary rib bilateral	2/2	-	2/2	1/1
rudimentary rib unilateral	4/4	6/6	9/5	4/2
rudimentary + extra rib	4/4	3/3	4/4	1/1
extra rib unilateral	13/9	12/9	11/8	5/4
extra rib bilateral	69/14	76*/15	115***/18	96***/14

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

The increased incidence of alterations in the normal ossification pattern of various structures in fetuses from the treated groups are considered minor variations.

Dosing Formulation Analysis: Dosing formulations were stable, within acceptable content range, and were homogenous.

3.3   Prenatal and Postnatal Development

3.3.1   JNJ-28431754-ZAE: Pre- and Post-Natal Development Study in the CD Rat by Oral Gavage Administration

Study #	TOX9382
Study report location	seq 0137
CRO/Laboratory name and location	(b) (4)
Date of study initiation	July 6, 2009
GLP compliance statement	yes (April 1, 2010)
GLP issues identified	none
QA statement	yes
Drug, lot #, and % purity	Sponsor Lot# ZR600348PFA021, purity 97.6%

Key Study Findings
- Dosages of 10, 30, or 100 mg/kg/day administered to rats to evaluate potential effects on pre- and post-natal development.

In F_0 animals:
- Clinical signs and reduced body weight gains present at MD/HD. Increased feed consumption observed in all groups during the last half of gestation and into the lactation period was an expected pharmacologic event.
- A dose related increase in the incidence in gastrointestinal track distention and abnormal gut contents seen in all treated groups. Minimal abdominal adipose tissue seen at HD.
- No effect on gestation length or rearing of litter to weaning.
- Exposure data were not collected as part of this study. However, at the same dosages from the developmental toxicity study, the exposures (AUC_{0-24}) at the end of dosing were approximately 42.5, 154.9, and 507.9 µg·hr/mL at 10, 30, and 100 mg/kg/day, respectively.

In F_1 animals
- Weight gains at the MD/HD were lower than control during lactation period, resulting in lower body weights at weaning at MD (-10%) and HD (-30%). Some recovery occurred post-weaning as body weights just prior to mating were within 10% of control at the HD.
- Delay in the age at which air righting and sexual maturation were demonstrated was evident in the HD pups; these effects are considered secondary to the body weight effects. There were no effects on development of other sensory reflexes, motor activity, or tests of learning and memory.
- There was no drug effect on the ability of F_1 animals to mate and produce litters. However, there were fewer corpora lutea and an increased incidence of pre-implantation loss leading to fewer implantations at the HD. As a result, there were fewer live embryos.
- There were no notable necropsy observations for F_1 pups.

Reviewer Comments:
The NOAEL for F_0 maternal animals was considered the LD of 10 mg/kg/day based on body weight and post-mortem observations at the higher dosages. For F_1 animals, the Sponsor identified two NOAELs. The dosage of 10 mg/kg/day was identified as the NOAEL for general effects on the F_1 animals based on lower body weights of pups at the MD/HD while 100 mg/kg/day was identified as the NOAEL for effects on functional development and reproductive performance, proposing that the observed delays on development of the air righting response and attainment of sexual maturation, and the fewer corpora lutea/decreased number of implantations at the HD are all secondary to the lower body weights of the F_1 animals. Regardless of the reason for the observed effects on F_1 endpoints, effects were evident at the

MD and/or HD. Consequently, the NOAEL for the F_1 generation in this study is also considered the LD.

Methods

Doses	10, 30 and 100
Frequency of dosing	once a day from gestation Day 6 (DG6) until lactation Day 20 (DL20)
Dose volume	10 ml/kg
Route of administration	oral gavage
Formulation/Vehicle	0.5% methocel (hydroxypropyl methylcellulose, F4 M premium grade)
Species/Strain	Crl:CD® (SD) rats
Number/Sex/Group	24/sex/group
Satellite groups	yes
Study design	standard Seg III study
Deviation from study protocol	Not notable

Dosages were selected based on the results of a range finding study (TOX9298) in which 0, 10, 30, or 100 mg/kg were administered from GD 6 through lactation day 6, with delivered offspring evaluated through day 7. Weight gain was reduced at the HD during the initial few days of treatment (74% lower) which led to decreased weight gain for the remainder of gestation, but showed increased weight gain during lactation. A similar pattern was seen for feed consumption. Gestation length, litter size and offspring survival were unaffected. Pup weights were similar between all groups on day 1, but weight gain d1 – 7) of HD pups was lower (25% and 34% lower in males and females, respectively), resulting in d7 body weights that were 13% ($\male$) and 16% ($\female$) lower than C. Based on the slight toxicities evident in this ranging study, the same dosages were selected for use in the pivotal study.

Observations and Results
F_0 Dams
Clinical Signs: Pale loose feces at MD and/or HD during both gestation and lactation, and abdominal distention seen in HD dams during lactation. Abdominal distention also occurred in a single LD and two MD dams during lactation. The distention was due to changes in the intestinal track (see Necropsy section below).

Body Weight: Decreased weight gain from GD 6 – 10 at MD led to statistically significant lower GD 10 body weights. Body weights at this dose remained lower than C through the remainder of gestation and into lactation, though the differences did not always attain statistical significance. Weight gain was increased (statistically significant) over controls during the remainder of the lactation period such that LD 21 weights were comparable to controls. At the HD, weight was lost during the first few days of treatment, and weight gain was lower thereafter for the remainder of gestation. During lactation, weight gain was increased over controls, particularly from LD 1-4 and 14-21, leading to LD 21 weights that were similar to controls.

Food Intake: Food intake was lower GD 6-10 at the HD. All treated groups had increased food consumption from GD 10-19 and continuing through LD 6, although the increases did not follow a dose-response. These periods of increased feed intake generally resulted in higher average feed consumption through both the gestation and lactation periods. The effect on feed consumption is expected based on the pharmacology of the compound.

Gestation/Parturition Assessment: There were no effects on the ability of animals to maintain their pregnancy, gestation length, parturition, litter size, or care of litters.

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Necropsy: Distention of various regions of the gastrointestinal track were noted in a few LD animals, about half the MD animals, and the majority of HD animals. The incidence and number of regions affected increased with dose. Associated with the distention were pale and soft feces, particularly at the HD. Rat at the HD also had minimal abdominal adipose tissue at the time of necropsy on LD 21 of lactation.

Maternal data summary
F_0 data are summarized in the tables below:

JNJ-28431754-ZAE: Pre- and Post-Natal Development Study in the CD Rat by Oral Gavage Administration

Species/Strain: Rat/ Crl:CD® (SD)   Duration of Dosing: F0 generation from Day 6 after mating to Day 20 of lactation

Age at First Dose: 11 weeks   Duration of Postdose: None
Date of First Dose: 6 July 2009   Route: Oral gavage
Vehicle/Formulation: Aqueous suspension containing 0.5% w/v Methocel (hydroxypropyl methylcellulose F4m premium grade)
Special Features:
No Observed Adverse Effect Level:

Daily Dose (mg/kg)	0 (Control)		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
	M:	F:	M:	F:	M:	F:	M:	F:
No. of Animals (F0)	-	24	-	24	-	24	-	24
No. of Animals (selectedF1)	24	24	24	24	24	24	24	24
F0 Females								
No. Pregnant		24		24		24		24
No. died or Sacrificed Moribund		0		0		0		0
No. Aborted or with total Res. of Litter		0		0		1		0
Clinical Observations (c)								
Gestation - Faeces Abnormal Colour, Pale		0		0		9		23
Faeces Loose		0		0		1		23
Increased Urine Output		0		4		8		17
Lactation - Faeces Abnormal Colour, Pale		0		2		10		23
Faeces Loose		0		0		2		18
Increased Urine Output		0		10		16		18
Abdominal Distention		0		1		2		19

a At end of gestation or lactation. For controls, group means are shown. For treated groups, multiples of controls are shown. Statistical significance is based on actual data (not   differences of Controls)   - No noteworthy findings

b Actual data are shown.   c number of animals shown

William's test * - p<0.05   ** - p<0.01; Steel's test ^^ - p<0.01; Shirley's test ++ - p<0.01
G = Gestation day   L = Lactation day

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Daily Dose (mg/kg/day)	0 (Control)		10		30		100	
	M:	F:	M:	F:	M:	F:	M:	F:
No. of Animals (F0)	-	24	-	24	-	24	-	24
No. of Animals (selectedF1)	24	24	24	24	24	24	24	24
Dosing Observations (c)								
Gestation - Chin Rubbing		0		0		0		12
Lactation - Chin Rubbing		0		0		0		9
Necropsy Observations		-		-		-		-
Gestation - Body Weight (a)		410g		x0.99		x0.97		x0.92
Body Weight Gain G6-20 (b)		120		119		106*		85**
Lactation - Body Weight (a)		358g		x1.02		x1.01		x1.03
Body Weight Gain L1 21 (b)		43		60**		57**		78**
Gestation Total Food Consumption (a)		31g/day		x1.12++		x1.06++		1.10++
Lactation − Total Food Consumption (a)		90g/day		x1.11^^		x0.99		X0.97

a At end of gestation or lactation. For controls, group means are shown. For treated groups, multiples of controls are shown.
Statistical significance is based on actual data (not differences of Controls)   - No noteworthy findings
b Actual data are shown.   c number of animals shown
William's test * - p<0.05 ** - p<0.01; Steel's test ^^ - p<0.01; Shirley's test ++ - p<0.01   - No noteworthy findings
G = Gestation day   L = Lactation day

F₁ Generation

Clinical Signs/Survival: There were no clinical signs in offspring related to maternal treatment. A statistically significant decrease in pups surviving to PND 4 at the HD was considered unrelated to treatment as the number of pups/litter was increased over control at day 1 and 4.

Body Weight: The body weights of pups at day 1 were significantly lower than controls (7% and 8% lower in ♂ and ♀, respectively). BW gain of F1 generation pups at MD and HD were lower than controls, resulting in absolute pups weights that were significantly lower than controls, with the effect appearing earlier at the HD than the MD. As a result, PND 21 pup (both ♂ and ♀) weights were ~10% and ~30% lower than controls at the MD and HD, respectively. Following weaning, pups at the MD and HD recovered somewhat as their weights prior to cohabitation were within 10% of controls (but still statistically lower at the HD). The body weight of HD females was ~95% of controls at the start of gestation and remained ~5% lower than controls through termination on GD 14. Pup weights at selected ages/intervals are shown in the reviewer created table below:

	Postnatal Body Weights (g)							
	Males				Females			
Day[a]	0	10	30	100	0	10	30	100
PND 1	6.8	6.9	6.8	6.3**	6.5	6.6	6.5	6.0**
PND 7	15.1	15.1	14.8	12.4**	14.7	14.6	13.9*	12.0**
PND 21	51.3	50.8	46.1**	36.2**	50.4	49.2	44.0**	35.2**
PND 1 – 21	44.4	43.9	39.3**	29.9**	43.9	42.6	37.5**	29.2**
PND 35[b]	129	128	122	102**	115	113	108	96**
PND 42[b]	194	196	185	160**	159	156	151*	139**
PND 57[b]	321	324	303	277**	216	212	207*	201**
PND 67[b]	391	395	373	345**	244	239	234*	229**
PND 90[b]	471	481	458	433**				
GD 0					258	256	248*	245**
GD 6					294	290	281*	278**
GD 14					342	339	326**	324**

a) PND = postnatal day, GD = gestation day

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b) approximate age as study data were presented as days after selection, with day
1 at ~5 weeks of age
*p<0.05, **p<0.01

Sensory/Activity/Learning and Memory: There were no effects considered directly treatment-related on any of these parameters. A slight delay in the age of surface righting and delay in the age of sexual maturation at the HD were considered secondary to the lower body weights at this dosage.

Offspring data summary (pre-mating)
F_1 generation data are summarized in the tables below:

Daily Dose (mg/kg/day)	0 (Control)		10		30		100	
	M:	F:	M:	F:	M:	F:	M:	F:
No. of Animals (F0)	-	24	-	24	-	24	-	24
No. of Animals (selected F1)	24	24	24	24	24	24	24	24
Abnormal Parturition		-		-		-		-
F1 litters (preweaning) (combined values presented centred)								
No. Litters Evaluated ([c])	24		24		23		24	
Mean No. of Implantations ([b])	15.3		15.0		15.5		15.8	
Mean No. Pups/Litter ([b])	14.0		14.3		13.9		15.0	
Mean No. Liveborn Pups/Litter ([b])	13.9		14.2		13.7		14.7	
Postnatal Survival to Day 4 of age (%[b])	100.0		99.3		99.7		98.9[#]	
Postnatal Survival to Weaning (%[b])	99.6		99.2		98.7		100.0	
No. of Total Litter Losses ([b])	0		0		1		0	
Bodyweight Change ([b]) Day 1-21 of age(g)	44.4	43.9	43.9	42.6	39.3[@@]	37.5[@@]	29.9[@@]	29.2[@@]
Bodyweight Change ([b]) Day 21-28 of age (g)	35.5	32.1	38.4	32.7	34.6	29.6	31.1[@@]	29.1[@]
Pup Sex Ratios (% males)	51.4		51.5		42.6		52.9	
Pup Clinical Signs	-		-		-		-	
Surface righting (Mean Day of age)	4.1		3.9		3.9		4.3	
Air righting (Mean Day of age)	17.0		17.0		17.3		17.9[@@]	
Pupil reflex (% pass)	100		100		100		99.6	
Startle response (% pass)	100		100		100		100	
Pup necropsy Obs.	-		-		-		-	

[b] Actual data are shown.   [c] number of animals shown
Log transformed data Williams test [@] - p<0.05, [@@] - p<0.01; Fisher's exact test [#] - p<0.05   - No noteworthy findings

Daily Dose (mg/kg/day)	0 (Control)		10		30		100	
	M:	F:	M:	F:	M:	F:	M:	F:
No. of Animals (F0)	-	24	-	24	-	24	-	24
No. of Animals (selectedF1)	24	24	24	24	24	24	24	24
Selected F1 males and females (post weaning)								
No. Animals Evaluated	24	24	24	24	24	24	24	24
No. Died or Sacrificed Moribund	1	0	0	0	0	0	0	0
Clinical Observations	-	-	-	-	-	-	-	-
Necropsy Observations	-	-	-	-	-	-	-	-
Bodyweight Change ([b]) (g) (D1-53)	342		353		336		330	
Premating Bodyweight Change[a] (g) (D1-32)		129		126		126		133
Gestation Bodyweight Change (g) (G0-14)		84		83		79		79
Sexual maturation (day of age sexual maturation attained)	44	35	44	35	44	35	46**	36*
Accelerating Rotarod (d)	210	208	201	208	198	199	193	200
Motor Activity (Beam Breaks High)	121.9	93.4	96.7	123.9	83.2	90.1	71.8	57.9
Motor Activity (Beam Breaks Low)	603.7	445.2	478.8	501.7	471.2	440.3	465.1	364.4
Learning and Memory (Morris Maze)	-	-	-	-	-	-	-	-

[b]   Actual data are shown.

d – Animals showed learning/memory as judged by marked improvements in performance between Days 1 and 4 of testing.
D = day of selected F1 generation (Day 1 = aprrox. 5 weeks of age)
William's test * - p<0.05   ** - p<0.01   - No noteworthy findings

Reproductive Performance: There were no effects on the time to mate, mating performance, or fertility of the F_1 animals. There were fewer corpora lutea and resultant numbers of implantations at the MD and HD, with the lower numbers attaining statistical significance at the HD. There was also an increased pre-implantation loss rate at the HD. As a result, the number of live embryos was lower (-12%; statistically significant) at the HD.

Reproductive Performance of F_1 Generation

Daily Dose (mg/kg)	0 (control)		10		30		100	
	M	F	M	F	M	F	M	F
No. of animals F_1	24	24	24	24	24	24	24	24
Pre-coital interval (% with 1 - 4 days)		92		100		100		92
Pre-coital interval (% with 5 - 8 days)		8		0		0		8
No. mated		24		24		24		24
No. fertile		23		22		24		23
No. corpora lutea (mean)		16.1		17.0		15.3		14.6*
No. implantations (mean)		16.0		16.0		14.6		13.6*
% Pre-implantation loss (mean)		1.0		5.8		4.7		7.8*
% Post-implantation loss (mean)		7.3		4.0		7.7		3.6
No. live embryos		14.8		15.3		13.8		13.0*

*p<0.05

Reviewer Comment: The Sponsor proposed that the effects on reproductive endpoints of the F_1 generation were secondary to the low F_1 body weights at mating (~5% lower) which would reduce the ovulation rate. However, this is speculative as no data or literature directly supporting this hypothesis was provided.

Postmortem examination: There were no necropsy observations considered related to maternal treatment. Notably, there were no effects on the offspring kidneys considered treatment related; the only renal system effects noted in the F_1 animals was enlargement of the kidney observed in a single C male and renal pelvis dilatation observed in 2 C and 1 MD male and in 1 MD and 1 HD female. No histopathology or organ weight information was collected.

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Dosing Formulation Analysis: The concentration, homogeneity, and stability of dosing formulations were acceptable.

4   Combination with Metformin

4.1   Embryonic Fetal Development

4.1.1   Oral Developmental Toxicity Study of JNJ-28431754-ZAE in the Rat

Study #	TOX9590
Study report location	seq 0210
CRO/Laboratory name and location	J&J Global Preclinical Development, Beerse Belgium
Date of study initiation	25 Feb 10
GLP compliance statement	yes
GLP issues identified	no
QA statement	yes
Drug, lot #, and % purity	Lot ZR600348PFA091; purity 97.5% (HPLC)

Key Study Findings

- JNJ/metformin dosages of 0/0, 10/300, 30/300, 60/300, 60/0, 0/300 mg/kg were administered.
- Dose-related reductions in weight gain were observed in all combination drug-treated groups during the initial treatment period. Reduced weight gains were also observed in the JNJ-28431754 and metformin alone groups during this same interval, with the magnitude of the effect being greater in the HD combination group relative to that seen with either entity alone. The effects during this early treatment period led to reductions in overall weight gain over the course of the study, with the largest effect observed in the HD combination group.
- Feed consumption reductions were seen in the MD and HD combination and both single treatment groups during the initial treatment period.
- No effects noted for Cesarean section endpoints. There were no significant effects on fetal development, although there were increased incidences of skeletal variations observed in the combination groups compared to that occurring in the control or JNJ-28431754 alone group. The effects on skeletal endpoints are considered transient and associated with the observed maternal toxicity.
- With combination treatment, t_{max} occurred earlier for JNJ-28431754 in combination with metformin as compared to that observed when JNJ-28431754 was administered by itself. The co-administration of metformin did not affect the C_{max} or AUC of JNJ-28431754. However, it did appear to increase the exposure (both C_{max} and AUC) to metformin.

Reviewer Comments: Maternal toxicity (reductions in weight gain and food consumption) was evident in groups administered the combination and JNJ-28431754 alone, although the magnitude of effects was increased with the combination in this study. Maternal weight gain was significantly lower (dose-related) during the initial treatment period in all but the lowest combination group, contributing to reduced corrected weight gains at the 30/300 and 60/300 mg/kg/day groups. Effects on feed consumption showed a similar profile. The tk profile showed a slight delay in t_{max} but no difference in exposure to JNJ-28431754 when combined with metformin. Conversely, exposure to metformin was lower when it was combined with JNJ-28431754 as compared to that observed when it was administered by itself. The combination did not affect maternal Cesarean section parameters. Regarding fetal development, treatment related effects were limited to alterations in fetal ossification, with the incidence of delayed ossification occurring in the combination groups appearing to be increased over the incidence seen with JNJ-28431754 alone in this study. These effects were likely related to the reductions

in maternal weight gain. Based on these results, the maternal NOAEL was the 10/300 mg/kg/day combination dosage while the NOAEL for fetal effects was the 60/300 mg/kg/dosage as the observed effects were considered secondary to the maternal toxicity.

Methods

Doses (drug/metformin)	0/0, 10/300, 30/300, 60/300, 60/0, 0/300 mg/kg
Frequency of dosing	once daily GD 6 – 17
Dose volume	10 mL/kg/day as 5 mL/kg/day of each formulation
Route of administration	oral gavage
Formulation/Vehicle	drug and metformin - aqueous suspension (drug) or solution (metformin) in demineralized water containing 0.5% w/v Methocel
Species/Strain	SPF Sprague-Dawley (Crl: CD) rat
Number/Sex/Group	22 presumed gravid females/group
Satellite groups	3 females/group for tk sampling
Study design	standard developmental tox study endpoints

Prior to the initiation of this study, a dose-ranging study was performed (TOX9521) using the same dose levels of the two drugs (different lot # of the JNJ-28431754) that were subsequently administered in the formal developmental toxicity study. There was no mortality or clinical signs. Weight gain was lower than controls during the initial treatment period in all combination groups and the group receiving the JNJ-28431754 alone, but the effect attained statistical significance only for the HD combination group. Food consumption was also reduced for this group during the same interval. During the remainder of the dosing period, body weights and food consumption were generally comparable between the various treated groups and control although there were periodic occurrences of statistically significant differences noted. For all drug treated groups, both weight gain and food consumption were increased during the post-dosing period. There were no treatment-related effects on Cesarean section parameters or on fetal external morphological development noted.

Observations and Results

Note: Dosages are shown as the JNJ-28431754 dose/metformin dose

Mortality: No treatment-related mortality.

Clinical Signs: No adverse clinical signs, although it was reported that water consumption was increased at JNJ-28431754 dosages $\geq$ 30 mg/kg alone or in combination with metformin.

Body Weight: Reduced weight gain was seen in all drug treated groups during the initial (GD 6-9) treatment period (-30%, -55%, -96%, -75%, -38% lower compared to controls in the 10/300, 30/300, 60/300, 60/0, and 0/300, respectively). The reductions were statistically significant with the exception of that occurring in the 10/300 group. Weight gain through the remainder of the treatment period was generally comparable between the groups through the end of the study with the exception of an additional period of lower weight gain in the 60/300 group at the end of treatment and during the post-dose period when animals in the 60/300 and 60/0 groups showed statistically significant increased weight gain relative to controls. Primarily as a result of the reduced weight gain during the early treatment period, overall weight gain [corrected for gravid uterine weight (comparable between groups)] was lower in all drug-treated groups (-10%, -24%, -32%, -15%, and -20% in the 10/300, 30/300, 60/300, 60/0, and 0/300, respectively), with the effects in the 30/300 and 60/300 groups reaching statistical significance. Note that absolute weight data were not provided. Data for selected intervals are tabulated below:

Weight Gain Data
Dosage (J&J compound/metformin in mg/kg/day)

Interval[a]	0	10/300	30/300	60/300	60/0	0/300
GD 6 - 9	20	14	9***	1***	5***	12*
GD 10 - 13	19	19	18	22	22	17
GD 14 - 17	45	48	41	35***	43	50
GD 6 - 17	84	81	68	58	70	79
Gravid uterus	98	102	93	95	98	104
Corrected weight gain	38	34	29*	26**	32	30

a) GD = gestation day
b) reviewer calculated, no stats
*p<0.05, **p<0.01, ***p<0.001

Feed Consumption: Feed consumption was 7%, 23%, 14%, and 7% lower than controls in the 30/300, 60/300, 60/0, and 0/300 groups, respectively, during the initial (GD 6-9) treatment interval, with the effects reaching statistical significance in all but the metformin-alone group. During subsequent intervals, periodic increases over controls were noted in many of the groups while all groups but the 0/300 group showing statistically significant increases (12% to 22%) over controls during the post-dosing period.

Toxicokinetics: The toxicokinetic data for both the JNJ-28431754 and metformin are summarized in the Sponsor tables below.

For JNJ-28431754, t_{max} occurred at a slightly earlier time-point when co-administered with metformin as compared to that observed following administration by itself (note: t_{max} ranged from 4 to 6 hours in the developmental toxicity study with JNJ-28431754 alone). Exposure to JNJ-28431754 increased dose-proportionally over the range of dosages evaluated, but there was no accumulation. The C_{max} and AUC were similar for JNJ-28431754 when administered alone or in combination (and were comparable to that seen in the developmental toxicity study with JNJ-28431754 alone).

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

Group	Compound Dosed	Dose	C_{max}	t_{max}	$AUC_{(0-24 h)}$
		(mg eq./kg/day)	(ng/mL)	(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)

For metformin, the dosage or presence of JNJ-28431754 did not alter t_{max}. However, both the C_{max} and AUC of metformin were higher (1.2 and 1.6x, respectively) when co-administered with JNJ-28431754 compared to that seen when administered by itself.

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

Group	Compound Dosed	Dose	C_{max}	t_{max}	$AUC_{(0-24 h)}$
		(mg eq./kg/day)	(ng/mL)	(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)

Necropsy: No treatment-related effects noted.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.): The number of pregnant animals and the numbers of corpora lutea, implantations, resorptions, live and dead fetuses, and pre/post-implantation loss were comparable between drug-treated groups and the controls.

Offspring (Malformations, Variations, etc.): There were no effects on fetal weight or sex ratio. There were no treatment-related effects on fetal morphological or visceral development. Skeletal examination revealed a number of minor ossification-related anomalies in the treated groups that are considered to be transient alterations.

There were increases in the number of fetuses with reduced ossification of various bones of the skull, vertebral column, sternum, and metatarsals in the combination groups as compared to the incidence in either the control or JNJ-28431754 alone groups. There was also an increase in the incidence of wavy ribs in the JNJ-28431754 alone group and the low dose combination group. Selected skeletal observations are tabulated below:

Select Skeletal Observations

Observation	0/1	10/300	30/300	60/300	60/0	0/300
			Dosage in mg/kg/day (J&J drug/metformin)			
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
* $p<0.05$; **$p<0.01$; ***$p<0.001$

Reviewer Comment: Statistically significant increases in the incidence of altered ossification tended to most frequently appear in the 60/300 combination group followed by the lower dose combination groups and the metformin alone group. Reduced ossification is generally regarded as an indication of delayed development resulting from maternal toxicity which occurred in the form of reduced weight gain in this study.

Dosing Formulation Analysis: Dosing formulations were stable, within acceptable content range, and were homogenous.

5   Additional Toxicology

5.1   Juvenile Toxicity

5.1.1   JNJ-28431754-ZAE: Juvenile Toxicity Study in the Crl:CD(SD) Rat by Oral Gavage Administration

Study #	TOX10286
Study report location	seq
CRO/Laboratory name and location	(b) (4)
Date of study initiation	24 Feb 12
GLP compliance statement	yes
GLP issues identified	no
QA statement	yes
Drug, lot #, and % purity	Lot ZR600348PFA271; purity 99.8% (HPLC)

Key Study Findings

- Dosages of 0, 4, 20, 65, or 100 mg/kg were administered from day 21 to 90 followed by a 4 week recovery period.
- No mortality. Soft/swollen abdomen present at $\geq$ 65 mg/kg/day.
- Dose-related reductions (reduced 10% to 18%) in weight gain were observed throughout the treatment period in all groups of males, appearing earlier during treatment at the higher dosages. In females, reduced weight gains (13% to 17% lower) were observed in females at $\geq$ 65 mg/kg/day during the first two weeks of treatment. Associated with the reduced weight gains were reductions in ulna growth and delays in the age of sexual maturation.
- Feed consumption was increased in all groups during the latter portion of the treatment period.
- Slight reductions in hemoglobin, hematocrit, and erythrocyte concentrations were observed in males and/or females at dosages $\geq$ 65 mg/kg/day, and reduced platelet counts in both sexes at 100 mg/kg/day. No effects seen at the end of recovery.
- Shortening activated partial thromboplastin time observed in males and females at 100 mg/kg/day; no effect at recovery.
- Clinical chemistry alterations included increases (up to 68%) in multiple parameters (eg, ALP, ALT, AST, CK, urea, Na, and Cl) in males and females in all groups. Decreases in various other parameters (reductions of less than 50%) were also noted in all groups. The observations did not necessarily follow a dose response, were observed at all dose levels for some of the parameters, and were generally reversible.
- Increases in urine output and changes in the composition of the urine were noted at all dosages, including glucose, protein, and all measured electrolytes.
- Postmortem examination revealed effects on GI track tissues, bone, kidney, and spleen. Thickening and/or distention of the jejunum, ileum, cecum, and duodenum were observed at dosages $\geq$ 20 mg/kg/day with histopathologic observations limited to hypertrophy/villous elongation of the duodenal mucosa. In bone there was a dose-related increase in the incidence and/or severity of hyperostosis of trabecular bone at $\geq$ 20 mg/kg/day. In the kidney pale areas and pelvic dilatation were evident; pelvic and tubule dilation were observed microscopically in males and/or females at all dosages. In the spleen, extramedullary hematopoiesis occurred at an increased incidence in females at dosages $\geq$ 65 mg/kg/day. All effects were reversible, with the exception of renal pelvic dilatation still noted in males at dosages $\geq$ 20 mg/kg/day.
- Exposure increased with dose in a greater than proportional manner.

Reviewer Comments: The administration of JNJ-28431754 to juvenile animals resulted in increased urine output including a dose-related increase in urinary excretion of glucose and concomitant glucosuria observed in all groups of treated males and females. A secondary dose dependent and progressive increase in food consumption was also observed in all groups. Even with the increased feed consumption, a dose-related reduction in weight gain occurred. Post-mortem observations included an increase in the incidence/severity of hyperostosis of trabecular bone in the sternum and stifle, distension and thickening of the intestinal track (associated with ante-mortem observation of the abdomen), and extramedullary hematopoiesis in the spleen. These effects were all reversible. In addition, renal pelvic and tubule dilatation were observed at all dosages. Although the tubule dilation reversed, pelvic dilatation was still evident at an increased incidence at $\geq$ 20 mg/kg/day in males. Based on the occurrence of treatment-related observations at all dosages, a NOAEL is not identifiable, although the Sponsor identified 4 mg/kg/day as the NOAEL based on the reversibility of observations at this dosage. At 4 mg/kg/day, the exposure in males was ~0.5x to 0.6x the exposure at the MRHD.

Methods

Doses	0, 4, 20, 65, 100 mg/kg
Frequency of dosing	once daily days 21 - 90
Dose volume	10 mL/kg/day
Route of administration	oral gavage
Formulation/Vehicle	aqueous solution (metformin) containing 0.5% w/v Methocel
Species/Strain	Sprague-Dawley Crl:CD(SD) rat
Number/Sex/Group	12/sex/group
Satellite groups	8/sex/group for recovery
Study design	standard developmental tox study endpoints

Dosages were selected based on the results of the 13 week and 6 month studies. In both studies, dosages of 4, 20, or 100 mg/kg/day were administered. In general, results were consistent in these two studies. At dosages $\geq$ 20 mg/kg/day in males and at 100 mg/kg/day, treatment-related effects often included decreased body weight, changes in food consumption, fecal alterations, and changes in body condition. Target organs included the skeleton (bone), urinary system, and adrenal glands. Increases in urine volume and marked increases in urinary glucose at all doses were related to the intended pharmacological activity of the compound. Urinary excretion of NAG, GGT, protein, sodium, calcium, and magnesium were increased in treated rats, particularly at high doses. Mineralization of the renal interstitium and pelvis, and trabecular hyperostosis (sternum, stifle) were also noted. The NOAEL was 4 mg/kg in males and 20 mg/kg in females. Similar observations were noted in the 6-month, with many effects that were associated with the pharmacologic activity of an SGLT2 inhibitor. In the kidney, tubular dilatation was noted at all doses, and an increased incidence and severity of transitional hyperplasia was noted at 100 mg/kg. In the bone, trabecular hyperostosis was observed in the femur/tibia at $\geq$4 mg/kg and in the sternum at $\geq$20 mg/kg. Based on these findings, the NOAEL was 4 mg/kg.

Observations and Results

Mortality: No mortality occurred. Individual animals in the 0, 4, and 20 mg/kg/day group were euthanized due to eye trauma related to the blood collection procedure and one 100 mg/kg/day animal died following the collection of week 4 clinical pathology samples.

Clinical Signs: A dose-related increased incidence of soft/swollen abdomen was apparent in males and females receiving 65 or 100 mg/kg/day during the treatment period. This sign was not evident during recovery.

Body Weights: Weight gain was reduced in all drug-treated groups of males. At dosages $\geq$ 65 mg/kg/day lower weight gains were noted during the first week of treatment while at lower dosages the effects were observed beginning during week 4. Over the entire treatment period (day 21 – 90), weight gains were 10%, 13%, 17%, and 18% lower than control at 4, 20, 65, and 100 mg/kg/day, respectively, resulting in absolute weights that were 9%, 12%, 15%, and 16% lower than controls at 4, 20, 65, and 100 mg/kg/day, respectively. During recovery, all groups of males gained more weight than controls, but absolute weights were still 8% to 13% lower than controls. These effects were statistically significant.

In females, weight gains were 13% to 17% lower than controls at 65 and 100 mg/kg/day, respectively, during the first two weeks of treatment, but thereafter were similar to controls. As a result of the initially lower weight gains, the absolute weights of animals in these two groups were approximately 10% lighter than controls through the first 3 weeks of the study before catching

back up to controls. There were no other effects noted during the remainder of the study in these groups nor were any effects observed in animals administered 4 or 20 mg/kg/day.

Feed Consumption: Food consumption was slightly lower during the first three days of treatment in males and females administered $\geq$ 65 mg/kg/day. Beginning during the 3rd week, statistically significant increases in food consumption were apparent in all treated groups. Feed consumption continued to remain higher than controls during the first week of recovery for all groups of females and for males at $\geq$ 65 mg/kg/day. Thereafter, food consumption was similar between the groups.

Ulna Growth: In males there were statistically significant reductions in ulna growth at dosages $\geq$ 20 mg/kg/day during the first 4 weeks of treatment. Reduced growth was also seen at 4 mg/kg/day, but the differences did not reach statistical significance. The reduced growth led to statistically significant reductions in the length of the ulna at each interval for males administered $\geq$ 20 mg/kg/day and for some of the intervals at 4 mg/kg/day. Although ulna growth was increased over controls at $\geq$ 20 mg/kg/day during the recovery period, the length of the ulna was still statistically significantly shorter than controls at the end of the recovery period at dosages $\geq$ 20 mg/kg/day.

In females, reductions in ulna growth were noted during the first half of the treatment period at all dosages, with those occurring at $\geq$ 20 mg/kg/day achieving statistical significance. The lower growth resulted in shorter lengths at 65 and 100 mg/kg/day for the entire treatment period. Some reversal was seen during the post-dosing period as there were no differences between groups at the end of the recovery period.

Sexual Maturation: There was a dose-related delay in the age of sexual maturation of both males and females at dosages $\geq$ 65 mg/kg/day. For the males, the mean age of balano-preputial separation was delayed by 2.2 days at 65 mg/kg/day and by 5.9 days in the 100 mg/kg/day group (both statistically significant), with the age of attainment at 100 mg/kg/day also being outside the historical range. In the 100 mg/kg/day group, the mean body weight at which sexual maturity was attained was significantly higher than controls which was not surprising given that they were older. In addition, the distribution of ages at completion of balano-preputial separation also showed a clear shift towards later attainment, with 90% of males attaining sexual maturity after Day 45 of age compared to only 5% of controls.

A similar pattern was seen for females. Vaginal patency was delayed (statistically significant) by 2.7 days at 65 mg/kg/day and by 3.6 days at 100 mg/kg/day and the age of attainment at 100 mg/kg/day was outside the historical range. The age distribution showed that 48% of the animals in these two groups achieved sexual maturity after day 35, compared to 0 in the controls.

Ophthalmoscopy: NA

ECG: NA

Hematology: Statistically significant slight reductions (<10%) in Hb and Hct concentrations were evident in females administered $\geq$ 65 mg/kg/day at week 4 and in all groups of females at week 10; RBC counts were similarly reduced at all dosages in females at week 10. In males, effects were limited to reductions (up to 45% lower) in erythrocyte concentrations at $\geq$ 20 mg/kg/day at week 4 and at $\geq$ 65 mg/kg/day at week 10. There also were statistically significant reductions in platelet counts (up to ~30% lower) in males and females receiving 100 mg/kg/day during week 4, and platelet counts were ~14% lower for males receiving 100 mg/kg/day in week

10. MCV were also increased at week 4 in females administered 100 mg/kg/day and at week 10 in males administered 100 mg/kg/day and at all dosages in females; although statistically significant, the increases were < 3% at all time points. Reticulocyte counts were decreased up to 30% in males and/or females at $\geq$ 65 mg/kg/day at week 10 (statistically significant in males). Reversal was noted by the end of the recovery period for these parameters.

Coagulation: A shortening of APTT time of ~2 sec was observed in males and females administered 100 mg/kg/day; reversal was noted.

Clinical Chemistry: In males, statistically significant increases (up to 50%) in ALP, ALT, AST, CK, urea, Na, and Cl were noted, as were decreases in Tri (-40%), Glu (-23%), total protein (-5%), and Glob (-11%). These effects did not necessarily follow a dose response, were observed at all dose levels for some of the parameters, observed at week 4 and/or 10, did not differ dramatically at the two evaluation time points; reversal occurred for the majority of effects, although a few parameters were still statistically different from control. GGT levels were also increased at $\geq$ 65 mg/kg/day.

In females, ALT, AST, CK, urea, Ca were increased (up to 68%), generally in all groups, while decreases in Creat, total protein, Tri, and ALB (up to 39% lower) were observed. GGT levels were increased at 100 mg/kg/day. As in males, these effects were statistically significant, were generally noted in all groups but not necessarily in a dose-related manner, were seen at 4 and/or 10 weeks, and showed evidence of reversal (although AST levels were still decreased by up to 19% at $\geq$ 65 mg/kg/day).

Urinalysis: Dose-related and statistically significant increases in urine output were observed in all drug-treated groups of males and females at both seeks 4 and 10. Associated with the increased volume were decreases in pH in the majority of groups. Changes in the composition of the urine were also noted, with statistically significant effects noted for virtually every parameter at both weeks 4 and 10, including dose-dependent increases in glucose, protein, and electrolytes, although urinary concentrations were predominantly lower. As a consequence of the changes in the composition of the urine, the specific gravity was higher than in controls in all treated groups. Data from week 10 are shown in the Sponsor tables below:

Males

Urinalysis - group mean values during Week 10 of treatment

Group	:	1	2	3	4	5
Compound	:	Control	JNJ-28431754-ZAE	JNJ-28431754-ZAE	JNJ-28431754-ZAE	JNJ-28431754-ZAE
Dose (mg/kg/day)	:	0	4	20	65	100

Group /Sex		Vol mL	pH	SG g/L	UCreat µmol/L	TCRR umol	Prot g/L	TPRR mg	TPCR g/mol
Statistical test:		Wi	Sh	Sh	Wi	Sh	Wi	Wi	Wi
1M	Mean	9.0	8.0	1018	3745	30.950	0.52	4.410	145.773
	SD	3.36	0.25	7.2	1426.6	9.7085	0.169	1.4602	37.867
	N	20	20	20	20	20	20	20	20
2M	Mean	12.6**	7.5**	1039**	2951	35.923	0.60	7.386**	202.976**
	SD	2.90	0.21	8.6	639.4	6.1386	0.215	3.0813	64.589
	N	20	20	20	20	20	20	20	20
3M	Mean	17.6**	7.2**	1038**	2165**	36.935	0.47	8.025**	215.475**
	SD	4.06	0.47	6.4	402.4	5.8872	0.143	2.3429	45.492
	N	20	20	20	20	20	20	20	20
4M	Mean	17.0**	7.2**	1042**	2098**	34.336	0.51	8.289**	245.022**
	SD	5.37	0.40	6.3	472.2	8.0277	0.156	2.5879	63.465
	N	20	20	20	20	20	20	20	20
5M	Mean	18.7**	7.0**	1042**	1863**	34.196	0.50	9.268**	268.712**
	SD	3.81	0.31	3.3	255.1	4.5987	0.098	2.1339	42.477
	N	20	20	20	20	20	20	20	20

Group	:	1	2	3	4	5
Compound	:	Control	JNJ-28431754-ZAE	JNJ-28431754-ZAE	JNJ-28431754-ZAE	JNJ-28431754-ZAE
Dose (mg/kg/day)	:	0	4	20	65	100

Group /Sex		UGLU mmol/L	TGLR umol	GLCR mmol/mol	U-Na mmol/L	TNAR mmol	NACR mol/mol	U-K mmol/L	TUKR mmol	KCR mol/mol
Statistical test:		Wi	Sh	Sh	lWi	Wi	Wi	Sh	Wi	Wi
1M	Mean	0.67	5.282	187	50.0	0.427	14.222	82.8	0.672	21.958
	SD	0.349	2.4480	100	26.66	0.2125	7.178	46.46	0.2342	6.0249
	N	20	20	20	20	20	20	20	20	20
2M	Mean	375.06**	4679.0**	127927**	49.6	0.634**	17.479	71.5	0.888*	24.670
	SD	135.02	1782.7	38038	13.39	0.2662	6.145	20.85	0.2834	7.1464
	N	20	20	20	20	20	20	20	20	20
3M	Mean	396.75**	6859.0**	186098**	54.0	0.960**	25.648**	60.6	1.043**	28.424**
	SD	102.57	2088.4	54868	17.82	0.4506	10.100	12.93	0.2524	6.8226
	N	20	20	20	20	20	20	20	20	20
4M	Mean	437.92**	7268.5**	212514**	52.2	0.926**	26.282**	59.0*	0.996**	28.476**
	SD	87.838	1997.9	34237	15.62	0.4594	10.368	15.21	0.3281	6.3728
	N	20	20	20	20	20	20	20	20	20
5M	Mean	440.97**	8209.4**	239179**	58.0	1.090**	31.709**	54.9**	1.025**	29.881**
	SD	46.395	1595.6	27786	17.30	0.4266	11.484	7.54	0.2393	5.2290
	N	20	20	20	20	20	20	20	20	20

Group		1	2	3	4	5
Compound	:	Control	JNJ-28431754-ZAE	JNJ-28431754-ZAE	JNJ-28431754-ZAE	JNJ-28431754-ZAE
Dose (mg/kg/day)	:	0	4	20	65	100

Group /Sex		U-Cl mmol/L	TCLR mmol	CLCR mol/mol	UCA mmol/L	TCAR umol	CACR mol/mol	UIP mmol/L	TIPR umol	IPCR mol/mol
Statistical test:		St	1Wi	Wi	1Wi	sWi	1Wi	1Wi	Wi	1Wi
1M	Mean	53.0	0.435	14.230	1.56	13.270	0.440	3.51	30.537	0.969
	SD	35.51	0.1959	6.144	1.176	11.619	0.3356	1.620	17.727	0.3797
	N	20	20	20	20	20	20	9	9	9
2M	Mean	46.8	0.608*	16.735	2.25*	28.193**	0.769**	8.29**	103.34**	2.914**
	SD	17.31	0.3313	8.173	1.393	19.326	0.4567	3.267	44.903	1.2493
	N	20	20	20	20	20	20	18	18	18
3M	Mean	49.1	0.870**	23.457**	2.19*	38.464**	1.046**	9.61**	167.95**	4.475**
	SD	16.38	0.4105	9.514	0.744	15.324	0.3758	4.880	102.28	2.5186
	N	20	20	20	20	20	20	17	17	17
4M	Mean	52.1	0.924**	26.207**	4.36**	78.082**	2.265**	7.27**	120.69**	3.598**
	SD	15.86	0.4600	10.601	1.994	54.887	1.3076	4.147	85.447	2.2743
	N	20	20	20	20	20	20	19	19	19
5M	Mean	56.2	1.050**	30.685**	6.56**	125.58**	3.629**	8.50**	159.27**	4.761**
	SD	17.41	0.3900	10.603	2.089	58.322	1.467	3.977	80.319	2.6699
	N	20	20	20	20	20	20	19	19	19

Group		1	2	3	4	5
Compound	:	Control	JNJ-28431754-ZAE	JNJ-28431754-ZAE	JNJ-28431754-ZAE	JNJ-28431754-ZAE
Dose (mg/kg/day)	:	0	4	20	65	100

Group /Sex		UMG mmol/L	TMGR umol/L	MGCR mol/mol	UGGT U/L	TGGR IU	GGTC U/mmol	UNAG U/L	TNGR U	NGCR U/mmol
Statistical test:		1Wi	Wi	Wi	Du	sWi	Sh	Du	Wi	1Wi
1M	Mean	11.19	93.167	3.074	1671	14	446.0	9.1	0.1	2.397
	SD	4.277	29.257	0.7666	731.5	4.2	82.96	4.10	0.04	0.4749
	N	20	20	20	20	20	20	20	20	20
2M	Mean	8.75	109.88	3.0245	2211*	26**	743.8**	12.8**	0.2**	4.366**
	SD	2.517	40.669	0.8312	697.3	5.3	141.76	3.44	0.05	0.8083
	N	20	20	20	20	20	20	20	20	20
3M	Mean	7.80*	134.42**	3.659*	1675	28**	776.4**	10.1	0.2**	4.697**
	SD	1.478	32.167	0.6873	537.7	8.1	231.92	3.19	0.08	1.3247
	N	20	20	20	20	20	20	20	20	20
4M	Mean	10.29	175.53**	5.061**	1523	24**	703.3**	10.6	0.2**	5.132**
	SD	1.550	57.100	1.0061	716.9	9.4	227.60	3.36	0.07	1.3902
	N	20	20	20	20	20	20	20	20	20
5M	Mean	11.41	211.65**	6.195**	1189	21**	631.3**	9.6	0.2**	5.175**
	SD	1.650	43.246	0.9635	496.3	6.9	209.01	2.44	0.06	1.2807
	N	20	20	20	20	20	20	20	20	20

Females

Group	:	1	2	3	4	5
Compound	:	Control	JNJ-28431754-ZAE	JNJ-28431754-ZAE	JNJ-28431754-ZAE	JNJ-28431754-ZAE
Dose (mg/kg/day)	:	0	4	20	65	100

Group /Sex		Vol mL	pH	SG g/L	UCreat µmol/L	TCRR umol	Prot g/L	TPRR mg	TPCR g/mol
Statistical test:		Wi	Wi	Wi	lWi	Sh	Wi	Sh	Sh
1F	Mean	8.5	7.8	1014	2711	18.673	0.06	0.418	22.155
	SD	5.22	0.46	5.3	1248.4	4.3485	0.025	0.1823	6.908
	N	20	20	20	20	20	20	20	20
2F	Mean	8.7	7.2**	1031**	2597	21.178	0.06	0.503	23.770
	SD	3.08	0.33	7.0	614.6	4.0030	0.023	0.1193	4.445
	N	20	20	20	20	20	20	20	20
3F	Mean	9.9	6.7**	1038**	2265	20.577	0.12*	1.212**	59.205**
	SD	3.44	0.42	10.9	654.3	3.7290	0.108	1.3467	64.755
	N	20	20	20	20	20	20	20	20
4F	Mean	11.5*	6.6**	1042**	2025*	21.531*	0.10*	1.140**	54.925**
	SD	3.62	0.45	10.0	579.6	3.6117	0.108	1.6039	83.368
	N	20	20	20	20	20	20	20	20
5F	Mean	13.1**	6.4**	1042**	1921*	24.204**	0.08*	1.105**	46.627**
	SD	3.41	0.45	8.5	336.8	2.8638	0.075	1.1383	50.731
	N	18	18	18	18	18	18	18	18

Group	:	1	2	3	4	5
Compound	:	Control	JNJ-28431754-ZAE	JNJ-28431754-ZAE	JNJ-28431754-ZAE	JNJ-28431754-ZAE
Dose (mg/kg/day)	:	0	4	20	65	100

Group /Sex		UGLU mmol/L	TGLR umol	GLCR mmol/mol	U-Na mmol/L	TNAR mmol	NACR mol/mol	U-K mmol/L	TUKR mmol	KCR mol/mol
Statistical test:		Wi	Sh	Sh	Sh	lWi	Wi	Wi	Wi	Wi
1F	Mean	0.56	4.687	251	41.2	0.299	16.362	54.3	0.409	22.030
	SD	0.389	4.3794	238	19.09	0.1160	5.839	21.07	0.1717	8.4397
	N	20	20	20	20	20	20	20	20	20
2F	Mean	250.21**	2065.5**	97391*	50.9	0.410*	19.662	54.6	0.456	21.413
	SD	76.556	697.75	27010	19.09	0.1451	6.432	13.30	0.1353	4.5210
	N	20	20	20	20	20	20	20	20	20
3F	Mean	352.18**	3297.3**	158076**	47.8	0.455**	21.865*	63.5	0.596**	28.494**
	SD	98.049	1041.2	29349	13.00	0.1569	5.881	20.08	0.1975	5.7776
	N	20	20	20	20	20	20	20	20	20
4F	Mean	388.95**	4253.0**	195947**	55.6	0.631**	28.699**	63.3	0.702**	32.284**
	SD	97.961	1278.5	38936	19.75	0.2848	10.588	15.44	0.2065	6.6954
	N	20	20	20	20	20	20	20	20	20
5F	Mean	396.69**	4999.0**	206880**	46.2	0.594**	24.319**	66.5*	0.851**	34.896**
	SD	84.592	873.33	29320	17.72	0.2458	9.124	14.78	0.2168	6.4076
	N	18	18	20	18	18	18	18	18	18

Group	:	1	2	3	4	5
Compound	:	Control	JNJ-28431754-ZAE	JNJ-28431754-ZAE	JNJ-28431754-ZAE	JNJ-28431754-ZAE
Dose (mg/kg/day)	:	0	4	20	65	100

Group /Sex		U-Cl mmol/L	TCLR mmol	CLCR mol/mol	UCA mmol/L	TCAR umol	CACR mol/mol	UIP mmol/L	TIPR umol	IPCR mol/mol
Statistical test:		Wi	Wi	Wi	lWi	Wi	Wi	Wi	lWi	lWi
1F	Mean	36.0	0.256	14.115	3.41	28.027	1.591	6.88	56.528	2.741
	SD	23.26	0.1155	6.681	2.360	23.385	1.3930	5.929	94.551	3.1530
	N	20	20	20	20	20	20	11	11	11
2F	Mean	44.4	0.352	16.724	4.99*	40.614	1.938	8.83*	76.368	3.571**
	SD	21.97	0.1873	7.894	2.253	17.120	0.8151	3.402	39.101	1.5424
	N	19	19	19	20	20	20	20	20	20
3F	Mean	44.2	0.431**	20.395*	5.28*	52.405*	2.447*	13.28**	118.43**	5.820**
	SD	15.08	0.2059	7.516	2.264	33.482	1.2078	6.946	47.883	2.2578
	N	20	20	20	20	20	20	20	20	20
4F	Mean	49.9	0.571**	25.866**	9.00**	100.11**	4.615**	12.65**	148.01**	6.670**
	SD	18.58	0.2807	10.193	2.626	34.744	1.3149	6.319	87.132	3.5458
	N	20	20	20	20	20	20	20	20	20
5F	Mean	40.2	0.518**	21.316**	11.21**	141.94**	5.904**	19.43**	236.53**	9.823**
	SD	14.04	0.2063	7.713	2.697	31.220	1.2595	8.799	77.067	3.2017
	N	18	18	18	18	18	18	18	18	18

Group	:	1	2	3	4	5
Compound	:	Control	JNJ-28431754-ZAE	JNJ-28431754-ZAE	JNJ-28431754-ZAE	JNJ-28431754-ZAE
Dose (mg/kg/day)	:	0	4	20	65	100

Group /Sex		UMG mmol/L	TMGR umol/L	MGCR mol/mol	UGGT U/L	TGGR IU	GGTC U/mmol	UNAG U/L	TNGR U	NGCR U/mmol
Statistical test:		sWi	Wi	Wi	lWi	Du	lWi	Du	Fe~	lWi
1F	Mean	8.62	62.421	3.403	420	3	144.5	5.8	0.0	2.180
	SD	3.568	19.408	0.9417	288.7	1.3	48.31	2.60	0.04	0.457
	N	20	20	20	20	20	20	20	20	20
2F	Mean	8.63	70.442	3.329	214**	2*	81.3**	9.6**	0.1**	3.756**
	SD	2.486	20.816	0.7110	87.0	0.6	17.44	2.21	0.00	0.6707
	N	20	20	20	20	20	20	20	20	20
3F	Mean	8.86	82.535*	3.977	237**	2	108.5**	9.4**	0.1**	4.176**
	SD	2.219	23.817	0.6996	95.9	1.3	49.54	3.07	0.00	0.8589
	N	20	20	20	20	20	20	20	20	20
4F	Mean	10.74*	117.43**	5.419**	240**	3	118.6**	8.2**	0.1**	4.241**
	SD	2.749	32.182	0.918	135.8	1.3	58.35	1.97	0.02	0.9920
	N	20	20	20	20	20	20	20	20	20
5F	Mean	11.98**	152.30**	6.310**	202**	3	101.1**	7.9*	0.1**	4.103**
	SD	2.582	32.794	1.2490	129.7	1.1	45.34	2.35	0.02	1.1048
	N	18	18	18	18	18	18	18	18	18

After 4 weeks of recovery, urinary changes demonstrated reversibility.

Gross Pathology: Observations in animals from all drug-treated groups included thickening and/or distention of the jejunum, ileum, cecum, and duodenum; in the kidney both pale areas and pelvic dilatation were evident. The occurrence of these effects generally followed a dose-response pattern. Following recovery, pelvic dilatation in the kidney was evident in males at dosages $\geq$ 20 mg/kg/day and in females at $\geq$ 65 mg/kg/day. Data are summarized below:

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Incidence of Key Treatment-Related Macroscopic Observations

Organ Observation	# examined	Dosage (mg/kg/day) 0	4	20	65	100
		12/12	12/12	12/12	12/12	12/11
GI Track						
Jejunum - thickened	M/F	0/0	5/1	8/4	11/5	9/10
Ileum - thickened		0/0	1/1	2/1	4/3	6/5
Cecum - distended		0/0	1/2	3/3	9/5	9/7
Duodenum - thickened		0/0	2/1	7/6	8/4	10/9
Kidney						
Pelvic dilation	M/F	0/0	4/3	6/4	8/4	10/6
Pale area	M/F	0/0	4/3	2/4	5/2	6/2
Recovery Kidney						
Pelvic dilation	M/F	0/2	0/0	3/0	2/3	2/3

Organ Weights: In males, kidney, liver, and testes weights were increased (~14%, up to ~19%, up to ~4%, respectively) in all groups, but the increases reached statistical significance only following adjustment for body weight. Adrenal weights were also increased (up to ~5%) at $\geq$ 20 mg/kg/day, reaching statistical significance after adjustment for body weight. Prostate weights were lower (up to 22% lower) in all groups, but the difference reached statistical significance only for the 100 mg/kg/day group following body weight adjustment. Following recovery, kidney weights (adj) were higher at all dosages, and liver weights were higher (adj) at $\geq$ 20 mg/kg/day. The increased adjusted weights were considered a reflection of the lower body weights.

In females, kidney and liver weights were increased (up to 36% and 41%, respectively) in all drug-treated groups. The increased kidney weights achieved statistical significance for all dosage levels following adjustment for body weight while the liver weights were statistically increased at $\geq$ 20 mg/kg/day following body weight adjustment. Spleen weights were also increased (up to 27%), with the adjusted weights reaching statistical significance at $\geq$ 65 mg/kg/day (correlated with microscopic effects).   Following recovery, absolute and adjusted kidney, liver, and spleen weights were still heavier than control, but the differences no longer were statistically significant.

Histopathology:
   Battery Considered Adequate? yes
   Peer Review Performed? Report says peer review was performed, but signature of peer
   reviewing pathologist not located in report.

Treatment related effects were observed in bone, kidney, spleen, and small intestine/duodenum of both sexes. Treatment-related observations and severity are summarized in the Sponsor table below.

In the bone (sternum and stifle) there was a dose-related increase in the incidence and/or severity of hyperostosis of the trabecular bone. Effects were seen in males at all dosages and in females at $\geq$ 20 mg/kg/day. The increased incidence of hyperostosis was statistically significant in males at $\geq$ 65 mg/kg/day and at all dosages in the stifle; in females the increased incidence in both

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structures attained statistical significance at $\geq$ 65 mg/kg/day. The hyperostosis was mainly located in the metaphysis (underneath the growth plate of femur and tibia). These bone changes were no longer apparent after 4 weeks of recovery.

Observations in the kidney included dilatation of the pelvis and/or tubules at all dosages. The incidence and severity generally increased with dosage in both sexes. The increased incidence of pelvic dilatation reached statistical significance at $\geq$ 20 mg/kg/day in males and at 100 mg/kg/day in females; the tubule dilation was statistically increased at $\geq$ 20 mg/kg/day in males and at all dosages in females. At the end of recovery the incidence of pelvic dilatation was considered increased at dosages $\geq$ 20 mg/kg/day in males; in females there was a low incidence in all groups, including controls. Recovery of tubule dilatation also occurred. The severity of the kidney observations at the end of the recovery period were of a reduced magnitude compared to that observed at the end of the dosing period, indicating partial reversibility. The kidney findings were considered related to the increased urine volume (glucose-related diuresis). There was no histological correlate to the pale areas observed macroscopically in the medullary region of the kidneys.

In the spleen, extramedullary hematopoiesis occurred in all groups of females, including controls. The incidence was considered increased at $\geq$ 65 mg/kg/day with the increase reaching statistical significance at 100 mg/kg/day. There was no association with drug-treatment in males. This observation correlated with the increased spleen weights. Recovery was considered to have occurred, although two animals at 65 mg/kg/day were affected.

In the GI track, histopathologic observations were limited to a dose-related increase in hypertrophy/villous elongation of the duodenal mucosa of males and females at dosages $\geq$ 20 mg/kg/day, with the increased incidence attaining statistical significance at $\geq$ 65 mg/kg/day in both sexes. These observations generally correlated with the thickened duodenal wall noted at macroscopic examination, although there was no histological correlate for the macroscopic thickening noted in the duodenum of some rats at 4 mg/kg/day or for the thickening noted in other intestinal segments (jejunum, ileum, and cecum). There was complete reversibility of these duodenal changes after the 4-week recovery period.

Tabulated overview of treatment-related findings

	Males					Females				
Dose (mg/kg/day)	0	4	20	65	100	0	4	20	65	100
Sternum: hyperostosis	0	3	2	7	12	0	0	2	9	9
- Grade 1		3	2	7	7			2	8	7
- Grade 2					5				1	2
Stifle: hyperostosis	0	4	4	11	10	0	0	2	10	11
- Grade 1		4	4	7	4			2	6	5
- Grade 2				3	5				4	6
- Grade 3				1	1					
end of recovery (bones)	0	0	0	0	0	0	0	0	0	0
Kidneys										
Dilated tubules	0	0	5	7	11	0	4	4	4	9
- Grade 1			5	7	9		4	4	4	8
- Grade 2					2					1
end of recovery (grade 1)	0	2	0	0	1	1	2	2	2	2
Dilated pelvis	1	3	6	8	7	0	2	2	2	6
- Grade 1	1	1	4	3	3		2	2	2	2
- Grade 2		2	2	4	4					3
- Grade 3				1						1
end of recovery	0	1	4	3	4	2	1	0	2	2
- Grade 1		1	1	1	3	1	1		2	1
- Grade 2			2	2	1					1
- Grade 3			1			1				
Spleen: extramedullary hematopoiesis						6	3	6	10	11
- Grade 1						6	2	5	8	4
- Grade 2							1	1	2	6
- Grade 3										1
end of recovery						0	0	0	2	0
Duodenum: hypertrophy/elongated villi (grade 1)	0	0	2	5	7	0	0	3	4	8
end of recovery	0	0	0	0	0	0	0	0	0	0

Yellow indicates statistically significant

Toxicokinetics: Exposure increased with dose at both day 1 and week 10 sampling in a greater than proportional manner. There was no accumulation as exposure at week 10 was similar to that at day 1. Peak plasma levels occurred 1 to 7 hours after dosing. Data are summarized in the Sponsor table below:

Interval/Day	Gender	Group	Dose (mg/kg/day)	C_{max} (ng/mL)	t_{max} (h)	$AUC_{0-\infty}$ (ng•h/mL)	AUC_{0-24h} (ng•h/mL)
1	Male	2	4	854	4.00	13100	11800
1	Male	3	20	5110	2.00	85300	73700
1	Male	4	65	15500	7.00	364000[a]	272000
1	Male	5	100	25700	4.00	530000[a]	380000
Week 10	Male	2	4	1230	1.00		11900
Week 10	Male	3	20	4800	2.00		63000
Week 10	Male	4	65	16100	2.00		198000
Week 10	Male	5	100	21300	4.00		328000
1	Female	2	4	841	4.00	13800	12100
1	Female	3	20	5070	7.00	90100	80000
1	Female	4	65	21700	2.00	356000	295000
1	Female	5	100	26600	4.00	658000[a]	459000
Week 10	Female	2	4	1410	2.00		16700
Week 10	Female	3	20	6960	1.00		105000
Week 10	Female	4	65	21800	2.00		284000
Week 10	Female	5	100	29700	7.00		491000

Day 1 of treatment, Day 21 of age and Week 10, Day 70 of treatment, Day 90 of age.
[a]: AUC% extrapolated greater than 25% of the total AUC, reported but excluded from discussion.

6   Integrated Summary and Safety Evaluation

Appropriately designed studies were performed to evaluate the potential effects JNJ-2843174 may have on fertility, embryo/fetal development, and on offspring growth and development. An additional study was also performed in juvenile animals to identify effects on young animals to determine if they show a difference in sensitivity to the test article.

Fertility
Regarding the fertility evaluation, there were no effects on the ability to mate and sire or maintain a litter, leading to identification of the HD as the NOAEL (100 mg/kg/day; ~14x and 18x the MRHD in males and females, respectively). However, it should be noted that there were minor alterations in a number of reproductive parameters at the highest dosage administered. In HD males there was decreased sperm velocity and a slight increase in the number of abnormal sperm while in HD females there were slightly fewer corpora lutea. There were also fewer implantation sites and smaller litter sizes at the HD. As the observed differences from control values were small (and within the range of publically available historical data), this reviewer agrees that the observed effects were not toxicologically relevant in this study.

Data from other studies also support the interpretation regarding the fertility endpoints. There was no cross-species evidence of toxicity to the reproductive organs based on the absence of drug-related microscopic effects on male or female reproductive organs or on absolute reproductive organ weights in the 2 or 13 week studies in either rats or dogs (the only effects noted were increases in the organ to body weight ratios occurring as a result of the lower terminal

body weights of treated rats). In addition, there was no evidence of a dose-response as these effects were only evident at the HD, a dosage associated with a minimal level of toxicity. In HD males, decreased weight gain resulted in terminal body weights that were approximately 10% lower than controls; effects on HD females weight gain were only noted during gestation, but the magnitude of the effect was small and not toxicologically meaningful. These effects on weight gain, as well as increased feed consumption observed in all drug-treated groups, were consistent with observations in the repeat-dose toxicity studies and are likely related to the pharmacologic activity of the compound. The reduced weight gain in males led to identification of 20 mg/kg/day (approximately 4x the MRHD) as the NOAEL for general toxicity in males; in females the general toxicity NOAEL remained at 100 mg/kg/day.

Of note, the effects on both sperm parameters and corpora lutea resulting in fewer implantation sites and smaller litter sizes were consistent with observations reported with some, but not all, SGLT2 inhibitors in development.

Given the minimal nature of these alterations on the reproductive performance of rats and the unknown relevance to humans, a brief statement summarizing the observed effects could be considered for inclusion in the fertility section of the label.

Embryo/fetal development
In both the rat and rabbit developmental toxicity studies with JNJ-28431754, maternal toxicity in the form of reduced weight gain and feed consumption during the first few days of treatment were evident, and treatment-related mortality occurred at the highest dosage administered in the rabbit study. There were no effects on embryo/fetal viability or the ability to maintain the pregnancy in either the rat or rabbit. Effects on fetal development included increases in the incidence of alterations in skeletal ossification (i.e., absent, misshapen, or atypical appearance of various skeletal structures), likely related to the slight maternal toxicity that was seen in the various studies. Since delays in ossification typically disappear as the animal matures, the observed effects are not considered to be an indication of risk to fetal development. Based on the observed effects, the maternal NOAEL was the LD (10 mg/kg/day) in the rat (~2x the exposure at the MRHD) and MD (40 mg/kg/day) in the rabbit (~3x the exposure at the MRHD). The NOAELs for fetal development were the highest dosages administered to both the rat (100 mg/kg/day, ~19x the exposure at the MRHD) and rabbit (160 mg/kg/day, ~19x the exposure at the MRHD).

In the rabbit study, it should be noted that there were 16 (15 with viable offspring), 15, 19, and 14 litters in the C, L, M, and H dose groups, respectively. Although the number of viable litters able to be evaluated at the C, L, and H dosage groups were below the target range of 16 to 20 litters with viable offspring identified in the ICH S5 guidance, this study is considered adequate for evaluating potential risk to fetal development. This assessment is based on the combination of the maternal data in this study being consistent with that reported in the dose-ranging study, the similar fetal observations (eg, alterations in ossification) in this study compared to those seen in the rat study, and the absence of a teratogenic signal in this study or in the rat study.

Similar effects were observed in maternal and fetal animals in a developmental toxicity study in which JNJ-28431754 and metformin were co-administered to rats. Maternal weight gain and food consumption was transiently reduced in the combination groups, with the effects being larger in magnitude in the combination groups than that seen with each drug by itself. The observed effects on weight gain were also more pronounced with the combo than that seen for the corresponding intervals with JNJ-28431754 alone or in comparison to that observed in the

original developmental toxicity study with JNJ-28431754 alone. A similar pattern was seen with feed consumption, and these effects led to the identification of the LD (10/300 mg/kg/day combination group) as the NOAEL for maternal toxicity (~2x the exposure at the MRHD for JNJ-28431754). Although there were no effects on pregnancy parameters, effects on fetal development were observed at maternally toxic dosages. Effects on ossification were observed to occur in more structures and at greater incidences in the combination treatment groups compared to those occurring with JNJ-28431754 alone in the same study or in the original developmental toxicity study, suggesting that the combination may lead to a slightly greater degree of toxicity to maternal animals and to an increased incidence of alterations in fetal skeletal ossification. The NOAEL for fetal development was considered the HD (60/300 mg/kg/day; ~11x the exposure at the MRHD for JNJ-28431754) as the observed effects on ossification were likely a result of the maternal toxicity. Regarding changes in exposure to the two components, the co-administration of JNJ-28431754 and metformin did not alter the exposure to JNJ-28431754 (other than to slightly reduce the time to t_{max}. In contrast, exposure to metformin was 1.2 (C_{max}) to 1.6 (AUC) fold higher in the combination groups compared to that in the metformin alone group.

The cross-species concordance for delays in ossification, likely secondary to maternal toxicity, suggest an increased risk for humans. However, given that there were no other meaningful effects on fetal development, the occurrence of ossification delays at a maternally toxic dosage, the absence of a clear and consistent dose-response, and moderately high exposure multiples relative to the exposure at the MRHD, suggest that JNJ-28431754 does not appear to increase the risk to human fetal development. The co-administration of metformin did not alter the overall fetal developmental toxicity profile, although the incidence of delays in ossification was increased in the combination group as compared to that seen with JNJ-28431754 alone.

Pre-/postnatal development
In the pre- and post-natal toxicity study with JNJ-28431754, minimal maternal toxicity analogous to that reported in the fertility and developmental toxicity studies was observed, with the addition of GI track changes noted during the postmortem examination, at the mid and high dosages leading to the identification of the LD (10 mg/kg/day), as the NOAEL (~2x the exposure at the MRHD based on the exposure data from the developmental toxicity study). There were no effects on gestation length or ability to rear a litter. In the F_1 animals, body weights were lower at the MD/HD beginning at birth and remained lower until after weaning. Corresponding delays in the age at which air righting and the timing of indices of sexual maturation were also noted. There was no effect on the ability of F_1 animals to mate and produce litters, although there were fewer corpora lutea and an increased incidence of pre-implantation loss leading to fewer implantations at the HD, resulting in fewer live F_2 embryos (note the similarity to the findings in the fertility study). In contrast to what has been reported with other SGLT2 inhibitors, gross postmortem examination of F_1 animals exposed to JNJ-28431754 revealed no indication of a drug-related effect on urinary track tissues (eg, no dilatation of renal pelvi or tubules).

Regarding the F_1 reproductive performance, the Sponsor proposed that the low F_1 body weights at mating (5% lower) explain the fewer corpora lutea and decreased number of implantations at the HD. The Sponsor further speculated that the cause of the reduced F_1 body weights was either that treated F_0 females had depleted their energy supplies to the point where they were not able to meet the physiological demands of the litter or that treatment altered the volume or composition of the milk, with the end result being that fetuses were under-nourished during both gestation and lactation leading to reduced growth. It was only after the pups were weaned that they were able to achieve proper nutrition and begin to catch up to the controls in terms of body weight. Supporting their hypothesis is literature indicating that nutritional alterations, particularly

during the early postnatal period, can affect subsequent reproductive performance. Although this may very well be the case, the Sponsor has not directly demonstrated that the F_1 pups are nutritionally deprived, especially as the easiest marker to monitor (body weight) was not dramatically different from controls by the time of mating. An additional possibility not mentioned by the Sponsor is a direct effect of the drug on the pup based on the milk excretion study that showed drug concentrations in milk are equivalent to that in maternal plasma (ratio ranged from 1.05 to 1.55 at all time points evaluated). Thus, the pups were directly exposed to test article during the nursing period and this may have contributed to the observed effects on pup growth. The Sponsor identified the HD as the NOAEL for offspring functional development and reproductive performance, attributing the observed effects on these parameters to the low body weights of the offspring. Regardless of whether the altered pup growth and reproductive endpoints are secondary to F_0 induced alterations or are an indication of a direct drug effect on the F_1, the observations signify an effect on the F_1 generation in this study. Consequently, the NOAEL for F_1 growth and development is considered the LD of 10 mg/kg/day (~2x the exposure at the MRHD).

Juvenile toxicity
There were no new toxicities identified following the administration of JNJ-28431754 to juvenile rats as the effects observed were consistent with those noted in repeated-dose studies in adult rats. The key observations in the juvenile animal were the postmortem examinations which revealed effects on kidney tissues and bone. In the kidney, increased kidney weights occurred at all dosages, showing complete recovery in females but not males. Macroscopically and microscopically, a treatment-related increase in the incidence and severity of tubular and/or pelvic dilatation were observed in all treated groups (at $\geq 0.5x$ the MRHD) although the increased incidence did not always attain statistical significance. The tubular dilatation reversed in all groups while the pelvic dilatation was reversed at all dosages in females and in low dose males, but not at higher dosages in males. Macroscopic and microscopic kidney findings in the juvenile animal are shown in the Sponsor tables below:

Incidence of Key Treatment-Related Macroscopic Observations
Dosage (mg/kg/day)

Organ		0	4	20	65	100
Observation	# examined	12/12	12/12	12/12	12/12	12/11
Kidney						
Pelvic dilation	M/F	0/0	4/3	6/4	8/4	10/6
Pale area	M/F	0/0	4/3	2/4	5/2	6/2
Recovery						
Kidney						
Pelvic dilation	M/F	0/2	0/0	3/0	2/3	2/3

Key Treatment-Related Microscopic Observations

Tabulated overview of treatment-related findings										
		Males				Females				
Dose (mg/kg/day)	0	4	20	65	100	0	4	20	65	100
Kidneys										
Dilated tubules	0	0	5	7	11	0	4	4	4	9
- Grade 1			5	7	9		4	4	4	8
- Grade 2					2					1
end of recovery (grade 1)	0	2	0	0	1	1	2	2	2	2
Dilated pelvis	1	3	6	8	7	0	2	2	2	6
- Grade 1	1	1	4	3	3		2	2	2	2
- Grade 2			2	2	4					3
- Grade 3				1						1
end of recovery	0	1	4	3	4	2	1	0	2	2
- Grade 1		1	1	1	3	1	1		2	1
- Grade 2			2	2	1					1
- Grade 3			1			1				

· Yellow indicates statistically significant

Kidney effects were also seen in adults in the 6 month study (see table below), but were not observed in the 1 or 3 month studies that utilized the same dosages. An important difference, however, is that pelvic dilatation occurred at an increased incidence at all dosages in the juvenile animal but only at the higher dosages in adult animals. Thus, the juvenile kidney was more sensitive to the test-article in that effects were induced at lower dosages and after a shorter treatment period in young animals as compared to adults (although it should be noted that single animals exhibited tubular or pelvic dilatation at higher dosages in the 2 week study but these effects were not considered drug related, likely based on the low incidence).

Kidney Observations (selected) in 6 Month Rat Study

Kidneys: treatment-related changes								
	Males				Females			
Group	V	L	M	H	V	L	M	H
Dose level (mg/kg)	0	4	20	100	0	4	20	100
Number examined	19	18	20	19	19	19	19	19
Dilatation, tubules, with debris – cortex and medulla								
minimal	0	8	16	12	0	6	10	9
slight	0	1	0	5	0	0	1	7
Total	0	9**	16**	17**	0	6**	11**	16**
Dilatation, pelvic								
minimal	0	1	3	6	1	0	0	5
slight	2	1	0	1	0	0	0	0
moderate	0	0	2	0	0	0	0	0
Total	2	2	5	7	1	0	0	5
One sided exact Fisher test: *) p<=0.05; **) p<=0.01								

These results are analogous to those seen with other SGLT2 inhibitors in development that also demonstrated increased incidences of tubular and pelvic dilatation at a lower dosage and/or after shorter exposure periods in juvenile animals as compared to adults. An additional postmortem observation was a dose-related increased incidence of hyperostosis in juvenile rats which was also consistent with effects observed at the same dose levels in adult rats. There was complete

recovery of hyperostosis by the end of the 4-week recovery period. Other effects observed in the study, such as the decreased weight gains, increased food consumption, increases in urine volume with increased glucose and electrolyte excretion, etc, were considered sequelae to the pharmacology of the compound. Based on the occurrence of treatment-related observations at all dosages, the exposure at the lowest dosage administered was ~0.5x the exposure at the MRHD. As a result, a NOAEL was not identified by the FDA. This conclusion is in contrast to that of the Sponsor which identified 4 mg/kg/day as the NOAEL based on the reversibility of observations at this dosage.

Conclusion
In summary, the Sponsor has performed all DART studies necessary to support the usage of canagliflozin in subjects of reproductive age and no additional studies are necessary. The results of the DART studies did not reveal effects on mating or fertility except at dosages associated with parental toxicity in which slight alterations in sperm parameters and implantation viability were noted. In the developmental toxicity studies, alterations in the pattern of ossification were noted at doses associated with maternal toxicity, effects that typically disappear as the fetus matures, while the perinatal/postnatal study revealed growth delays also at dosages associated with maternal toxicity. Importantly, the juvenile animal study revealed renal tubule and pelvic dilatation occurring at an increased incidence at lower dosages and/or after a shorter period of dosing than that seen in adults. As the period of dosing in the juvenile animal study is characterized by active morphological and functional development of the kidney and is analogous to events occurring during the last half of gestation and continuing until approximately 2 years of age in humans, these data suggest caution for use in the second half of pregnancy and in nursing women, especially combined with the data demonstrating that JNJ-28431754 is excreted in milk at a level comparable to that measured in maternal plasma. As effects on kidneys in the juvenile animal study were seen at all dosages (equivalent to exposures 0.5x the exposure at the MRHD and above) and a NOAEL was not identified by the FDA. Based on the kidney observations, appropriate statements will need to be incorporated into the label to discontinue treatment during late gestation and while nursing (lactation).

Clinical Safety Margins

Toxicity	Species	Sex	NOAEL (Dose)	AUC$_{0-24}$ (µg·hr/mL)	Clinical Safety Margin (Based on AUC*)
Fertility	rat	M (toxicity)	20	74.4	3x
		M (fertility)	100	365	14x
		F (fertility)	100	470	18x
Developmental Toxicity	rat	maternal	10	42.6	2x
		developmental	100	508	19x
	rabbit	maternal	40	84.9	3x
		developmental	140	487	19x
Pre- and Postnatal Development	rat	F$_0$	10	same exposure information as in rat developmental toxicity at this dosage	
		F$_1$	10		
Juvenile Toxicity	rat	M	ND**	11.9	0.5x
		F	ND**	16.7	0.6x
Developmental Toxicity - Combination	rat	maternal	10/300	46.8	2x
		developmental	60/300	300	11x

*Human 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

** Not determined as effects on kidney were noted at all dosages. This interpretation differs from Sponsor in that the Sponsor identified the LD as the NOAEL based on reversal data

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/s/

DANIEL R MINCK
01/31/2013

TODD M BOURCIER
02/01/2013
DART review of 204042
Reviewer recommends AP with labeling changes

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:	204042
Supporting document/s:	000
Applicant's letter date:	June 30, 2012
CDER stamp date:	June 30, 2012
Product:	Canagliflozin (INVOKANA®)
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:	Jena Weber

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1   Executive Summary

1.1   Recommendations

1.1.1   Approvability: The nonclinical data supports Approval of NDA 204042.

1.1.2   Additional Nonclinical Recommendations: No new studies are recommended.

1.1.3   Labeling Recommendations

13 NONCLINICAL TOXICOLOGY
13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility (draft)

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 100mg/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.

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.

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 (~14x and 18x the MRHD 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.

1.2   Brief Discussion of Nonclinical Findings

Canagliflozin (JNJ-28431754) is a selective sodium-glucose co-transporter 2 (SGLT2) inhibitor designed to lower blood glucose by reducing renal threshold for glucose reabsorption, independent of insulin. Although canagliflozin is approximately 160 fold more selective to

SGLT2 (IC_{50} 4.1 nM) than SGLT1 (IC_{50} 664 nM), at high concentrations, it is capable of inhibiting intestinal SGLT1. Canagliflozin is pharmacologically active in all species. The order of activity was rats > > dogs > rabbits > mice. The chronic toxicity of canagliflozin was tested in rats and dogs while reproductive toxicity was assessed in rats and rabbits. The carcinogenicity of canagliflozin was determined in 2-year studies conducted in mice and rats.

Two key target organs, the renal system and bone, were identified in the nonclinical studies. As expected, canagliflozin increased urinary glucose and associated diuresis in animals leading to lower blood glucose and substantial weight loss in all animal models, especially in rats. In rats, canagliflozin resulted in dose-dependent increase in renal tubule, pelvic and urinary tract dilatation. The dilations of the renal and urinary tract were considered an adaptive response to chronic diuresis.   The dilatations were absent or limited to very high doses in mice and dogs, likely due to limited pharmacological activity in these animals. Whether renal tubule cells with SGLT2 were directly impacted by canagliflozin is not clear. Most of the available data seem to suggest an indirect effect, i.e. mineralization (excess Ca absorption) and an adaptive response to polyuria induced by osmotic effect of glucose in the tubules.

Based on potential underlying causes and morphological characteristics, canagliflozin's hyperostotic effect on bone could be considered somewhat two distinct adverse findings: an acute effect noted in the trabecular bone and a chronic effect noted in the compact bone. The acute effect on trabecular bone was seen only in rats and caused by hypercalcium absorption, while the decrease in compact bone density and strength that occurred in older rats and dogs appeared to be related to weight loss and Ca metabolism. There was no evidence of hyperostosis in mice, dogs, or humans, again likely the result of a much lesser or no effect on carbohydrate absorption and calcium homeostasis in these species.   Hyperostosis in rats occurred in as few as 4 days of high dose canagliflozin (≥12x the MRHD) and over time at lower doses (<1x the MRHD). The effect was more pronounced in young rats undergoing rapid bone growth and marked by significant calciuria and greater suppression of Ca and bone biomarkers. Canagliflozin-induced increase in Ca absorption appeared to be the primary driver of hyperostosis based on a) direct evidence canagliflozin transiently increased Ca absorption from the gut with each dose, b) preventing carbohydrate malabsorption induced by canagliflozin also prevented hyperostosis and calciuria, c) reducing the dietary Ca content to $1/10^{th}$ of normal levels substantially prevented bone hyperostosis and calciuria.

Compact bone density and strength declined in both rats and dogs receiving high doses of canagliflozin (≥12x the MRHD).   The decreases in density were generally marked by significant decreases in bone biomarkers. However, the most remarkable correlate was the decrease in body weight in rats (M: - 26%, F: -20%) and in male dogs. A similar relationship was also observed in humans. Interestingly, there was no change in bone density or BW in female dogs. The weight of evidence suggests that body weight loss had played a substantial role. Since Ca is fundamental to bone density and strength, any potential canagliflozin induced imbalance in Ca metabolism may have long-term consequence on bone density and strength in humans.

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The carcinogenicity of canagliflozin 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 (table 1).

Table 1: Incidence of Neoplasms in Sprague Dawley rats administered canagliflozin *(n=65/group)*		Canagliflozin, mg/kg			
		0	10	30	100
Multiple of Clinical Exposure			*1-2x*	*5-7x*	*12-21x*
Adrenal Gland Pheochromocytoma, *(adenoma, carcinoma combined)*	Male	4	4	7	28*
	Female	2	1	3	7
Kidney, Renal tubule *(adenoma, carcinoma combined)*	Male	0	0	2	12*
	Female	0	0	0	8*
Testes, Leydig cell adenoma	Male	1	8*	20*	24*

 * *Statistically significant, trend and pair-wise comparison vs. control*

The emergent tumors in SD rats are frequently observed with compounds known to inhibit carbohydrate absorption from the intestine, such as acarbose. Since preventing carbohydrate malabsorption prevented acarbose-induced increase in renal and adrenal tumors, the sponsor hypothesized that canagliflozin might be inhibiting intestinal carbohydrates via SGLT1. High incidence of bloating, soft stool, and diarrhea in animals appeared to support the carbohydrate malabsorption hypothesis. The sponsor proposed that at high doses, canagliflozin may overcome its selectivity and inhibit intestinal SGLT1 resulting in carbohydrate malabsorption, similar to acarbose. Exactly how carbohydrate malabsorption leads to renal and adrenal tumors is not clear. The sponsor hypothesized that hypercalcium absorption was the link between carbohydrate malabsorption and tumors in rats (see Figure 1).

Figure 1. Proposed carbohydrate malabsorption and calcium hyperabsorption hypothesis
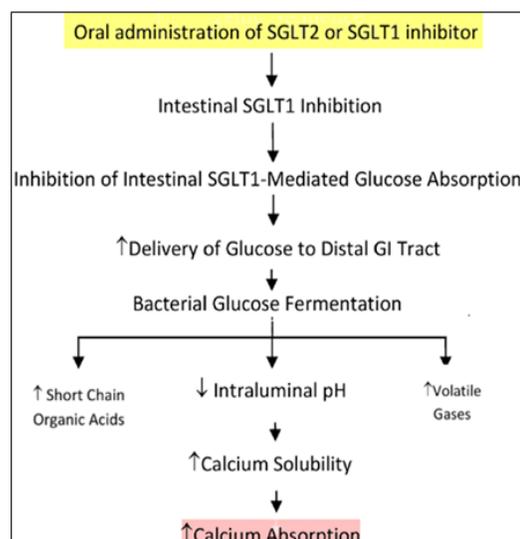
When rats were maintained on a high fructose diet (HF, 40%), the absorption of which is unaffected by SGLT1 inhibition, there was a substantial decrease in renal and adrenal cell proliferation biomarkers, suggesting that preventing carbohydrate malabsorption was likely responsible for renal and adrenal tumors observed in rats at 100 mg/kg. However, the sponsor failed to prove a link between hypercalcium absorption and tumorigenesis in rats. Rats maintained on low Ca diet (0.1% LC diet vs. 1% Ca normal diet) had actually greater renal and adrenal cell proliferation biomarkers, in spite of reduced urinary Ca, thereby confounding interpretation of the study

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for the renal and adrenal endpoints.   Although the LC diet study failed to show hypercalcium absorption as the proximal step in the canagliflozin induced tumorigenesis, it provided evidence that bone hyperostosis was caused by hypercalcium absorption.

Rat testicular Leydig cells are known to have up to 10 fold more luteinizing hormone (LH) receptors than humans. Therefore, Leydig cell tumors in rats are linked to heightened sensitivity to small increases in luteinizing hormone (LH). The sponsor's hypothesis that LH was responsible for Leydig cell tumors was supported by the 2-fold increase in LH levels observed during the first 5 months of canagliflozin treatment. Since there was no increase in LH in the clinical studies, the testicular tumors in rats were therefore not relevant at clinical doses of canagliflozin.

Overall, carbohydrate malabsorption was accepted as a reasonable mode of action for the renal and adrenal tumors in rats. With a 5- to 7 fold safety margin and an absence of any reported clinical signs of carbohydrate malabsorption at therapeutic doses of canagliflozin, the risk to humans was considered minimal. Testicular tumors were caused by the increase in LH in rats. With no change in LH in humans, testicular tumors were considered not a human risk at clinical doses of canagliflozin.

Canagliflozin in humans is extensively metabolized to two highly water soluble inactive *O*-glucruonide metabolites, M5 and M7. Both metabolites are present in all nonclicnal studies but at significantly lower levels. Since they are readily eliminated by the kidneys, there was no safety concern for the two metabolites.   Impurities in the drug product were within ICH specifications. A genotoxic mutagenic degradant (b) (4) identified during the 18-month stability study was present in the drug susbtance and drug product up to (b) (4) (b) (4) per 300 mg tablet), significantly greater than 1.5 µg/day allowed under guidance for genotoxic impurities. Further analysis showed a (b) (4) moeity in the degradant as the cause of the genotxicity.

Nonclinical safety issues that might be relevant to clinical use
The most prominent non-clinical finding with potential clinical relevance is the canagliflozin related decrease in bone density and strength seen in rats and dogs (12x the MRHD). At sufficiently high doses, canagliflozin can result in carbohydrate malabsorption. In animals, this leads to calcium disruption, tissue calcification, bone effects, and renal/adrenal tumors. Intentional alteration of carbohydrate absorption in humans can increase Ca absorption (Cashman 2006, Scholz-Ahrens, 2007), making the pathway clinically relevant. The available clinical data show no substantial canagliflozin-related carbohydrate malabsorption (e.g. no disruption in calcium) at therapeutic doses of canagliflozin. Although the clinical risk is minimal, the potential imbalance in Ca metabolism caused by slight carbohydrate malabsorption under chronic conditions has the potential to have long-term consequences on bone health in humans.

Cashman KD (2006) A prebiotic substance persistently enhances intestinal calcium absorption and increases bone mineralization in young adolescents. Nutri Reviews 64(4), 189-196

Scholz-Ahrens KE et al. (2007) Prebiotics, probiotics, and synbiotics affect mineral absorption, none mineral content, and bone structure. J Nutri 137, 838s-846s.

2   Drug Information

2.1   Drug: INVOKANA®

2.1.2   Generic Name: Canagliflozin

2.1.3   Code Name: JNJ-28431754 hemihydrate (R600348, TA-7284)

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

2.1.5   Molecular Formula/Molecular Weight: C24H25FO5S,   MW 444.5

2.1.6   Structure:
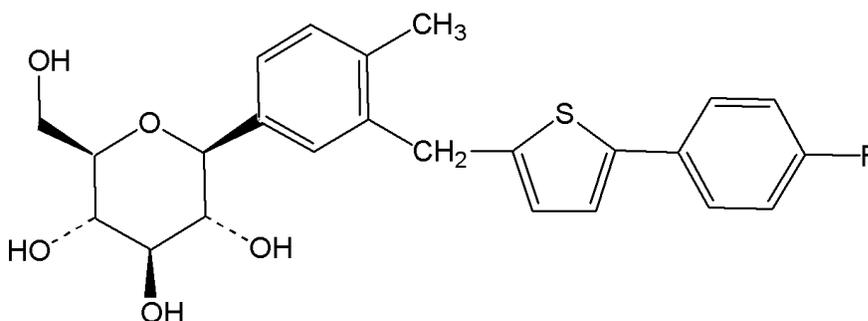
2.1.7   Pharmacologic class: Sodium glucose co-transporter 2 (SGLT2) inhibitor

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

NDA 204042 was developed under IND 76479

2.3   Clinical Formulation

2.3.1   Drug Formulation

Active ingredient: 102 mg of JNJ-28431754 hemihydrate in the 100 mg tablet
                        306 mg of JNJ-28431754 hemihydrate in the 300 mg tablet
Inactive ingredients: Microcrystalline Cellulose, Lactose Anhydrous, Hydroxypropyl
                        Cellulose, Croscarmellose Sodium, Magnesium Stearate, and
                                            (b) (4)   .

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The concentrations of excipients are listed below. The yellow and white tablets contain 100 and 300 mg canagliflozin.

Quantitative Ingredient Statement per 100-mg Unit Dose
(b) (4) 28431754-ZAE-CA-005 Yellow Filmcoated Tablets)

Component	Quality Reference	Role	% w/w	mg/unit
Core Tablet				
JNJ-28431754-ZAE	In-house	Active	(b) (4)	102.00[a]
Filmcoating				
(b) (4)	In-house USP		(b) (4)	(b) (4)
		Total Tablet Weight		208.00

[a] Quantity of the active ingredient (a hemihydrate) equivalent to the labeled amount of JNJ-28431754.

[d] A commercially available mixture consisting of Polyvinyl Alcohol-Partially Hydrolyzed, Ph.Eur./USP; Titanium Dioxide, Ph.Eur./USP; Macrogol/PEG (b) (4) Ph.Eur./NF; Talc, Ph.Eur./USP; and Iron Oxide Yellow, NF.

Quantitative Ingredient Statement per 300-mg Unit Dose
(b) (4) 28431754-ZAE-CA-006 White Filmcoated Tablets)

Component	Quality Reference	Role	Theoretical % w/w	mg/unit
Core Tablet				
JNJ-28431754-ZAE	In-house	Active	(b) (4)	306.00[a]
Filmcoating				
(b) (4)	In-house USP			(b) (4)
		Total Tablet Weight		618.00

[a] Quantity of the active ingredient (a hemihydrate) equivalent to the labeled amount of JNJ-28431754.

[d] A commercially available mixture consisting of Polyvinyl Alcohol-Partially Hydrolyzed, Ph.Eur./USP; Titanium Dioxide, Ph.Eur./USP; Macrogol/PEG (b) (4) Ph.Eur./NF; Talc, Ph.Eur./USP.

2.3.3   Comments on Impurities/Degradants of Concern

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). 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.

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Degradants

In the 4-month safety update during the NDA review cycle, the sponsor notified the agency of presence of a weak genotoxic degradant ▆▆▆▆▆▆ (b) (4) in stability studies. The maximum exposure to the degradant was ▆▆ (b) (4) significantly greater than the 1.5µg/day limit set in the guidance for genotoxic impurities. Genotoxicity of the degradant was confirmed by in-silico SAR analysis using DEREK software and Ames test. The maximum exposure to ▆ (b) (4) ▆▆▆▆ levels in the 18-month stability studies are shown below (▆▆▆▆▆ (b) (4) /day for the 300mg tablet).

Overview of 18-Month ▆▆▆▆ (b) (4) Values for 100-mg Canagliflozin Stability Batches Manufactured in July 2010 with Corresponding Data on the Drug Substance Lots Used for Manufacture of the Drug Product

Study ID	DP Batch	Storage Condition	Package	DP Result (ppm)	DS Result (ppm)	DS Lot ZR600348
H01003	0HG2281-X	25 °C/60% RH	Bottle			PUA071
H01003	0HG2281-X	30 °C/75% RH	Bottle			PUA071
H01008	0HG2282-X	25 °C/60% RH	Bottle			PUA081
H01008	0HG2282-X	30 °C/75% RH	Bottle			PUA081
H01013	0HG2283-X	25 °C/60% RH	Bottle			PUA091
H01013	0HG2283-X	30 °C/75% RH	Bottle			PUA091
H01005	0HG2281-X	25 °C/60% RH	Blister			PUA071
H01005	0HG2281-X	30 °C/75% RH	Blister			PUA071
H01010	0HG2282-X	25 °C/60% RH	Blister			PUA081
H01010	0HG2282-X	30 °C/75% RH	Blister			PUA081
H01015	0HG2283-X	25 °C/60% RH	Blister			PUA091
H01015	0HG2283-X	30 °C/75% RH	Blister			PUA091

Overview of 18-Month ▆▆▆▆ (b) (4) Values for 300-mg Canagliflozin Stability Batches Manufactured in July 2010 with Corresponding Data on the Drug Substance Lots Used for Manufacture of the Drug Product

Study ID	DP Batch	Storage Condition	Package	DP Result (ppm)	DS Result (ppm)	DS Lot ZR600348
H01018	0HG2278-X	25 °C/60% RH	Bottle			PUA071
H01018	0HG2278-X	30 °C/75% RH	Bottle			PUA071
H01024	0HG2279-X	25 °C/60% RH	Bottle			PUA081
H01024	0HG2279-X	30 °C/75% RH	Bottle			PUA081
H01030	0HG2280-X	25 °C/60% RH	Bottle			PUA091
H01030	0HG2280-X	30 °C/75% RH	Bottle			PUA091
H01021	0HG2278-X	25 °C/60% RH	Blister			PUA071
H01021	0HG2278-X	30 °C/75% RH	Blister			PUA071
H01027	0HG2279-X	25 °C/60% RH	Blister			PUA081
H01027	0HG2279-X	30 °C/75% RH	Blister			PUA081
H01033	0HG2280-X	25 °C/60% RH	Blister			PUA091
H01033	0HG2280-X	30 °C/75% RH	Blister			PUA091

The genotoxicity of ▆▆▆▆▆ (b) (4) was attributed to the ▆▆▆▆ (b) (4) moiety. The ▆▆▆▆ (b) (4) moiety was similar but significantly lower than the ▆▆▆▆ (b) (4) in edible oils and other products used by humans on daily basis. The agency consulted the computational toxicology and the genotoxicity subcommittee at the agency. The computational chemistry group determined that the genotoxicity of ▆▆▆▆▆ (b) (4) rose from the ▆▆▆▆ (b) (4) moiety and the degradant was a weak genotoxicant and unlikely to be carcinogenic in rat or mouse study. After reviewing the genotoxicity subcommittee's recommendation and the

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computational chemistry report, the reviewer concluded � (b) (4) as weak genotoxicant, unlikely to cause cancer in humans. Since the highest detected level of (b) (4) ▓▓▓▓▓▓▓ was ▓▓▓▓▓▓▓ (b) (4)) in the 18-month stability study, the division limited the amount of the degradant in the 100 and 300 mg tablet to ▓▓▓▓▓ (b) (4) , respectively.

100mg drug product (tablet): ▓▓▓▓▓▓▓ (b) (4)
300mg drug product (tablet): ▓▓▓▓▓▓▓ (b) (4)

2.4   Proposed Clinical Population and Dosing Regimen

Type II diabetic patients, 100 or 300 mg QD

2.5   Regulatory Background

Canagliflozin (JNJ-28431754) application was submitted to the division on April 25, 2007. The initial IND was supported by nonclinical and clinical studies (up to 800 mg) carried out overseas. In humans, canagliflozin dose-dependently increased urine glucose with minimal change in urine volume. ▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓ (b) (4)
▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓▓

On Feb 1, 2011, the sponsor submitted a 15-day nonclinical safety report showing a significant treatment related increase in the incidence of renal tubular (adenoma and carcinoma) and adrenal tumors in both male and female rats at 100 mg/kg and testicular Leydig cells tumors in males at all doses of canagliflozin (10, 30 and 100 mg/kg). There were no tumors in mice at doses and plasma exposures similar to those used in the rats. In addition to renal tubule tumors in rats, there was a treatment-related increase in the incidence of renal tubule hyperplasia. The preliminary analysis appeared to show the incidences of these tumors to be significantly above the concurrent and historical controls in SD rats. Carbohydrate malabsorption is known to cause renal, adrenal and testicular tumors in SD rats treated with acarbose, an antidiabetic drug designed to block carbohydrate absorption from the intestine. The sponsor therefore proposed carbohydrate malabsorption as the likely mode of action. The proposed mechanism was considered reasonable but the agency requested mechanistic studies supporting the proposed mechanism. Three 6-month and a 15-month mechanistic study protocols were submitted and reviewed by the agency. The findings of the 6-months studies were submitted on May 31, 2012. The ongoing 15-month study will likely be available in late 2013. In January of 2012, the agency also recommended a juvenile rat study to assess renal development.

This IND and NDA were submitted electronically (eCTD) and followed all the required information pursuant to 21 CFR 312.23 and is in accordance with the April 2006 FDA Guidance for Industry *Providing Regulatory Submissions in Electronic Format - Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications.*

3   Studies Submitted

3.1   Studies Reviewed

- Study # DD10318: JNJ-28431754 On Renal Glucose Threshold In ZDF Rats
- Study # DD10319: JNJ-28431754 on Renal Glucose Excretion in ZDF Rats During Hyperglycemic, Euglycemic and Hypoglycemic Condition
- Study # DD08322: Effect of JNJ-28431754 Metabolite (M5) on Human SGLT1 and SGLT2 Activity *In-vitro*
- Study # DD08324: In Vitro Activity Studies of a Glucuronide Metabolite (M7) of the SGLT2   Inhibitor JNJ-28431754
- Study # FK5827: In vitro Metabolism of JNJ-28431754 in Cryopreserved Rat, Dog, and Human Hepatocytes
- Study # FK5983: In vitro Metabolism of JNJ-28431754 in Human Liver Cytosol and Microsomal Tissue Fractions
- Study # FK5904: Evaluation of Transport Mechanism of [14C] JNJ-28431754 in MDR1 and MRP2 Expressed MDCKII Cell Lines
- 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 # FK7483: Interaction of M5 and M7 with transporters using cRNA-injected oocytes
- Study # FK7446: An In Vitro Investigation of the Potential of JNJ-41488525 (M7) and JNJ-41980874 (M5), O-Glucuronide Metabolites of JNJ-28431754, to Inhibit CYP450 Isoforms in Human Liver Microsomes
- Study # FK7558: Evaluation of the Toxicokinetics of M8 and M9, Metabolites of JNJ-28431754 Following Two-Week Oral/Subcutaneous Administrations of JNJ-28431754-ZAE in Support of a Toxicity Study in Rats
- Study # FK6375: Evaluation of the PK of JNJ-28431754 Following a Single Oral dose of canagliflozin in three different formulations to male beagle dogs.
- Study # FK6251: In-vivo metabolism of JNJ-28431754 in human
- Study # FK6419: The Analyses of Radioactive Biological Samples Collected During Clinical Trial 28431754-NAP-1006, Investigating in vivo Metabolism of 14C-JNJ-28431754 After a Single Oral Dose of 200 mg in Healthy Male Subjects
- Study # FK7526, The biliary excretion of C^{14}-canagliflozin in male SD rats
- Study # TOX8574: 6-Month Repeated Dose Oral Toxicity Study of JNJ-28431754 (canagliflozin) in Rats
- Study # TOX8446: 12-Month Oral Toxicity Study of JNJ-28431754 in Dogs
- Study # TOX8799: 2-Year Oral Gavage Carcinogenicity Study of JNJ-28431754-ZAE in CD-1 Mice
- Study #TOX8986: 24-Month Repeated Dose Oral Carcinogenicity Study of JNJ-28431754-ZAE in the Rat.

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- Study # TOX10093: 6-Month repeated dose oral toxicity study of JNJ-28431754 in the male rat under standard diet and fructose diet feeding conditions with a 1-month and 3- month interim kill
- Study # TOX10168: 6-Month repeated dose oral and subcutaneous toxicity study of JNJ-28431754 in the male rat under standard diet and low calcium diet feeding conditions with a 1-month and 3-month interim kill
- Study # TOX10108: 7-Month Mechanistic Repeated Dose Oral Toxicity Study of JNJ-28431754 in the male rat (hormonal evaluation).
- Study #TOX 8707: Four-Week Oral Bone Biomarker Investigative Toxicity Study of JNJ-28431754 in Female rats
- Study # TOX10359: 1-Month repeated dose oral toxicity study of ▓▓▓▓▓▓ (b) (4) (dapagliflozin) in the male SD rat
- Study# TOX10446: In vitro Bacterial Reverse Mutation test with ▓▓▓▓▓ (b) (4) in Salmonella typhimurium

3.2   Studies Not Reviewed

Non-GLP studies were not reviewed except when they provided valuable data to reach a conclusion.

3.3   Previous Reviews Referenced

Acute and subchronic toxicology studies were reviewed under canagliflozin IND 76,479. The reproductive toxicology studies were reviewed separately by Dr. Mink.

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4   Pharmacology

4.1   Primary Pharmacology

Canagliflozin is a selective, reversible inhibitor of SGLT2 transporter with minimal SGLT1 inhibitory activity. The IC_{50s} 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 IC_{50} data suggests a 160 fold selectively to SGLT2, the intestinal exposure after oral dosing can reach high

enough to inhibit SGLT1 in the small intestine in rats (soft feces, bloating, gas) and possibly in humans (minor bloating) to prevent glucose absorption from the GI tract.

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 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

The IC_{50} for rat SGLT2 and SGLT1 were 3.7 nM and 555 nM, respectively. In mice the IC_{50s} 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 IC_{50} 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 which may explain the absence of renal, adrenal or bone findings in mice.

Canagliflozin is extensively metabolized in humans to two prominent glucuronide metabolites. 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 IC_{50} for M5 and M7 were nearly 5000 times less than canagliflozin for SGLT2 and greater than 1000 fold for SGLT1 transporter. The Cmax for M7 and M5 after single dose of 200 mg were 1159 and 703 ng/ml in normal subjects, respectively. It should be noted that neither metabolite is likely to inhibit SGLT2 at Cmax.

In vitro inhibitory activity of canagliflozin and its major human glucuronide metabolites, M5 and M7	Human SGLT2 IC_{50}	Human SGLT1 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

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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 IC_{50} for mouse and SD rat was similar, the ED dose was significantly lower in SD rats.

Overview of In Vivo Effects of Canagliflozin in Nonclinical Pharmacology Studies

In Vivo Effects	ED^a (mg/kg)
Urinary Glucose Excretion Studies	
C57BL/6J mice (non-diabetic)	10
db/db mice (diabetic)	1
SD rats (non-diabetic)	1
Zucker Diabetic fa/fa rats (diabetic)	0.3
Beagle dog (obese, non-diabetic)	3
OGTT Studies	
SD rats (non-diabetic)	0.3
Zucker Diabetic fa/fa rats (diabetic)	1
Beagle dog (obese, non-diabetic)	10
Fed Blood Glucose Lowering	
db/db mice (diabetic)	1
Zucker Diabetic fa/fa rats (diabetic)	1
Chronic Treatment for Blood Glucose Lowering	
Zucker Diabetic *fa/fa* rats (diabetic)	3
Chronic Treatment for Body Weight Control	
C57Bl/6J diet induced obese (DIO) mice (insuiln resistant)	30
ob/ob mice (diabetic)	30
SD rat (non-diabetic)	30
Zucker Fatty Rat (diabetic)	3
Renal Glucose Threshold Study	
Zucker Diabetic *fa/fa* rats (diabetic)	1

[a] Effective dose where a significant difference was observed versus placebo for AUC_{glu} after OGTT.

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
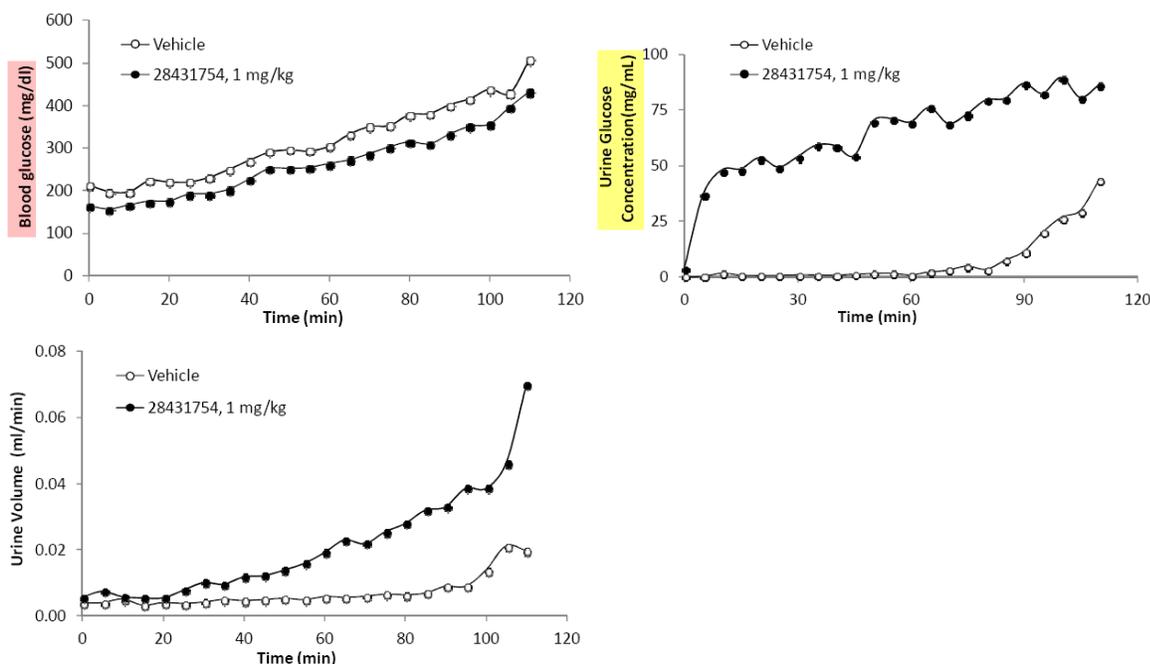
When the study was repeated under hyperglycemic, euglycemic and hypoglycemic conditions in ZDR rats (DD10319), canagliflozin (1 mg/kg) significantly increased urinary glucose excretion at blood glucose levels ≥ 90 mg/dl. However, as blood glucose decreased, there was a marked decrease in urinary glucose excretion in both canagliflozin and placebo treatments suggesting that urinary excretion of glucose depends on the prevailing glucose concentration.
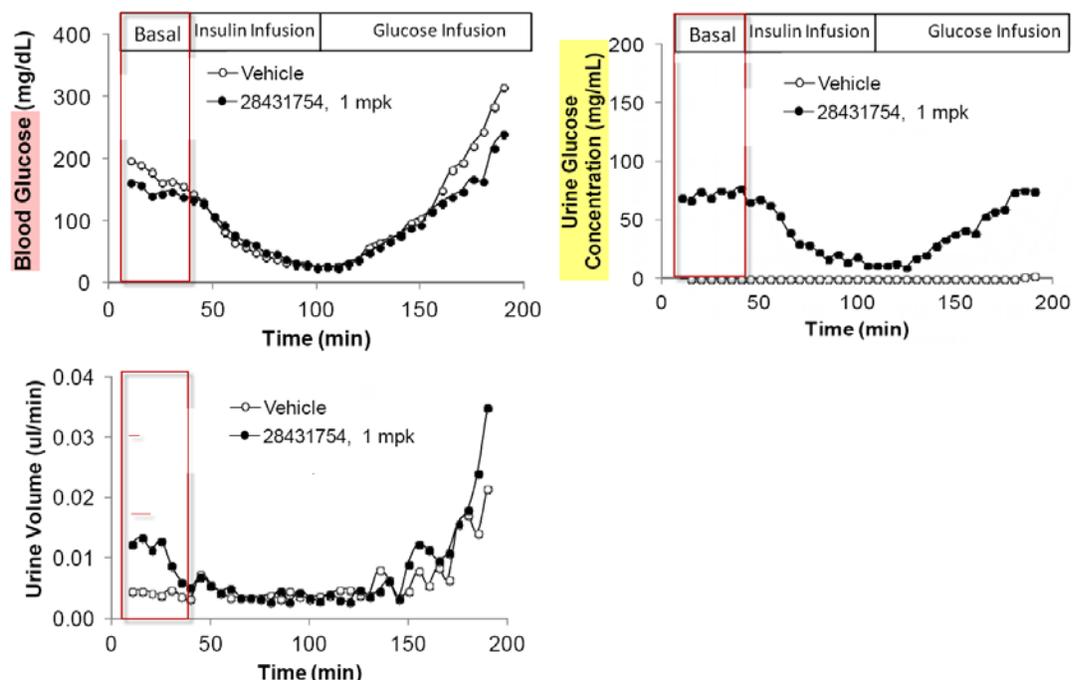
The effects of canagliflozin on other receptors were evaluated in the [redacted] (b) (4) receptor screen. Canagliflozin had slight inhibition of norepinephrine transporter (34% inhibition) and $5HT_{2A}$ receptors (37% inhibition). Other receptors/transporters were unaffected by canagliflozin.

4.2   Secondary Pharmacology

Canagliflozin-induced glucose excretion resulted in persistent polyuria in rats caused by the osmotic effect of glucose. 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.

The effect of canagliflozin on body weight was investigated in several animal models. Administration of canagliflozin (10, 30 and 100 mg/kg, PO) reduced body weight in SD rats but the weight loss generally occurred at significantly higher doses than glucosuric effect. In DIO and ob/ob mice, administration of JNJ-28431754 (3, 10 and 30 mg/kg, PO) caused a significant decrease in BW only at 30 mg/kg at the end of a 4-week treatment period while significant increase in urine glucose occurred at 3 mg/kg and blood glucose at 10 mg/kg.   The decrease in

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BW was not directly related to food intake (20.6 g in control vs. 19 g at 100 mg/kg in SD rats) or energy expenditure but rather due to lost glucose calories in urine. These studies suggest that a change in BW in the clinical setting may occur at the highest therapeutic dose range.

Mean Effect of JNJ-28431754 on Body Weight Gain (g) in SD Rats During a 14-Day Treatment.

Day of Treatment	Body Weight Gain (g)			
	Vehicle	10 mg/kg	30 mg/kg	100 mg/kg
4	32.0 ± 5.8	24.0 ± 2.6	17.4 ± 3.0*	3.3 ± 2.5*
8	65.3 ± 7.5	57.3 ± 2.9	49.0 ± 5.2*	27.1 ± 6.1*
11	92.3 ± 8.3	84.3 ± 6.0	66.4 ± 6.2*	54.6 ± 8.3*
15	123.1 ± 9.5	110.4 ± 9.7	97.6 ± 12.1*	73.8 ± 12.4*

*: $P<0.05$, compared with that of vehicle treated group at the same date.
Values represent the mean $\pm$ S.E.M.; n=7-8/group, g=grams

4.3   Safety Pharmacology

Canagliflozin is pharmacological active at renal SGLT2 and, at higher doses, at intestinal and renal SGLT1. Therefore, it is predicted to have minimal direct effect on CNS, cardiovascular, and respiratory system.   Any effect observed on blood pressure or HR is likely to be due to diuretic effect of urinary glucose. Canagliflozin administration to dogs did not produce any change in blood pressure.

Neurological
The behavioral effect of single dose canagliflozin was evaluated before and at 2, 4, 6 and 24 hrs post dose on Day 1 and on Day 8 in male rats using modified Irwin's method. There were no deaths after single doses of canagliflozin in rats. Canagliflozin had no notable effect on body temperature or neurobehavioral activity in rats.   At doses ≥250 mg/kg, canagliflozin was associated with decreased / soft feces. Watery feces were noted at higher doses of canagliflozin, likely due to intestinal carbohydrate inhibition. A slight decrease in BW was noted at all doses of JNJ-28431754 in rats. Single doses of JNJ-28431754 up to 1000 mg/kg had no notable CNS effect in male rats.

Concentration	Noteworthy Effects
Rat (FOB), CRL:CD® (SD), Neurobehavioral activity was assessed using a modified Irwin's method after a single oral dose; GLP Study (TR TOX7637 2007)	
(n = 5 males per group; vehicle = 0.5% hypromellose/suspension)	
0, 250, 500, and 1,000 mg/kg, p.o.	No neurological effects seen; no change in body temperature noted
≥250	↓ and soft fecal output, feces stained coat; ↓ body weight
≥500	Watery feces and unkempt coat.

Key: CD = cumulative dose; ECG = electrocardiogram; F = female; M = male; NOAEL = no observed adverse effect level; ↓ = decrease; ↑ = increase; i.v. = intravenous; p.o. = per os, by mouth; GLP = good laboratory practices; FOB = functional observation battery; SD=Sprague-Dawley

Cardiovascular and Pulmonary function
The cardiovascular and pulmonary effects of canagliflozin were evaluated in beagle dogs instrumented with telemetry prior to the study. Oral administration of canagliflozin (4, 40, and 400 mg/kg, PO) did not produce any test article-related effects on body weight, blood pressure,

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heart rate, or the ECG in dogs. Drug-related clinical signs included emesis, soft or watery green feces nearly at all dose. Oral administration of 400 mg/kg was associated with mild decrease in body temperature. Since there were no notable changes in the cardiovascular or pulmonary parameters at the highest dose tested, 400 mg/kg was considered NOAEL.

Concentration	Noteworthy Effects
Conscious dog, beagle, M; cardiovascular and pulmonary effects; GLP Study (TR TOX7722 2007) (n = 4; vehicle = 0.5% hypromellose) (Escalating doses at least 1 week apart for cardiovascular assessment and a second identical series for assessment of respiratory parameters)	
0, 4, 40, and 400 mg/kg p.o.	No test article-related effects on body weight, blood pressure, heart rate or the ECG. Clinical findings of emesis and/or soft/watery green feces following all test article doses. At 400 mg/kg: mild decrease in body temperature, not considered adverse. NOAEL = 400 mg/kg; estimated mean plasma concentration based on a pharmacokinetic bridging study (PKR FK7262 2009): 49,800 at 1 h, 59,400 ng/mL at 3 h, 30,100 ng/mL at 24 h

The effect of canagliflozin on cardiovascular parameters and ECG were evaluated using anesthetized Guinea pigs (0.16 mg/kg to 5 mg/kg, IV).   Intravenous cumulative administration of canagliflozin did not produce a significant dose-related increase in blood pressure or heart rate relative to the vehicle. A slight change in QRS duration was noted at cumulative intravenous dose of 1.11, 2.36 and 4.86 mg/kg according to the sponsor. The mean plasma concentration at 5 mg/kg was 12µg/ml (5 min post dose).

Concentration	Noteworthy Effects
Anesthetized guinea-pig, F, escalating i.v. doses (PHR DD06329 2006) (n = 6 for compound and n = 6 for vehicle; vehicle = 10% Solutol in 5% dextrose in water)	
0.16 to 5 mg/kg i.v. CD 9.86 mg/kg i.v.	Relative to vehicle: No dose-related statistically significant effects on mean arterial blood pressure, heart rate or on the electrocardiogram. At 0.63, 1.25, and 2.5 mg/kg i.v.: statistically significant difference in QRS duration, but largely due to changes in the vehicle group (+2%, +3%, and +5%, respectively, versus -5%, -5%, and −7% with vehicle). Mean plasma concentrations at 5 min postdose:

Dose (mg/kg)	0.16	0.32	0.63	1.25	2.5	5
Plasma level (ng/mL)	166	425	1,141	3,229	6,363	12,749

The effect of canagliflozin on hERG and isolated rabbit heart (Langendorff preparation) was also tested. Canagliflozin (10^{-7}, 3×10^{-7} and 3×10^{-6} M) and the reference (astemizole, 3×10^{-9}, 10^{-8} and 3×10^{-8} M) effect on membrane K+ current (IKr) in hERG-transfected HEK293 cells were evaluated. Canagliflozin had no significant effect on the K+ current in the hERG channel study. The positive control significantly inhibited the hERG-mediated current.

Summary of In Vitro Safety Studies

Concentration	Noteworthy Effects
I_{Kr} current in hERG-transfected HEK293 cells (PHR CPF1344 2006) (n = 3 for compound; n = 3 for vehicle; n = 3 for astemizole; vehicle = 0.1% DMSO)	
0.1, 0.3, and 3 μM	No inhibition of I_{Kr} current. No analysis of the locally applied drug concentration. Astemizole reduced I_{Kr} current significantly already at nanomolar concentrations.
Electrophysiological effects in isolated Langendorff-perfused female rabbit hearts (PHR HPC51 2006) (n = 6 for compound and n = 6 for vehicle; vehicle = 0.1% DMSO)	
0.1, 0.3, 1, 3, and 10 μM	No notable effect on triangulation, instability and reverse-use dependence of the action potential, on an index of proarrhythmia and intraventricular conduction time. No induction of EADs, ventricular tachycardia, ventricular fibrillation or torsades de pointes. At 3 and 10 μM: shortening of APD_{60} (-6% and –16%, respectively, versus +6% and +3% with vehicle; $p < 0.05$). At 10 μM: tendency to increase coronary flow (+18% versus +2% with vehicle; $p > 0.05$).

Key: I_{Kr} = rapidly activating delayed-rectifier potassium current; APD_{60} = duration of the action potential at 60% repolarization; DMSO = dimethyl sulfoxide; EADs = early after depolarization - type of cardiac arrhythmias.

Renal: Not evaluated
Gastrointestinal: Not examined
Abuse Liability: Not evaluated due to drug pharmacology and absence of CNS signal

5   Pharmacokinetics/ADME/Toxicokinetics

5.1   PK/ADME

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). Early *in-vitro* studies had identified large number of metabolites. The two prominent metabolites (M5 and M7) were not present in the *in-vitro* mouse, rat, dog and human hepatocyte metabolism studies. The *in-vitro* profile of canagliflozin in rats and dogs was similar to that in humans. *In-vivo* metabolism of canagliflozin in rats and dogs found metabolites that were not seen in the *in-vitro* 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 into urine and feces, there was no safety concern. Furthermore, analysis of bile in rats found far greater exposure to M7 (12%) and M5 (4%) than observed 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 (IC_{50} of 8.5 µg/mL) and MRP2 (IC_{50} of 9.5 µg/mL),) transporters. Whether these transporters are contributing to the difference in drug exposure observed in animals is unknown.

Summary of Mass balance Studies in Mice, Rats and humans

The sponsor had carried out *in-vivo* mass balance studies using single dose of metabolically stable C^{14}-JNJ-28431754 in mice, rats and humans. JNJ-28431754 was extensively metabolized in all species. In humans, JNJ-28431754 was moderately metabolized to *O*-glucuronide conjugates. Both M5 and M7 were the major metabolites in human plasma, urine and feces. There was some exposer to M5 and M7 in mouse urine and feces but not in rat. It is not clear whether absence of M5 and M7 in rats were due to hydrolysis back to parent form or low dose of C^{14} canagliflozin (100 mg/kg in mice vs. 3 mg/kg in rats and 200 mg in humans).

The metabolic profile of JNJ-28431754 (canagliflozin) in urine and feces from mice, rats and humans are shown below. Overall, most of the canagliflozin metabolites (o-glucuronide metabolites) in humans were also seen in toxicology studies, however, at much lower concentrations.

The Mass balance of unchanged JNJ-28431754 (UD) and its metabolites in urine and feces of mice, rats and humans after a single oral administration of ^{14}C-JNJ-28431754 of 100 mg/kg, 3 mg/kg and 200 mg, respectively

Figures represent the percentage of the dose radioactivity

Species	Matrix	Sex	UD[a]	M1	M2	M4	M5	M6	M7	M8	M9	M10	M11
Mouse	Urine	M	0.25	0.7	0.3	---	---	---	1.25	0.24	2.3	---	---
(FK6592		F	0.2	0.14	---	0.4	0.1	---	1.1	1.0	3.3	---	0.1
	Feces	M	32.5	---	---	2.7	0.8	1.8	6.4	13.6	27.6	---	---
		F	10.1	---	---	9.3	2.4	3.7	14.0	17.3	29.0	---	---
Rat	Urine	M	---	---	---	---	---	0.94	---	2.7	0.4	---	---
(FK6169)		F	0.20	---	---	---	---	0.40	---	4.1	0.6	---	---
	Feces	M	3.50	5.5	---	---	---	10.10	---	51.9	17.6	2.60	---
		F	5.30	18.0	---	---	---	7.90	---	58.8	2.1	1.40	---
Human	Urine	M	---	---	---	---	13.3	---	17.17	---	---	---	---
(FK6419)	Feces	M	41.47	---	---	---	---	---	3.17	---	6.95	---	---

[a] UD = unchanged drug   --- = not detected,

Analysis of pooled plasma from the three species found the parent drug being the primary plasma constituent. The metabolite levels in the mouse, rat and human plasma were less than 10% of the radioactivity. There were no O-glucuronide metabolites in rat plasma. It is not clear why JNJ-28431754 was positive in mouse lymphoma assay under metabolic activation (rat S9 mix), since no notable metabolite was identified in rat plasma.

Pooled (0-24 h) Plasma Levels of JNJ-28431754 (UD) and its Metabolites in Mice, Rats and Humans after a Single Oral Administration of ^{14}C-JNJ-28431754 of 100 mg/kg, 3 mg/kg and 200 mg, respectively

			Figures represent the percentage of the exposure radioactivity										
Species	Matrix	Sex	UD[a]	M1	M2	M4	M5	M6	M7	M8	M9	M10	M11
Mouse (FK6592)	Plasma	M	94.2	---	---	---	---	---	2.60	---	1.60	---	
		F	93.9	---	---	---	---	---	1.60		2.20	---	
Rat (FK6169)	Plasma	M	97.7	---	---	---	---	---	---	---	---	---	---
		F	96.5	---	---	---	---	---	---	---	---	---	---
Human (FK6419)	Plasma	M	84.3	---	---	---	---	---	3.56	---	4.77	---	3.73[b]

[a] UD = unchanged drug
[b] M11 was not detected in one sample, which was included in the calculation with a value of 0%.
--- = not detected.

Distribution of C^{14}-JNJ-28431754 was carried out after single dose of 5 mg/kg in pigmented Long Evans male rats. Since kidney is the primary target organ for pharmacological effect of JNJ-28431754, high radioactivity was detected in kidney cortex. In fact, the kidney exposure was nearly 12.5x greater than blood exposure. Protein binding studies found no significant differences among species (>98.3%).

AUC of ^{14}C labeled JNJ-28431754 in Tissues of Pigmented (Long Evans) Rats Administered a Single Oral Dose of 5 mg/kg

Organ/Tissue	AUC_{0-24h} of ^{14}C-JNJ-28431754	Tissue/blood ratio of AUCs
Small Intestine	223	13.1
Harderian Gland	203	12.0
Kidney Cortex	212	12.5
Liver	153	9.0
Adrenal Gland	119	7.0

Canagliflozin is eliminated primarily to feces via liver metabolism (plus unabsorbed drug in the gut) and urine in all three species but to a different extent. Nearly 92% of the radioactivity was recovered by 24 hrs in mice. Approximately 98 and 92% of the radioactivity was recovered by 120 and 168 hrs post dose in rats and humans.

Route and Extent of Excretion of ^{14}C-JNJ-28431754 in Mouse, Rat and Man

Species	Sex	Excretion (% of TR)		Total Excretion	
		Urine	Feces	Total time	Total %
Mouse (FK6592)[a]	M	5.0	85.4	24 h	90.4
	F	6.3	85.8	24 h	92.1
Rat (FK6169)[b]	M	4.0	88.5	120 h	96.8
	F	5.3	5.1	120 h	98.4
Man (FK6410)	M	32.5	60.4	168 h	92.9

[a] data from male metabolism samples collected over 24 h
[b] data from male mass balance samples collected over 120 h
TR = total radioactivity

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Metabolism of canagliflozin in animals and humans

Early *in-vitro* and *in-vivo* studies had identified several glucuronide metabolites in humans (M1A-C) that were not present in significant concentrations in rats and dogs. In the new metabolism studies in humans, the primary glucuronide metabolites were identified as M7 and M5. Similar to earlier studies, exposure to these two metabolites were very high in humans but relatively limited in mice, rats and dogs.
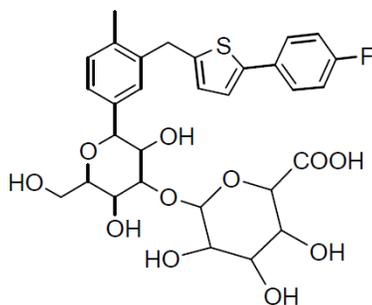
JNJ-41488525
M7
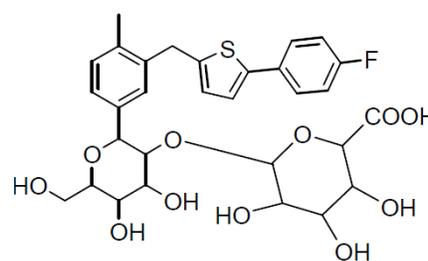
JNJ-41980874
M5

Canagliflozin is metabolized by uridine diphosphate glucuronosyltransferase isozymes (UGT). The UGT enzymes responsible for glucuronidaton of M7 and M5 were UGT1A9 and UGT2B4, respectively. Glucuronyl transferase is a microsomal enzyme involved in phase 2 metabolic reactions, forms a conjugate between the glucuronic acid and canagliflozin leading to glucuronide metabolites, M5 and M7. Glucuronide conjugates are known by the attachment site of glucuronic acid (N-, O-, COO-, S- and C- Glucuronides). Potential exhaustion of the enzyme may lead to accumulation or the parent or alternate pathway.

In-vitro metabolism

In-vitro metabolism of canagliflozin was determined using hepatocytes from rats, dogs and humans. Canagliflozin was metabolized via hydroxylation, alcohol oxidation and o-glucuronidation. Although the amount of each metabolite varied, all the metabolites identified in humans were also found the early in-vitro human hepatocyte studies. Parent drug was the most abundant product.

Metabolites of JNJ-28431754 Produced In Vitro by Cryopreserved Hepatocytes from Rat, Dog, and Human Incubated with JNJ-28431754 at a Concentration of 10 μM

Compound/Metabolite	Rat	Dog	Human
JNJ-28431754	46	55	56
Glucuronide (M1A)	<1	<1	11
Glucuronide (M1B)	2	<1	<1
Glucuronide (M1C)	7	6	31
Carboxy (M2)	28	37	1
Mono-oxygenated (M3A)	7	<1	<1
Mono-oxygenated (M3B)	<1	ND	ND
Mono-oxygenated (M3C)	9	2	ND
Total detected (%)	~100	~100	~100

ND = Not detected

In-vivo metabolism of canagliflozin

Since plasma levels of the O-glucuronide metabolites (M5 and M7) were very low in the toxicology studies, specifically in rats, a new in-vivo mass balance study was carried out in male SD rats. Exposure to M5 and M7 in bile, plasma and urine were determined after single and 14

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days (3 and 100 mg/kg PO, C[14]-canagliflozin, Study#FK7526, May 27, 2010, Janssen Research and Development, Belgium). After single dose of 3 mg/kg, the 24-hr excretion of canagliflozin in bile, feces and urine were 52%, 22% and 4%, respectively. The excretion profile after single dose of 100 mg/kg was similar to multiple doses at 100 mg/kg (48% bile, 18% feces and 4% urine). Major metabolites in bile were M6, M6B, M7, M5, M5B and M8. _M7 accounted for 12% of the total administered dose while M5 and its isomer, M5B accounted for 5 to 6% of the total administered dose_ suggesting that exposure to M5 and M7 are dose proportional and much higher than previously detected. It is highly possible that the glucuronide metabolites were hydrolyzed in the intestine in rats, leading to very low plasma levels of M5 and M7. Based on this new data, exposure to M5 and M7 were significantly greater than previously observed in the 6-month rat toxicology study (M7:0.8% and M5:0.07% of parent drug exposure).

Comparison of the abundance of unchanged drug and its metabolites in bile (expressed as % of the administered dose) after 24 h of bile collection following 3 mg/kg administration (single dose) and 100 mg/kg (multiple dose)
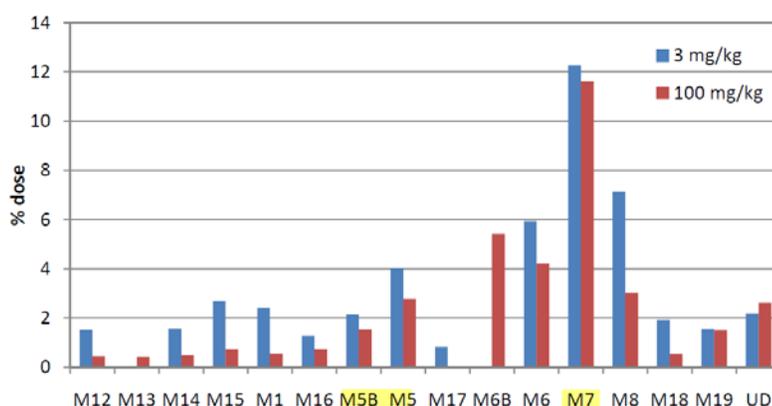
Excretion of total radioactivity (% of administered dose) in pooled rat bile and feces per metabolite following oral administration of JNJ-28431754 at 3 mg/kg.

Metabolite Code	Description	BILE						FECES*
		% dose administered						% dose administered
		0-4h	4-8h	8-12h	12-16h	16-24h	0-24h	0-24h
M12	UD + 2O -2H	0.1	0.56	0.43	0.26	0.17	1.52	-
M14	UD + O + Glucuronide	0.48	0.38	0.28	0.3	0.12	1.56	-
M15	UD + O + Glucuronide	0.54	0.67	0.56	0.38	0.54	2.69	-
M1	O- glucuronide of M8 or M9	-	0.75	0.91	0.43	0.32	2.41	-
M16	oxidation and glucuronidation	-	0.34	0.46	0.17	0.3	1.27	-
M5B	UD + O + Glucuronide	0.45	0.44	0.52	0.43	0.29	2.13	-
M5	UD + glucuronide	0.71	0.85	0.98	0.61	0.86	4.01	-
M17	UD + glucuronide	0.29	0.2	0.14	0.19		0.82	-
M6	Carboxylated Metabolite	0.54	1.73	1.58	1.13	0.95	5.93	-
M7	UD + glucuronide	2.26	2.59	2.95	2.09	2.37	12.26	-
M8	UD + O	0.74	1.57	1.79	1.48	1.54	7.12	0.94
M18	UD + O	0.1	0.54	0.4	0.35	0.54	1.93	-
M19	UD + O	0.15	0.22	0.46	0.34	0.38	1.55	-
UD	JNJ-28431754	0.48	0.42	0.45	0.56	0.26	2.17	7.09
	Sum reported metabolites (dose %)	6.84	11.26	11.91	8.72	8.64	47.37	8.03
	Sum observed metabolites (dose %)	7.88	13.07	13.74	10.26	9.38		10.41
	Column Recovery %	106.3	108.5	105.2	110.7	90		62.2
	Total radioactivity (LSC)	7.41	12	13.1	9.27	10.4	52.2	22.4

* Extraction efficiency of feces: 75%; recovery after UPLC sample preparation: 95%

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In-vivo metabolism in humans

In an early *in-vivo* metabolism study in health human volunteers (FK6251, 200 mg canagliflozin), *O*-glucuronide metabolites (M1A-C) were considered the primary metabolites in plasma (30 to 61%) and in urine (95 to 98%). Parent drug constituted 39 to 70% of the total in plasma and 1 to 3% in urine. These o-glucuronide metabolites are now appear to be reclassified as M7, M5 and M5B (Study#NAP-1006, see below)

Pharmacokinetics: Metabolism *In Vivo*

Test Article: JNJ-28431754

| Compound | m/z | RRT | SAD Study in Healthy Male Human Subjects | | | | | |
| | | | Plasma | | | Urine | | |
			0.25-6 hr	8-24 hr	48-96 hr	0-7 hr	7-24 hr	24-96 hr
JNJ-28431754	462	1.00	48	39	70	1	1	3
O-Glucuronide (M1A)	638	9.60	18	28	14	36	36	37
O-Glucuronide (M1B)	638	10.2	2	2	ND	2	3	3
O-Glucuronide (M1C)	638	10.9	33	32	16	61	60	57
Carboxy (M2)	476	0.803	ND	ND	ND	trace	trace	ND
Monooxygenated (M3A)	478	0.864	ND	ND	ND	trace	trace	Trace
Monooxygenated (M3B)	478	ND	ND	ND	ND	ND	ND	ND
Monooxygenated (M3C)	478	ND	ND	ND	ND	ND	ND	ND
Monooxygenated (M3D)	478	ND	ND	ND	ND	ND	ND	ND
Monooxygenated (M3E)	478	ND	ND	ND	ND	ND	ND	ND
Monooxygenated (M3F)	478	ND	ND	ND	ND	ND	ND	ND
O-Glycoside (M4A)	624	ND	ND	ND	ND	ND	ND	ND
O-Glycoside (M4B)	624	ND	ND	ND	ND	ND	ND	ND
O-Glucuronide of M3A (M5)	654	0.682	ND	ND	ND	1	2	2

Clinical Protocol: JNJ-28431754NAP1001
ND: Not detected
RRT: Retention time relative to unchanged drug
The percent of each component is estimated from integrated peak areas in reconstructed ion chromatograms with assumption of equal ionization efficiencies and no suppression of ionization by co-eluting components from matrix. Data is only for qualitative use only.

In a recent clinical study (FK6419, clinical study # NAP-1006), the metabolite profile of C^{14}-canagliflozin (200 mg) was determined again in healthy male volunteers. Approximately 92.9% of the administered radioactivity was recovered in urine (32.5±51%) and feces (60.4±5.7%) at one week. The two prominent o-glucuronide metabolites in plasma, urine and feces were identified as M7 (JNJ-41488525) and M5 (JNJ-41980874). M9 (JNJ-42023566) was a minor metabolite in plasma.

Plasma: The mean AUC_{0-24h} for canagliflozin (JNJ-28431754) was 32.5% of total plasma radioactivity AUC_{0-24h}. Unchanged plasma concentrations ranged from 45.4% to 98.7% of the total radioactivity during the first 24 hrs. The remaining drug-related radioactivity during the first 12 hrs in plasma were two O-glucuronides (M7:16 to 28% and M5:1.9 to 29.6%) and a hydroxylated metabolite (M9:2.4 to 3.7%).

Urine constituted 32.5% of the radioactivity was comprised of M5 (13.3%) and M7 (17.2%). There was no parent drug activity in urine.

Feces which constituted 60% of the total radioactivity, was made up of parent drug (41.5%) and M9 (7%) and M7 (3.2%). It is highly possible that parent drug was excreted unchanged or hydrolyzed biliary excretion of M7 product.

Percentage of Total Radioactivity of JNJ-28431754 and its Metabolites in Pooled Time-Points from 6 Healthy Male Subjects Following Oral   Administration of 14C-JNJ-28431754 (200 mg/subject)

Metabolite	% Total Radioactivity				
	Pooled Plasma from Each Time-point (hr) Across Subjects (1001-1006)				
	1.5	4	8	12	24
Unchanged Drug (UD) (JNJ-28431754)	62.7	47.1	45.4	65.8	98.7
O-glucuronide of UD (M5, JNJ-41980874)	18.6	29.6	24.1	1.89	-
O-glucuronide of UD (M7, JNJ-41488525)	16.0	18.3	24.7	28.8	-
Hydroxylated UD (M9, JNJ-42023566)	2.42	3.70	2.58	2.83	-
Total	99.7	98.7	96.8	99.3	98.7

The percent of each component is calculated from integrated peak areas in radiochromatograms

Canagliflozin Pk and metabolism profile in patients with renal disease

The exposure for canagliflozin and its prominent inactive O-glucuronide metabolites, M7 (JNJ-41488525) and M5 (JNJ-41980874) were determined in patients with varying end stage renal disease in a single dose PK study (Protocol #28431754-DIA-1003).   Canagliflozin AUC exposure increased in patients with mild, moderate and severe renal impairment by as much as 17%, 63% and 51%, respectively. Since both M7 and M5 are rapidly clearly by the kidneys, the exposure to both M7 (41%, 130% and 103% in mild, moderate and sever renal impairment) and M5 (27%, 151% ad 161%) also increased with increase in severity of renal impairment, however the increase in the M7 and M5 exposure were significantly higher than the increase in canagliflozin exposure. The study suggests that, a) patients with end stage renal impairment will have significant increase in exposure to both parent and M5 and M7 metabolites, b) the exposure to inactive M7 and M5 is likely to be as much as 2-fold higher than the rise in parent drug AUC. Protein binding of the parent drug, total plasma protein and albumin under varying renal conditions were similar. However, alpha-1 AGP increase with decreasing renal function.

Arithmetic Mean (SD) Plasma and Urine Pharmacokinetic Parameters Following a Single 200-mg Oral Dose of Canagliflozin in Subjects With Renal Function Ranging From Normal to End-Stage Renal Disease (Study 28431754DIA1003, Pharmacokinetic Data Analyses Set)

PK Parameters	Normal Renal Function (≥80 mL/min)	Mildly Impaired (50 to <80 mL/min)	Moderately Impaired (30 to <50 mL/min)	Severely Impaired (<30 mL/min)	ESRD Pre-Dialysis[b]	ESRD Post-Dialysis[b]
C_{max} (ng/mL)	1475 (669)	1574 (482)	1773 (439)	1834 (732)	1433 (509)	1287 (277)
t_{max} (h)[a]	1.75 (0.5-5.0)	3.5 (1.5-5.0)	1.75 (1.0-5.0)	1.5 (1.0-5.0)	2.25 (1.0-6.0)	2.00 (1.5-5.0)
AUC_∞ (ng.h/mL)	14345 (3605)	16719 (3721)	23311 (5475)	21596 (5485)	14205 (3648)	13587 (3216)
AUC_{last} (ng.h/mL)	14114 (3597)	16363 (3635)	22876 (5517)	21154 (5336)	13758 (3322)	13271 (3019)
$t_{1/2}$ (h)	17.4 (5.4)	22.7 (10.3)	21.7 (10.9)	21.6 (9.2)	21.4 (12.0)	17.2 (4.9)
Vd/F (L)	359 (113)	414 (212)	287 (172)	294 (105)	428 (205)	365 (68.8)
CL/F (L/h)	14.8 (3.93)	12.5 (2.65)	9.03 (2.23)	9.81 (2.53)	15.0 (4.05)	15.4 (3.22)
Ae (% dose)	0.58 (0.26)	0.32 (0.09)	0.33 (0.15)	0.14 (0.04)	NA	NA
% Dialyzed	NA	NA	NA	NA	0.026[c]	NA
CL_R (L/h)	0.092 (0.068)	0.039 (0.014)	0.032 (0.021)	0.015 (0.007)	NA	NA

[a] t_{max} expressed as median (range)
[b] Pre-dialysis (dosed 2 hours before HD) or post-dialysis (1 hour after HD)   [c] n=7
Key: ESRD=end-stage renal disease; NA= Not applicable
Note: N=8 for each renal function group

Mean (SD) Metabolite Pharmacokinetic Parameters Following a Single 200-mg Oral Dose of Canagliflozin in Subjects With Renal Function Ranging From Normal to End-Stage Renal Disease (Study 28431754DIA1003, Pharmacokinetic Data Analyses Set)

Parameters	Normal Function (≥80 mL/min)	Mildly Impaired (50 to <80 mL/min)	Moderately Impaired (30 to <50 mL/min)	Severely Impaired (<30 mL/min)	ESRD Pre-dialysis[e]	ESRD Post-dialysis[e]
			JNJ-41488525 (M7)			
C_{max} (ng/mL)	1159 (380)	1831 (712)	2437 (1179)	2216 (1565)	2313 (1372)	2038 (1197)
t_{max} (h)[a]	3.5 (2.0-6.0)	3.5 (2.0-6.0)	3.5 (2.0-6.0)	3.5 (2.0-6.0)	3.52 (2.00-6.00)	3.00 (2.00-6.00)
AUC_∞ (ng.h/mL)	15949 (4717)	22435 (6972)	41103 (19486)	39743 (38407)	35120 (29380)	32229 (25524)
AUC_{last} (ng.h/mL)	15259 (4732)	22098 (7037)	40622 (19347)	38907 (38534)	34111 (27895)	31744 (25079)
$t_{1/2}$ (h)	19.1 (9.9)	22.7 (9.6)	18.0 (5.2)	25.3 (16.5)	22.5 (12.8)	17.9 (7.1)
C_{max} Molar Ratios[c]	0.63 (0.22)	0.89 (0.35)	1.07 (0.63)	0.91 (0.56)	1.20 (0.61)	1.22 (0.80)
AUC_∞ Molar Ratios[c]	0.82 (0.17)	1.05 (0.46)	1.39 (0.81)	1.31 (1.03)	1.84 (1.42)	1.79 (1.31)
Ae (% dose) [d]	17.7 (3.6)	14.2 (2.45)	8.4 (3.9)	4.4 (2.9)	NA	NA
%Dialyzed	NA	NA	NA	NA	0.502	NA
			JNJ-41980874 (M5)			
C_{max} (ng/mL)	703 (175)	1023 (383)	1431 (591)	1651 (1135)	1628 (689)	1725 (687)
t_{max} (h)[a]	4.0 (2.0-8.0)	4.0 (2.0-6.0)	4.5 (3.0-24.1)	4.0 (4.0-12.0)	6.00 (6.00-6.00)	6.00 (5.00-6.00)
AUC_∞ (ng.h/mL)	13467 (6211)[b]	16019 (3922)	36297 (23661)	40046 (31479)	32238 (22600)	33625 (19683)
AUC_{last} (ng.h/mL)	13551 (5830)	15755 (3967)	35863 (23592)	39275 (31424)	31198 (21422)	32975 (19106)
$t_{1/2}$ (h)	14.0 (3.2)[b]	22.4 (11.2)	18.3 (4.4)	22.4 (9.7)	19.4 (9.7)	16.9 (6.2)
C_{max} Molar Ratios[c]	0.40 (0.17)	0.48 (0.09)	0.62 (0.29)	0.67 (0.40)	0.87 (0.39)	1.04 (0.50)
AUC_∞ Molar Ratios[c]	0.70 (0.23)	0.72 (0.19)	1.15 (0.64)	1.30 (0.85)	1.65 (1.07)	1.84 (0.96)
Ae (% dose) [c]	8.5 (2.5)	7.6 (2.5)	4.4 (2.4)	3.2 (3.8)	NA	NA
%Dialyzed	NA	NA	NA	NA	1.18	NA

[a] Median (Range)   [b] n=7

[c] Molar Ratio = [Parameter(metabolite)/Molecular weight(metabolite)]/[Parameter(parent)/Molecular weight(parent)]; Molecular weights: canagliflozin (454 g/mole); JNJ-41488525 (M7)and JNJ-41980874 (M5) (620.6 g/mole)
[d] Ae (% dose) adjusted for molecular weight
[e] Pre-dialysis (dosed 2 hours before HD) or post-dialysis (1 hour after HD)

In vivo metabolism profile of canagliflozin in mouse, rat, dog and humans

In Vivo Metabolism of [^{14}C]Canagliflozin After a Single Oral Dose in Mouse (100 mg/kg), Rat (3 mg/kg), Dog (4 mg/kg) and Human (196 mg): Percent Radioactivity

	Mouse, Swiss CD-1 (PKR FK6592 2008)						Rat, Sprague-Dawley (PKR FK6169 2008)						Dog, Beagle (PKR FK6183 2008)			Human (PKR FK6419 2008[a])						
	Plasma 0-24h		Urine 0-48h		Feces 0-48h		Plasma 0-24h		Urine 0-48h		Feces 0-48h		Plasma 0-24h	Urine 0-48h	Feces 0-72h	Plasma					Urine 0-48h	Feces 0-48h
	M	F	M	F	M	F	M	F	M	F	M	F	M	M	M	1.5h M	4h M	8h M	12h M	24h M	M	M
UD	94.2	93.9	0.25	0.2	32.5	10.1	97.7	96.5	--	0.20	3.50	5.30	96.9	0.25	11.1	62.7	47.1	45.4	65.8	98.7	--	41.5
M1	--	--	0.7	0.14	--	--	--	--	--	--	5.50	18.0	--	--	--	--	--	--	--	--	--	--
M2	--	--	0.3	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
M4	--	--	--	0.4	2.7	9.3	--	--	--	--	--	--	--	0.40	11.2	--	--	--	--	--	--	--
M5	--	--	--	0.1	0.8	2.4	--	--	--	--	--	--	--	--	--	18.6	29.6	24.1	1.89	--	13.3	--
M6	--	--	--	--	1.8	3.7	--	--	0.94	0.40	10.1	7.90	--	--	--	--	--	--	--	--	--	--
M7	2.6	1.6	1.25	1.1	6.4	14.0	--	--	--	--			--	--	7.13	16.0	18.3	24.7	28.8	--	17.2	3.2
M8	--	--	0.24	1.0	13.6	17.3	--	--	2.70	4.10	51.9	58.8	--	0.81	41.8	--	--	--	--	--	--	--
M9	1.6	2.2	2.3	3.3	27.6	29.0	--	--	0.40	0.60	17.6	2.10	--	0.51	22.5	2.42	3.70	2.58	2.83	--	--	7.0
M10	--	--	--	--	--	--	--	--	--	--	2.60	1.40	--	--	--	--	--	--	--	--	--	--

[a] Data for clinical study 28431754NAP1006 2009;
-- = not detected; UD = unchanged drug
Note: 196 mg of ^{14}C-canagliflozin is equivalent to 200 mg of canagliflozin-ZAE, the hemi-hydrate form of canagliflozin.

New proposed *in-vitro* metabolic pathway in mouse, rat, dog, rabbit and human hepatocytes

Canagliflozin in vitro metabolism charted out early in the development were inconclusive and not well characterized.   Some of the metabolites identified in the latest in vitro studies were not detected in the in vivo studies.

Test Article: JNJ-28431754 (Batch ID: 30178757)
^{14}C-JNJ-28431754 (Batch : DCH-21245-166)
Study No. FK6320
Proposed *in vitro* Metabolic Pathways of JNJ-28431754 in Rat, Dog, Mouse, Rabbit and Human hepatocytes
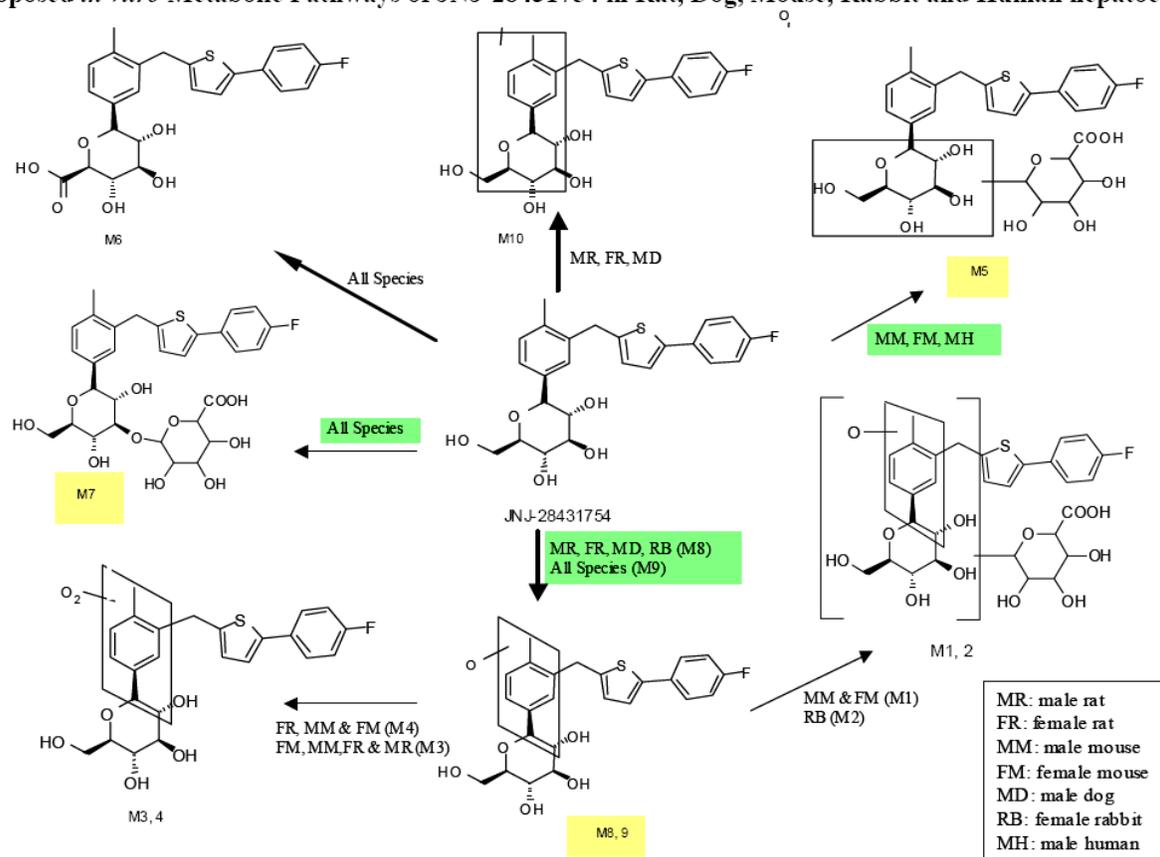
New proposed *in-vivo* metabolic pathway in mouse, rat and humans

The most recent proposed in-vivo metabolic pathway for canagliflozin (JNJ-28431754) in mouse, rat and human is shown in figure below. The prominent human o-glucuronide metabolites (M7 >M5) were also present in mouse and rat plasma, however they were quantitatively significantly less than humans. However, when bile levels were measured in a mass balance study in rats, significantly higher levels of both M7 (12%) and M5 (4%) were present in bile suggesting that rats form both M7 and M5 but they might be hydrolyzed.

Metabolic pathway of ^{14}C-JNJ-28431754 in mouse, rat and human
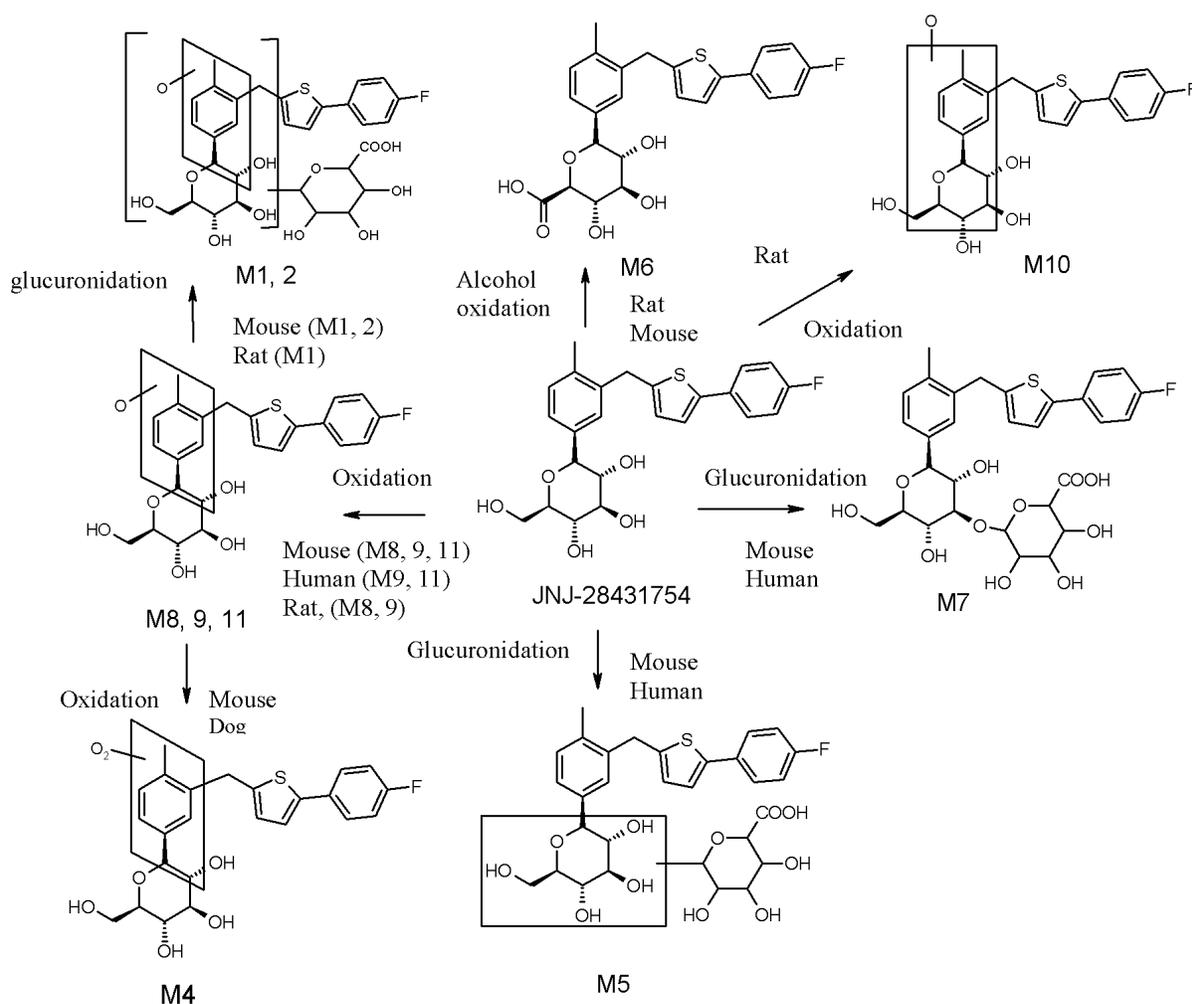
CYP Induction and inhibition properties of canagliflozin

Canagliflozin was not an inducer of CYP 3A4, 2C9, 2C19 and 1A2 and it appeared to be a weak (<2x) inhibitor of Cyp3A4 (IC50 27 μM= 12 μg/ml) and CYP2C9 (IC50 of 80 μM =35 μg/ml).

Neither M5 nor M7 had any inhibitory effect in CYP isoforms (IC$_{50}$ > 100 µM) except for CYP2B6 and CYP2C8 for M7 metabolite. M7 was a weak inhibitor of CYP2B6 (IC$_{50}$ 55 µM) and CYP2C8 (IC$_{50}$ of 64 µM) and likely to have low potential for drug-drug interaction.

Canagliflozin Effect on drug transporters
The ability of canagliflozin to cross the intestinal wall was tested using Caco-2 cells. Based on this study, canagliflozin had intermediate permeability in the human intestine (apical to basolateral transport, A→B).   The transport was affected by an apically located efflux pump (B→A was higher than A→B).
Canagliflozin was a substrate to and a weak inhibitor of efflux transporter protein MDR1 (pg-P, IC$_{50}$ of 19.3 µM or 8.5 µg/ml) and multidrug resistant protein MDR2 (IC$_{50}$ of 21.5µM=9.5 µg/ml). Canagliflozin was not substrate for hNTCP, hOAT1, hOAT3, hOATP1B1, or hOCT1 cRNA transporters. There was no inhibition of hOAT1, hOAT3 or hOCT1 transporters (FK6238, 2007).

M5 and M7 (o-glucuronide metabolites) on drug transporters
As major plasma constituents (up to 28%), the effect of M5 and M7 on transporters were determined (FK7483, May 23, 2011).   There was no uptake of M5 or M7 into water-injected control, or human NTCO, OAT1, OATP1B1 (OATP2), OCT1 or OCT2-expressing oocytes.   M5 was not substrate or inhibitor of human NTCP, OAT1, OATP2, OCT1 or OCT2. M7 was not substrate for human NTCP, OAT1, OATP2, OCT1 or OCT2 and did not inhibit OAT1, OCT1 or OCT2 except at super pharmacological concentrations (100 µM) where M7 showed a positive inhibition of NTCP (86%), OAT3 (54%) and OATP2 (65%).

Inhibition of human NTCP, OAT3, OATP2 and OCT2-meidated uptake activity

Probe Substrate	Inhibitor	pmol probe substrate in water-injected control oocytes at t=60 min			pmol probe substrate in NTCP-expressing oocytes at t=60 min			Transporter-specific uptake (transporter-control)	Uptake Ratio[3]	Inhibition of substrate uptake[4]
		mean	SD[1]	n[2]	mean	SD	n	mean		
2 µM [³H]-TCA (taurocholic acid)	-	0.014	0.0052	10	2.4	0.86	10	2.4	169	-
	200 µM taurochenodeoxycholic acid	0.024	0.0058	10	0.051	0.011	10	0.027	2.1	99%
	100 µM M5 (JNJ-41980874)	0.020	0.0053	10	2.1	0.57	8	2.1	105	12%
	100 µM M7 (JNJ-41488525)	0.040	0.0089	10	0.37	0.16	7	0.33	9.4	86%

Probe Substrate	Inhibitor	pmol probe substrate in water-injected control oocytes at t=60 min			pmol probe substrate in OAT3-expressing oocytes at t=60 min			Transporter-specific uptake (transporter-control)	Uptake Ratio[3]	Inhibition of substrate uptake[4]
		mean	SD[1]	n[2]	mean	SD	n	mean		
2 µM [³H]-E3S (estrone-3 sulfate)	-	0.060	0.011	10	1.8	1.4	10	1.8	31	-
	200 µM bumetanide	0.066	0.0087	10	0.19	0.066	10	0.12	2.9	93%
	100 µM M5 (JNJ-41980874)	0.060	0.010	10	2.0	1.0	10	1.9	33	-9.3%
	100 µM M7 (JNJ-41488525)	0.057	0.0076	10	0.88	0.41	10	0.82	15	54%

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Inhibition of human NTCP, OAT3, OATP2 and OCT2-meidated uptake activity

Probe Substrate	Inhibitor	pmol probe substrate in water-injected control oocytes at t=60 min			pmol probe substrate in OATP1B1 (OATP2)-expressing oocytes at t=60 min			Transporter-specific uptake (transporter-control)	Uptake Ratio[3]	Inhibition of substrate uptake[4]
		mean	SD[1]	n[2]	mean	SD	n	mean		
2 µM [3H]-E3S (estrone-3 sulfate)	-	0.15	0.075	8	1.2	0.28	9	1.0	7.9	-
	200 µM rifamycin	0.21	0.061	9	0.26	0.072	9	0.050	1.2	95%
	100 µM M5 (JNJ-41980874)	0.18	0.051	8	1.3	0.27	7	1.1	7.4	-11%
	100 µM M7 (JNJ-41488525)	0.20	0.084	9	0.56	0.21	9	0.36	2.8	65%

Probe Substrate	Inhibitor	pmol probe substrate in water-injected control oocytes at t=60 min			pmol probe substrate in OCT2-expressing oocytes at t=60 min			Transporter-specific uptake (transporter-control)	Uptake Ratio[3]	Inhibition of substrate uptake[4]
		mean	SD[1]	n[2]	mean	SD	n	mean		
10 µM [14C]-TEA (tetraethyl ammonium acid)	-	0.32	0	10	14	5.8	8	14	44	-
	300 µM cimetidine	0.32	0	10	2.2	0.89	8	1.9	6.9	86%
	100 µM M5 (JNJ-41980874)	0.32	0	10	13	4.4	8	13	40	8.8%
	100 µM M7 (JNJ-41488525)	0.33	0	10	13	5.9	8	12	38	11%
	100 µM JNJ-28431754	0.33	0	10	8.0	2.7	8	7.7	25	44%

[1] SD: standard deviation   [2] n: number of oocytes included in the mean   [3] Uptake Ratio: (Uptake Activity Transporter / Uptake Activity Control)
[4] Inhibition = [1- (Uptake Activity with inhibitor) / (Uptake Activity without inhibitor)] x 100

Comparing Different Formulations of Canagliflozin in Dogs

The PK profiles of five different formulations of canagliflozin were tested in fasted male beagle dogs. There were no meaningful differences in AUC exposure among 5 different canagliflozin formulations.

Pharmacokinetic Characteristics of 5 Formulations of JNJ-28431754 in Fasted Male Dogs					
Formulation[a]	1	2	3	4	5
Dose	10 mg/kg	100 mg	100 mg	100 mg	100 mg
Pharmacokinetic Parameters					
C_{max} (ng/mL)	9668	11328	11475	11318	12800
t_{max} (h)	1.00	2.13	2.25	1.38	1.38
$AUC_{0-\infty}$ (ng•h/mL)	162046	177446	191001	157052	176133
$t_{1/2}$ (h)	12.9	10.1	10.7	8.08	8.95
CL/F (mL/h)	802	602	529	647	568

(b) (4)

5.2   Toxicokinetics

Oral gavage toxicology studies were carried with canagliflozin suspended in 0.5% hypromellose solution except for the rat carcinogenicity 0.5% methocel served as vehicle.   Since canagliflozin was metabolized primarily to O-glucuronide metabolites M5 and M7, the exposure to M5 and M7 were determined in plasma samples collected from nonclinical and clinical studies. TK parameters for canagliflozin, M5 and M7 in mice, rats and dogs are shown tables below.

Canagliflozin Exposure in 3-Month Mouse, and Chronic Rat and Dog Toxicity Studies and Human Exposure at 100 and 300 mg QD Clinical Doses

	Sex	Dose (mg/kg/day)	C_{max} (μg/mL)	t_{max} (h)	AUC^{a} (μg.h/mL)
3-Month Mouse Toxicity (TOX8262), n = 3/sex/dose/timepoint					
Day 1	M	30	8.09	1.00	67.0
	F		7.41	1.00	99.9
	M	100	25.5	1.00	285.0
	F		29.5	1.00	344.0
	M	300	69.9	2.00	876.0
	F		76.9	2.00	1,270.0
Month 3	M	30	8.80	1.00	65.9
	F		10.4	1.00	102.0
	M	100	28.9	2.00	235.0
	F		35.9	1.00	354.0
	M	300	73.9	2.00	651.0
	F		86.3	2.00	736.0
6-Month Rat Toxicity (TOX8574), n=6/sex/dose					
Day 0	M	4	0.93	4.00	12.9
	F		0.83	4.00	13.7
	M	20	4.36	4.00	72.3
	F		4.19	7.00	73.9
	M	100	25.7	4.00	479.0
	F		25.0	4.00	636.0
Month 6	M	4	1.11	4.00	14.1
	F		1.56	0.500	21.6
	M	20	4.02	7.00	68.5
	F		5.51	4.00	88.3
	M	100	19.3	7.00	321.0
	F		29.8	4.00	379.0
1-year Dog Toxicity (TOX8446), n = 4/sex/dose					
Day 1	M	4	3.20	2.25	54.5
	F		3.05	1.50	50.6
	M	30	15.2	6.00	*444.0
	F		17.3	2.75	*437.0
	M	100	26.8	1.75	*533.0
	F		23.4	3.13	*901.0
Week 52	M	4^{b}	4.19	1.33	52.2
	F		5.67	0.875	67.0
Week 52	M	30	21.4	1.75	262.0
	F		20.2	1.00	264.0
Week 52	M	100	39.3	1.50	529.0
	F		38.6	0.875	503.0
Human Exposure (pooled data from DIA1007 and DIA1023; Mod2.7.2\App2.4)					
		100 mg QD	0.98	1.5^{c}	6.97
		300 mg QD	4.12	1.5^{c}	26.1

[a] $AUC_{0-\infty}$ for single dose and AUC_{0-24} for repeated doses   [c] Data for DIA1023
[b] n = 3 due to animal death

* = $AUC_{0-\infty}$ extrapolated value was greater than 25% of AUC_{0-24}; F = female; M = male; n = number of animals

Mean Canagliflozin Exposure in the 2-year Mouse and Rat Carcinogenicity Studies and at 100 and 300 mg Clinical Doses

	Sex	Dose (mg/kg/day)	C_{max} (µg/mL)	AUC[a] (µg.h/mL)
2-year Mouse Carcinogenicity (TOX8799), n = 18/sex/dose				
Day 0	M	10	1.18	8.17
	F	10	1.34	12.8
	M	30	8.84	62.0
	F	30	12.3	93.2
	M	100	57.2	324.0
	F	100	47.6	344.0
Month 6[b]	M	10	2.38	11.3
	F	10	3.7	36.4
	M	30	10.3	47.7
	F	30	13.1	102.0
	M	100	27.6	194.0
	F	100	37.0	353.0
2-year Rat Carcinogenicity (TOX8986), n = 4/sex/dose				
Day 0	M	10	1.82	27.7
	F	10	1.37	20.8[c]
	M	30	5.30	83.6
	F	30	4.67	69.8
	M	100	21.82	370.8[c]
	F	100	18.70	408.9[d]
Month 6[b]	M	10	3.11	38.4
	F	10	4.71	62.0
	M	30	8.28	117.7
	F	30	14.93	188.4[c]
	M	100	23.39	316.0
	F	100	36.9	559.9
Human Exposure (pooled data for DIA1007 and DIA1023; Mod2.7.2\App2.4)				
		100 mg	0.98	6.97
		300 mg	4.12	26.1

[a] $AUC_{0-\infty}$ for single dose and AUC_{0-24} for repeated doses
[b] Last time point
[c] n = 3
[d] n = 2

F = female; M = male; n = number of animals

M5 and M7 Exposure in the Chronic Rat and Dog Toxicity Studies, the Mouse Carcinogenicity Study, and in Humans

	Sex	Dose (mg/kg/day)	M5 C_{max} (µg/mL)	M5 AUC_{0-24h} (µg.h/mL)	M7 C_{max} (µg/mL)	M7 AUC_{0-24h} (µg.h/mL)
6-Month Rat (TOX8574)						
Month 6	M	4	BQL	BQL	0.017	0.116
	F	4	BQL	BQL	0.031	0.115
	M	20	0.013	0.048	0.087	0.559
	F	20	0.082	0.009	0.074	0.606
	M	100	0.053	0.460	0.432	4.020
	F	100	0.055	0.446	0.595	5.480
1-year Dog (TOX8446)[a]						
Week 52	M	100	0.050	0.440	0.515	5.670
	F	100	0.052	0.483	0.541	5.860
2-year Mouse Carcinogenicity (TOX8799)[b]						
Month 6	M	10	NC	NC	0.170	0.695
	F	10	0.213	0.088	0.581	1.950
	M	30	0.158	0.071	0.400	1.590
	F	30	0.374	0.166	1.740	6.250
	M	100	0.677	0.261	2.160	9.530
	F	100	0.121	0.909	1.570	11.100
Rat Pharmacokinetic Study (FK10046)[c]						
Day 1	M	2000	2.05	186[c]	2.33	673[c]
Human Exposure[d] (DIA1023)						
Day 7		100 mg	0.559	6.003	1.276	10.819
		300 mg	1.900	21.911	3.122	28.110

[a] Toxicokinetic parameters determined only for the high dose group.

[b] Systemic exposure assessed at 6 months (after 182 doses on Day 181).

[c] $AUC_{0-\infty}$ values presented for a single-dose study at 2000 mg/kg, the high dose in the in vivo genotoxicity studies.

[d] The M5 and M7 metabolites were not measured in the clinical study DIA1007.

BQL = below quantification limit; F = female; M = male; NA = not applicable; NC = not calculated

Safety margin for canagliflozin (JNJ-28431754)

Species	Daily Dose, (mg/kg)	Canagliflozin AUC_{0-24} (μg.h/ml)	NOAEL, (mg/kg) M/F	AUC Safety margins, (Animal /Human)	
				male	Female
13-Week mouse Study	30	M:65.9 F:102		2	3
	100	M:235 F:354	100	9	14
	300	M:651 F:736		25	28
13-Week rat study	4	M:12.6 F:21.3	M: 4	<0.5	<0.8
	20	M:74.4 F:94.6	F:20	3	4
	100	M:365 F:470		14	18
6-Month rat study	4	M:14.1 F:21.6	< 4	<1	<1
	20	M:68.5 F:88.3		3	3
	100	M:321 F:379		12	14
12-Month Dog Study	4	M: 52.2 F: 67		2	2.6
	30	M: 262 F: 264	M:30	10	10
	100	M: 529 F: 503	F:100	20	19
104-Week Mouse Carci Study	10	M:11.3 F:36.4		<1	1
	30	M:47.7 F: 102		2	4
	100	M:194 F:353	100	**7**	**14**
104-Week Rat Carci Study	10	M:38.3 F:62		2	3
	30	M:118 F:188	30	5	7
	100	M:316 F:560		**12**	**21**
Maximum Clinical Dose: Canagliflozin , 300 mg		**26.1**			

AUC exposures for ==M7 metabolite== (JNJ-41488525) in animals relative to humans:

Species	Dose, mg/kg/day	AUC_{0-24}, ng.h/ml	Safety margins based on AUC (Animal/Human)
6-Month rat study	30	M: 116 F: 115	M:0.000 F:0.00
	100	M: 559 F: 606	M: 0.024 F: 0.026
	300	M: 4,020 F: 5,480	M: 0.17 F: 0.24
12-Month dog study	100	M: 5,670 F: 5,860	M: 0.25 F: 0.25
2-Year mouse study	10	M:695 F: 1,950	M: 0.03 F:0.08
	30	M:1,590 F: 6,250	M:0.06 F: 0.27
	100	M:9,530 F:11,100	M: 0.42 F: 0.48
MRHD, 300 mg BID*		**22,888**	

* The AUC for M7 was estimated after single dose of 200 mg canagliflozin in healthy subjects

AUC exposures for ==M5 metabolite== (JNJ-41980874) in animals relative to humans:

Species	Dose, mg/kg/day	AUC$_{0-24}$, ng.h/ml		Safety margins based on AUC (Animal/Human)	
6-Month rat study	100	M: 48.3	F: 9.12	M: 0.002	F: 0.0004
	300	M: 460	F: 446	M: 0.022	F: 0.02
12-Month dog study	100	M: 440	F: 483	M: 0.02	F: 0.023
2-Year mouse study	10	M:NC	F: 87.9	M: NA	F: 0.004
	30	M:71.2	F: 166	M: 0.003	F: 0.008
	100	M: 261	F: 909	M: 0.013	F: 0.04
MRHD, 300 mg QD*		**20,326**			

* The AUC for M5 was estimated after single dose of 200 mg canagliflozin in healthy subjects

TK/PK Data Manipulation by Two Chemists

The sponsor-initiated evaluation of the bioanalytical (BA) validation methods (July 18, 2008) uncovered two chemists that inappropriately manipulated bioanalytical validation methods and sample analysis for four projects. One of the projects the two chemists had worked with dealt with several nonclinical and clinical studies with canagliflozin. After the discovery of the BA data manipulation, the sponsor performed a detailed review of all the canagliflozin nonclinical and clinical studies on March 27, 2009. The sponsor's report was submitted to the agency on May 12, 2009 (eCTD# 0049). The manipulation of the BA data had occurred between 2005 and 2007. The two chemists had committed the following activity:

- Failed analytical runs were forced to pass the acceptance criteria by re-injecting quality control samples (QCs) or calibration standards (STDs) multiple times and then using selected QCs and STDs.
- Failed analytical runs were forced to pass the acceptance criteria by applying individual integration parameters (different from the rest of the samples in the same run) to failed QCs to make them pass
- STDs which met acceptance criteria were deactivated in order for QCs to pass acceptance criteria.

Nonclinical canagliflozin bioanalytical studies the chemists had worked with included the 2- and 13-week rat and dog studies. Additional studies affected were rat and dog plasma HPLC validations studies and drug concentrations in the genotoxicity assays. The chronic 6-month rat, 12-month dog and 13-week mouse studies were done later and unaffected by the two chemists. The overall canagliflozin exposure from the affected studies appears to be similar to AUC data from the 6-month rat and 12-month dog studies performed later by validated method.

6   General Toxicology

6.1   Single-Dose Toxicity

Mice tolerated single canagliflozin doses up to 2000 mg/kg. In rats, however, deaths occurred after oral administration to female rats (2000 mg/kg, 1/5 F) and intraperitoneal administration to male SD rats (500 mg/kg, 3/5M and 250 mg/kg, 1/5 M).

6.2   Repeat-Dose Toxicity

Study title: 6-month Repeated Dose Oral Toxicity Study of JNJ-28431754 in the Rat

Study no.:	TOX8574 (EDMS-PSDB-8597342)
Study report location:	JNJ, Raritan, NJ
Conducting laboratory and location:	Global Preclinical Development, Beerse Belgium. PK data was evaluated at JNJ facility in Raritan US. Bone biomarkers and pathology were evaluated at [(b) (4)] and histopath and histoprocessing at [(b) (4)]
Date of study initiation:	Oct 11, 2007
GLP compliance:	yes
QA statement:	Yes
Drug, lot #, and % purity:	ZR600348PFA021, 97.6% (purity by HPLC assay value, %W/W)

Key Study Findings

Findings at ≥ 4 mg/kg

- There were no treatment-related deaths.
- Clinical signs included polydipsia at all doses (≥ 4 mg/kg).
- Canagliflozin dose-dependently reduced BW and BW gain at ≥ 4 mg/kg. At 100 mg/kg BW gain was reduced by 26% in males and 22% in females.
- Dose-related decrease in plasma glucose, increase in BUN, ALP in both sexes and increase in ALT and AST were noted in males. Slight decrease in serum Ca was seen only in females.
- Canagliflozin significant increased urine volume, Ca and P and decrease urine pH (fasted animals). There was no change or increase in urine pH in nonfasted.
- Significant increase in kidney weight was noted in both sexes.
- Histopath findings included trabecular hyperostosis in femur/tibia, renal tubular dilatation with cellular debris and acute erosion of stomach.
- Bone biomarkers such as calcitonin (Males), serum osteocalcin (Females) were reduced.

Findings at ≥ 20 mg/kg

- Serum Trig was slightly decreased (M:54% to 143%, F:64% to 145%).

- The severity and incidence of renal <u>tubular dilatation</u>, <u>hyperostosis</u> (femur/tibia and sternum) was greater at ≥ 20 mg/kg. There was also evidence of bladder dilatation and pale discoloration of kidneys.
- Canagliflozin increased kidney, liver and adrenal weight.
- Canagliflozin reduced bone biomarkers (1,25 dihydroxyvitamin D3, crosslinked C-telopeptide type 1 collagen in males and PTH in females).
- DXA scan found decreased bone area of proximal femur in males.

Findings at 100 mg/kg
- The severity and incidence of changes observed at lower doses were increased with the 100 mg/kg canagliflozin.
- Slight decreases in WBC in both sexes and RBC counts in females were observed.
- Canagliflozin decreased Na and increased in Chol and Trig in males.
- <u>Renal pelvic calculus</u>, enlarged adrenal glands, lower thymus weight and thymus atrophy severity increased in both sexes at 100 mg/kg.
- <u>Canagliflozin caused renal transitional hyperplasia</u> and dilatation and distention of ureters.
- Additional bone and Ca biomarkers included decrease in deoxypyridinoline/creatinine in both sexes.
- Canagliflozin decreased femur bone area (proximal, mid shaft, distal) in both sexes (DXA scan) and whole femur mineral content in males and proximal femur in females was noted in females.
- Canagliflozin decreased bone strength test (maximal load and energy in femoral shaft in both sexes).

PK
- The AUC and Cmax increased in dose-proportional manner.
- Multiple dosing increased AUC (1.36-1.58x) and Cmax (1.9 to 2x) up to 20 mg/kg. The AUC at 100 mg/kg was lower after multiple dosing (30 to 40% lower AUC).
- Canagliflozin exposure was <u>greater in female rats than male</u> SD rats.
- Tmax ranged from 0.5 to 7 hrs
- Due to minimal incidence of hyperostosis and tubular dilatations, the NOAEL was considered to be less than 4 mg/kg. The exposure multiples at 4 mg/kg in female and male rats were 1 and 0.7x the clinical AUC at 300 mg QD (21.1 µg.h/ml), respectively.

Reviewer Comments:
Canagliflozin 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 increase in kidney weight was associated with dose-dependent increase in tubule and pelvic dilatation to pelvic calculi (100 mg/kg). Bladder and tubule dilatations were attributed to exaggerated pharmacological activity on renal tubules (glucosuria and diuresis). Although hyperplasia was noted in renal transitional cells, there was no evidence of renal tubule hyperplasia.

Dose-dependent increase in trabecular bone hyperostosis and increased urinary Ca and significant suppression of Ca and bone biomarkers were indicative of significant change in Ca homeostasis even though plasma Ca was generally unchanged. Mechanistic studies found hypercalcium absorption to be responsible for bone hyperostosis. While hyperostosis occurred acutely in the trabecular bone, the decrease in bone mass density and strength in dense bone occurred over time suggesting divergent effect of chronic canagliflozin treatment. Although both related to Ca mobilization, the latter may have been also impacted by the decrease in body weight. The NOAEL for the study was less than the lowest dose (4 mg/kg) due to slight increase in hyperostosis and renal tubule dilatation.

Methods

Doses:	**4, 20 and 100 mg/kg**
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	5 ml/kg/day
Formulation/Vehicle:	0.5% w/v aqueous Methocel, final pH 5.1-5.8
Species/Strain:	SD rats (Crl:CD® (SD) IGS), ▨▨▨▨ (b) (4)
Number/Sex/Group:	20/sex/dose (wire mesh housing, 5/cage/sex,)
Age:	6 weeks old at the time of treatment
Weight:	136-217 g for female and male rats
Satellite groups:	3 to 6/sex/group (housed 3/cage/sex)
Unique study design:	Additional analysis included bone biomarkers (1,25-dihydroxy vitamin D, 25-hydroxy vitamin D3, calcitonin, intact PTH, osteocalcin, crosslinked C-telopeptide of type 1 collagen in serum, deoxypyridinoline) and creatinine in urine, bone mineral content, area and density (DXA scan) and biomechanical bone strength of lumbar vertebra and femur and histomorphometry of the metaphysis of the proximal tibia.
	10 rats/sex/group from the control and HD were used to determine the effect of 6-8 hr food deprivation/fed state on urinary pH and sediment formation at the end of the study (WK 25).
Deviation from study protocol:	Minor, not relevant

Additional description of study protocol:
SD rats were housed 5/cage/sex in wire mesh cages with had lib access to water and food (certified R/M-H pelleted rat feed, Germany, Oct 11, 2007). All rats were deprived of food for hematological, coagulation and clinical chemistry and urinalysis prior to scheduled termination. TK animals were not fasted before sample collection. At the end of the study, rats were anesthetized with isoflurane and killed by exsanguination (April 15-18, 2008).

Canagliflozin dose, concentration and dose volume are shown below:

Dosage groups:	V	L	M	H
mg eq./kg/day	00	4	20	100
mg eq./ml	0	0.8	4	20
ml/kg/day	5	5	5	5

Dose selection was based on the 13-week rat study (4, 20 and 100 mg/kg) where canagliflozin increased food intake, increased ALT, AST, BUN (M only), decreased serum glucose (LD males), increased urine glucose and volume.   Other findings included renal hypertrophy and mineralization, discoloration of glandular stomach, trabecular bone hyperostosis of stifle bone (LD females) in male and female SD rats. Additional findings noted at ≥ 20 mg/kg included decrease in BW and BW gain (F), decrease in urinary pH, increased adrenal wt. (MD females), increased liver wt. (F), trabecular hyperostosis of sternum (MD males), increased hematopoietic cells in bone femur and tibia (MD males) and mineralization of renal pelvis (F). All findings were reversed at the end of 8-WK recovery phase except for decreased BW in female and interstitial mineralization of the kidney. Due to hyperostosis, the NOAEL in the 13-WK study was 4 mg/kg (12 µg.h/ml) in females and 20 mg/kg (94.6 µg.h/ml) in males. The dose selection was appropriate since these doses were able to identify target organs and NOAEL with adequate exposure multiples relative (11-18x) to the maximum clinical dose of 300 mg.

Observational endpoints/timing

Clinical Findings	Once a day
Body weights	Weekly
Food consumption	Weekly
Ophthalmoscopy	Day -1, and Day 176 on first 10 animals of each sex in HD rats
EKG	Not done
Hematology	At WK 14 and 26-27
Clinical chemistry	Under fasted conditions at 3- and 6-months
Urinalysis	Under fasted (food deprivation) at 3- and 6-months
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 Ca homeostasis hormone, bone biomarkers and femoral bone strength

Observations and Results

Mortality

- There were 2 deaths in the main study (LD male on WK 22 and HD male on WK18) due to apparent gavage error.
- There were no deaths in the TK study.

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Clinical Signs

- All rats treated with canagliflozin doses ≥ 4 mg/kg displayed polydipsia from the beginning to the end of the study.
- HD males and females had soft feces in the first week.

Body Weights

- Canagliflozin produced a dose-dependent decrease in BW and BW gain in rats. The decrease in BW gain tended to be greater in males (HD: -26%) than females (HD: -20 to -22%) even though drug exposure was nearly 2-fold greater in females.
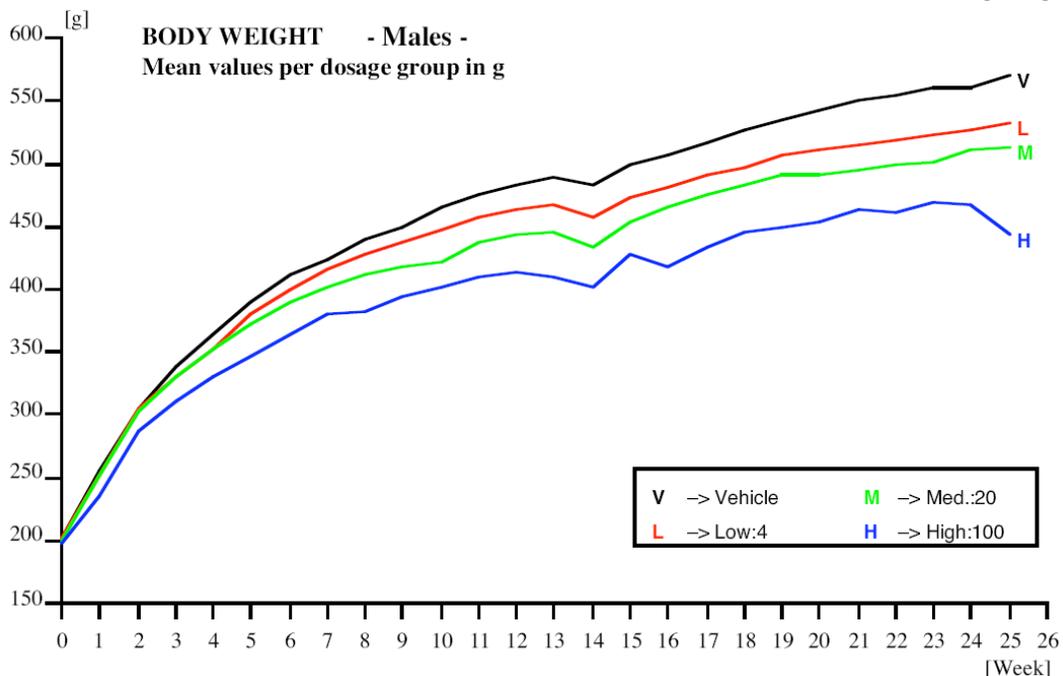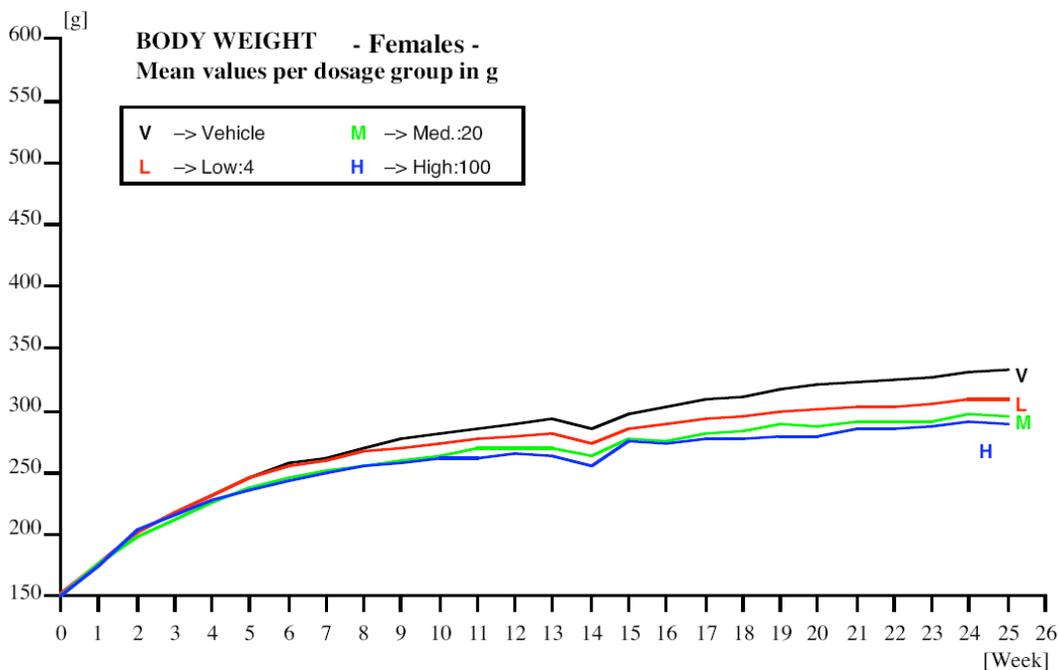
Feed Consumption

- There was a dose-dependent increase in food intake (10 to 20%) within the first week due to heavy glucosuria.
- Food intake was significantly increased in LD (10 to 20%), MD (15 to 30%) and HD (25 to 40%) relative to vehicle.   The increase in food intake was not sufficient to compensate for the decreased BW caused by urinary loss of glucose.

Ophthalmoscopy: There were no drug related ophthalmic findings.

Hematology

- There was no hematological change at 4 mg/kg.
- The 20 mg/kg resulted in slight to moderate decrease in eosinophil count in both sexes. There were minimal decreases in WBC, lymphocyte and monocyte counts in males. There was slight decrease in RBC in females at WK 26.
- At 100 mg/kg a slight to moderate decrease in WBC, eosinophil, lymphocytes (M, F), monocytes (M) was noted. In females, the decrease in RBC and hematocrit was accompanied by slight increase in MCV and MCH.
- There was a small decrease in partial thromboplastin time in HD males and females at WK 14 and 26.

HAEMATOLOGY
Mean values per dosage group   Terminal, recorded in week 26 till 27

Parameter	Unit	MALES Vehicle	Low:4	Med.:20	High:100	FEMALES Vehicle	Low:4	Med.:20	High:100
Act. Part. Thromb. Time	sec	16.58 (0.40)	16.00 (0.30)	16.43 (0.35)	15.18 (0.45) *	15.12 (0.24)	14.80 (0.29)	14.89 (0.30)	14.21 (0.35) *
Prothrombin Time	sec	15.60 (0.27)	15.47 (0.25)	15.67 (0.21)	15.23 (0.28)	12.77 (0.18)	13.29 (0.20)	13.57 (0.14)	12.86 (0.14)
White blood cells	10^3/µl	8.9 (0.5)	9.3 (0.4)	7.6 (0.4)	6.6 (0.4) **	6.0 (0.3)	6.1 (0.3)	6.4 (0.4)	5.2 (0.3) *
Red blood cells	10^6/µl	8.76 (0.07)	8.72 (0.08)	8.74 (0.07)	8.49 (0.09) *	7.95 (0.07)	7.90 (0.10)	7.72 (0.07) *	7.65 (0.08) **
Mean cell haemoglobin	pg	17.9 (0.1)	18.2 (0.1)	18.3 (0.2)	18.6 (0.1) **	19.0 (0.1)	19.4 (0.1)	19.5 (0.2)	19.5 (0.2) *
Mean cell volume	fl	52.8 (0.4)	53.1 (0.4)	53.5 (0.3)	54.9 (0.3) ***	54.9 (0.2)	55.7 (0.4)	55.7 (0.3) *	56.0 (0.3) *
Mean cell haemoglobin	pg	17.9 (0.1)	18.2 (0.1)	18.3 (0.2)	18.6 (0.1) **	19.0 (0.1)	19.4 (0.1)	19.5 (0.2)	19.5 (0.2) *
Thrombocytes	10^3/µl	1080 (31)	1063 (37)	1106 (27)	1057 (30)	968 (25)	1059 (26) *	1078 (27) **	1071 (38) *
Lymphocytes	10^3/µl	6.93 (0.43)	7.19 (0.35)	5.64 (0.35)	4.70 (0.33) ***	4.78 (0.28)	4.74 (0.26)	5.02 (0.28)	3.87 (0.24) *
Eosinophils	10^3/µl	0.14 (0.01)	0.13 (0.01)	0.08 (0.01)	0.07 (0.01) ***	0.14 (0.01) ***	0.11 (0.01)	0.10 (0.01) *	0.08 (0.01) ***

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

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Clinical Chemistry

- Per pharmacology, canagliflozin produced a dose-dependent decrease in plasma glucose. The decrease in plasma glucose ranged from 23% to 48% in males and 15% to 50% in females.
- Minor changes especially at the highest dose was noted in serum Na, Cl, albumin (F), total protein (F), total bilirubin and GGT (gamma glutamyl transferase). The significance of slight (p<0.05) decrease in serum Ca in LD, MD and HD female and MD male rats was not clear. It is possible that rapid renal excretion of Ca between the meals was exceeding Ca absorption between the meals.
- Canagliflozin significantly increased BUN in a dose dependent manner (up to 2 fold at highest dose) in both male and female rats. The increase in AST and ALT was less than 2-fold in males (all doses).
- Slight to moderate increase in Trig was noted at ≥ 20 mg/kg in males (54% to 143%) and female (64% to 145%).
- There was a slight decrease in creatinine and total Bilirubin in male and females likely due to increased renal filtration and polyuria.
- At 20 mg/kg, there was a decrease in serum Cl in males and increase in gamma glutamyl transferase (GGT) in females.
- The 100 mg/kg dose increased in Cholesterol and Trig in males.

CLINICAL CHEMISTRY
Mean values per dosage group   Periodical, recorded in week 14

Parameter	Unit	MALES Vehicle	Low:4	Med.:20	High:100	FEMALES Vehicle	Low:4	Med.:20	High:100
Sodium	mmol/l	143 (0)	143 (0)	143 (0)	142 (0) ***	143 (0)	142 (0) *	143 (0)	142 (0) *
Chloride	mmol/l	103 (0)	103 (0)	101 (0) ***	101 (0) ***	103 (0)	103 (0)	103 (0)	104 (0) *
Calcium	mg/dl	9.8 (0.0)	9.7 (0.1)	9.6 (0.1) *	9.7 (0.1)	10.0 (0.0)	9.7 (0.1) ***	9.7 (0.1) ***	9.6 (0.1) ***
Inorg. phosphorus	mg/dl	6.7 (0.1)	6.5 (0.1) *	6.5 (0.1)	6.9 (0.1)	5.5 (0.1)	5.6 (0.1)	5.5 (0.1)	5.9 (0.1) **
Total protein	g/dl	6.4 (0.0)	6.4 (0.1)	6.3 (0.1)	6.2 (0.1) *	7.0 (0.1)	6.8 (0.1) *	6.7 (0.1) *	6.3 (0.1) ***
Albumin	g/dl	4.0 (0.0)	4.0 (0.0)	4.0 (0.0)	4.1 (0.0)	4.6 (0.1)	4.3 (0.1) *	4.3 (0.1) *	4.1 (0.1) ***
Glucose	mg/dl	108 (3)	83 (2) ***	65 (2) ***	57 (1) ***	106 (3)	90 (2) ***	70 (2) ***	53 (2) ***
Cholesterol	mg/dl	45 (2)	35 (2) **	43 (2)	55 (2) **	65 (3)	50 (3) **	46 (4) ***	55 (4) *
Triglycerides	mg/dl	35 (3)	32 (3)	54 (4) ***	85 (8) ***	33 (2)	28 (1)	54 (5) ***	81 (9) ***
Urea nitrogen	mg/dl	13.7 (0.3)	18.3 (0.6) ***	26.0 (0.6) ***	33.9 (0.8) ***	15.8 (0.4)	18.8 (0.4) ***	25.3 (0.6) ***	33.1 (0.6) ***
Creatinine	mg/dl	0.27 (0.01)	0.25 (0.00)	0.21 (0.00) ***	0.24 (0.00) *	0.34 (0.01)	0.30 (0.01) *	0.28 (0.01) ***	0.27 (0.01) ***
Total bilirubin	mg/dl	0.10 (0.01)	0.10 (0.01)	0.09 (0.01)	0.09 (0.00)	0.17 (0.01)	0.14 (0.01) *	0.13 (0.01) **	0.12 (0.01) ***
Alk. phosphatase	U/l	83 (4)	93 (5)	102 (3) **	138 (7) ***	37 (2)	46 (2) **	48 (4) *	51 (4) ***
Aspartate aminotransferase	U/l	128 (6)	148 (6) *	157 (8) **	169 (8) ***	132 (11)	136 (9)	119 (5)	123 (4)
Alanine aminotransferase	U/l	38 (1)	52 (3) ***	56 (3) ***	63 (4) ***	52 (11)	43 (6)	40 (2)	47 (2) **
Gamma glutamyl transferase	U/l	1 (0)	1 (0)	1 (0) *	2 (0) ***	2 (0)	2 (0)	2 (0) *	3 (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

CLINICAL CHEMISTRY
Mean values per dosage group   Terminal, recorded in week 26 till 27

Parameter	Unit	Dosage Groups (mg eq. / kg) MALES				FEMALES			
		Vehicle	Low:4	Med.:20	High:100	Vehicle	Low:4	Med.:20	High:100
Sodium	mmol/l	144 (0)	143 (0)	144 (0)	142 (0) ***	142 (0)	141 (0) **	142 (0) *	141 (0) **
Chloride	mmol/l	104 (0)	103 (0) *	101 (0) ***	102 (0) **	103 (0)	103 (0)	102 (0)	103 (0)
Calcium	mg/dl	10.3 (0.0)	10.1 (0.1)	10.0 (0.0) ***	10.1 (0.1)	10.8 (0.1)	10.5 (0.1) ***	10.5 (0.1) ***	10.4 (0.1) ***
Albumin	g/dl	4.1 (0.0)	4.1 (0.0)	4.1 (0.0)	4.4 (0.0) ***	5.1 (0.1)	4.8 (0.0) **	4.9 (0.1)	4.8 (0.1) **
Glucose	mg/dl	109 (3)	80 (2) ***	65 (2) ***	53 (1) ***	108 (2)	95 (2) ***	74 (3) ***	57 (2) ***
Cholesterol	mg/dl	55 (4)	42 (3) **	51 (3)	63 (3) *	80 (5)	63 (4) *	64 (5)	80 (5)
Triglycerides	mg/dl	55 (3)	46 (4) *	62 (4)	89 (6) ***	40 (2)	38 (2)	69 (7) ***	93 (10) ***
Urea nitrogen	mg/dl	13.5 (0.4)	18.7 (0.5) ***	27.4 (0.8) ***	31.2 (0.9) ***	15.0 (0.4)	18.5 (0.5) ***	24.9 (0.5) ***	32.7 (1.1) ***
Creatinine	mg/dl	0.27 (0.01)	0.25 (0.01)	0.23 (0.01) ***	0.26 (0.01)	0.32 (0.01)	0.31 (0.01)	0.29 (0.01) *	0.28 (0.01) **
Total bilirubin	mg/dl	0.15 (0.01)	0.12 (0.01) *	0.13 (0.00) *	0.11 (0.01) **	0.20 (0.01)	0.19 (0.01)	0.16 (0.01) **	0.14 (0.01) ***
Alk. phosphatase	U/l	65 (3)	77 (4) *	88 (4) ***	115 (5) ***	25 (1)	33 (2) ***	33 (3) *	39 (3) ***
Aspartate aminotransferase	U/l	117 (4)	142 (8) **	143 (5) ***	167 (8) ***	127 (10)	132 (6)	131 (9)	135 (7)
Alanine aminotransferase	U/l	41 (2)	57 (5) ***	57 (2) ***	74 (4) ***	58 (9)	45 (2)	51 (5)	55 (5)
Gamma glutamyl transferase	U/l	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (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

- Canagliflozin dose-dependently increased urine glucose, Ca and inorganic phosphate excretion in both male and female SD rats.
- The specific gravity and volume was increased and pH was decreased at all doses under fasting conditions. Urinary pH under fed conditions actually increased.

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- Urine triple phosphate levels decreased in males.
- Canagliflozin at 20 mg/kg dose increased ketone levels in urine in males while 100 mg/kg increased ketone levels in both sexes.
- The 100 mg/kg dose decreased in urinary creatinine in both sexes. The clinical significance of the decrease is not clear since there was no notable change in serum creatinine.

6-Month Repeated Dose Oral Toxicity Study in the Rat

Daily Dose (mg/kg)	0(Vehicle)		4		20		100	
No. of Animals	M:20	F:20	M:20	F:20	M:20	F:20	M:20	F:20
Urinalysis[a]								
Terminal (Week 26/27)								
Specific gravity ()	1.028	1.029	1.017 ***	1.016 ***	1.018 ***	1.015 ***	1.014 ***	1.016 ***
pH ()	8.0	-	0.888 ***	-	0.825 ***	-	0.788 ***	-
Volume (ml)	14.2	9.6	1.739 ***	1.385 **	2.303 ***	2.063 ***	3.035 ***	2.292 ***
Glucose (Score)	0.00	0.00	3.74 ***	3.95 ***	3.50 ***	3.95 ***	3.84 ***	4.00 ***
Ketones (Score)	1.10	1.05	-	-	2.364 ***	-	3.064 ***	2.667 ***
Calcium (mg/mg Crea)	0.041	0.260	3.268 ***	1.650 ***	5.780 ***	2.485 ***	18.512 ***	5.054 ***
Phosphate (mg/mg Crea)	0.90	1.54	1.978 ***	1.357 ***	3.544 ***	2.026 ***	4.033 ***	2.721 ***
Glucose (mg/mg Crea)	0.08	0.12	1650***	953***	2687***	1597 ***	3162 ***	2072 ***
Triple phosphate crystals	2.67	-	0.79	-	0.43	-	0.16	-
Amorphous material	0.00	0.67	-	-	-	-	0.74[b]	2.61
Creatinine (mg)	14.24	7.69	-	-	-	-	0.877 **	0.887 **

Daily dose (mg/kg)	0 (Vehicle): Fasted		0 (Vehicle): Non-fasted		100: Fasted		100: Non-fasted	
Urinalysis fasted/non-fasted (week 24/25)[c]	M:10	F:10	M:10	F:10	M:10	F:10	M:9	F:10
pH	6.6	5.8	7.9***	7.2	5.4	5.2	7.1***	6.9***
Triple phosphate crystals	0.83	N.A.	1.89	1.80	0.20	0.11	0.22	0.20
Amorphous material	0.00	N.A.	0.10	0.00	0.00	0.00	1.78***	1.40*

[a]   At end of dosing period. For controls, group means are shown. For treated groups, multiples of vehicle are shown. Statistical significance is based on actual data (not on the multiples of control/baseline).
[b]   Absolute value.
[c]   For controls and treated groups, means are shown. Statistical significance is based on actual data.
* - No noteworthy findings; N.A.= Not Applicable
- p<0.05, ** - p<0.01; *** - p<0.001

Gross Pathology

- Canagliflozin caused dilatation of renal pelvis urinary tract (bladder and ureters) and adrenal gland at ≥ 20 mg/kg.

6-Month Repeated Dose Oral Toxicity Study in the Rat

Daily Dose (mg/kg)	0 (Vehicle)		4		20		100	
No. of Animals	M:20	F:20	M:20	F:20	M:20	F:20	M:20	F:20
Gross Pathology								
Terminal sacrifice group Animals Examined	20	20	19	20	20	20	19	20
ADRENAL GLANDS								
- Swollen	0	0	–	–	–	–	1	4
KIDNEYS								
- Discoloration: pale	0	0	–	–	–	1	1	7
- Pelvic dilatation	0	1	–	–	3	–	4	1
- Swollen	0	0	–	–	7	–	2	1
- Calculus in pelvis	0	0	–	–	–	–	–	1
THYMUS								
- Small	17	17	–	–	–	–	19	19
URETER(S)								
- Dilatation	0	0	–	–	–	–	4	3
URINARY BLADDER								
- Dilatation	0	0	–	–	2	–	8	–

- No noteworthy findings
* - p<0.05, ** - p<0.01

Organ Weights

- Canagliflozin increased kidney weight (10 to 25%) at all doses in both male and female rats; however, the increase did not appear to be dose-dependent. Maximal renal effect of canagliflozin may have been achieved at 4 to 20 mg/kg.
- Canagliflozin doses ≥ 20 mg/kg was associated with an increase in adrenal gland and liver weight.
- The decrease in thymus weight at 100 mg/kg was correlated with thymic atrophy.

6-Month Repeated Dose Oral Toxicity Study in the Rat

Daily Dose (mg/kg)	0 (Vehicle)		4		20		100	
No. of Animals	M:20	F:20	M:20	F:20	M:20	F:20	M:20	F:20
Organ Weights[a,b]								
Terminal sacrifice group	19	19	18	19	19	19	18	19
MEAN WEIGHT								
ADRENAL GLANDS	0.05715	0.07504	–	–	–	–	1.078	1.162 **
KIDNEYS	3.22	2.01	1.143 **	1.100 *	1.255 **	1.154 **	1.130 **	1.184 **
LIVER	12.08	7.28	–	–	–	–	–	1.107 **
THYMUS	0.23304	0.18086	–	–	–	–	0.637 **	0.815
MEAN % BODY WEIGHT								
ADRENAL GLANDS (%body)	0.01077	0.02461	–	–	1.186 *	–	1.396 **	1.411 **
KIDNEYS (%body)	0.60267	0.65841	1.243 **	1.211 **	1.438 **	1.337 **	1.468 **	1.441 **
LIVER (%body)	2.25	2.39	–	–	–	1.184 **	1.204 **	1.343 **
THYMUS (%body)	0.04325	0.05942	–	–	–	–	0.839 *	–
MEAN % BRAIN WEIGHT								
ADRENAL GLANDS (%brain)	2.59	3.8	–	–	–	–	1.143	1.171 **
KIDNEYS (%brain)	146	101.8	1.163 **	1.099 *	1.286 **	1.163 **	1.198 **	1.196 **
LIVER (%brain)	548.2	368.5	–	–	–	–	–	1.117 *
THYMUS (%brain)	10.55	9.18	–	–	–	–	0.677 **	0.818

[a] At end of dosing period. For controls, group means are shown. For treated groups, multiples of vehicle are shown. Statistical significance is based on actual data (not on the multiples of control/baseline).
[b] Both absolute and relative weights differed from controls in the direction indicated. Number indicates multiple of control for the absolute organ weights.
- No noteworthy findings
* - p<0.05, ** - p<0.01

Histopathology

Adequate Battery: Yes
Peer Review: Yes

Histological Findings
- Drug-related increase in incidence and severity (minimal to slight) of hyperostosis of trabecular femur/tibia at ≥ 4 mg/kg and sternum at ≥ 20 mg/kg of canagliflozin in both male and female SD rats.
- Minimal to slight dose-dependent increase in renal tubular dilatation was noted at all doses in both sexes.
- Minimal to slight increase in renal transitional hyperplasia was seen in HD rats. There was no renal tubule hyperplasia in the end of the 6-month study in rats.
- A non-dose dependent increase in acute stomach erosions at every dose level had no apparent long-term consequence and thus not likely to be clinically relevant. The mechanistic studies found stomach erosion in both control and canagliflozin rats. The sponsor attributed them to stress and anesthesia.
- Ureter distention in the HD male and female rats was likely an adaptation to high urine flow at 100 mg/kg of canagliflozin.

6-Month Repeated Dose Oral Toxicity Study in the Rat

Daily Dose (mg/kg)	(Vehicle)		4		20		100	
No. of Animals	M:20	F:20	M:20	F:20	M:20	F:20	M:20	F:20
Histopathology								
BONE, STERNUM	19	19	–	–	19	19	18	19
- Hyperostosis, trabecular	0	0			2	2	16**	14**
minimal	0	0			2	2	9	7
slight	0	0			0	0	7	7
BONE, STIFLE JOINT (FEMORAL TIBIAL METAPHYSIS)	19	19	18	19	19	19	18	16
- Hyperostosis, trabecular	0	0	2	3	5*	6**	10**	12**
minimal	0	0	2	3	5	5	9	5
slight	0	0	0	0	0	1	1	7
BONE MARROW, FEMUR	19	–	–	–	19	–	18	–
- Adipose tissue	19				19		18	
minimal	0				0		1	
slight	2				12		15	
moderate	13				7		2	
marked	4				0		0	
BONE MARROW, STERNUM	19	–	–	–	19	–	18	–
- Adipose tissue	19				19		18	
minimal	3				8		6	
slight	15				11		12	
moderate	1				0		0	
KIDNEY	19	19	18	19	20	19	19	19
- Dilatation, tubules	0	0	9**	6**	16**	11**	17**	16**
minimal	0	0	8	6	16	10	12	9
slight	0	0	1	0	0	1	5	7
- Hyperplasia, transitional	1	0	–	–	–	–	3	6**
minimal	1	0					3	3
slight	0	0					0	3
STOMACH	19	19	18	19	19	19	18	19
- Erosion	0	0	1	2	7**	5*	3	1
minimal	0	0	1	2	1	1	2	0
slight	0	0	0	0	4	3	0	0
moderate	0	0	0	0	2	1	1	1
THYMUS	20	20	–	–	–	–	19	20
- Atrophy	16	20					8	20
minimal	16	13					8	12
slight	4	7					10	7
moderate	0	0					1	1
URETER	19	18	n/a	n/a	n/a	n/a	19	19
- Distention	0	0					9**	4

· No noteworthy findings; n/a = Not Applicable
* - p<0.05, ** - p<0.01

Special Bone Biomarker and Bone Biomarker Evaluations

- Bone morphology, strength and bone biomarkers were evaluated due to dose-dependent increase bone hyperostosis and excess urinary Ca excretion.
- Canagliflozin caused a decrease in Ca homeostasis hormones which were not always dose-dependent:
  - Decrease in 1,25 hydroxyvitamin D were reduced in MD (M) and HD (M,F)
  - Decrease in 25 hydroxyvitamin D were reduced in HD *M, F)
  - Decrease in Calcitonin were reduced in LD and MD males
  - Decrease in intact PTH was reduced in MD and HD females
- Bone turnover biomarkers were generally lower than control.
  - Decrease in osteocalcin in LD(F), MD(M,F), HD (M, F)
  - Decrease in crosslinked C-telopeptide type 1 collagen in MD males
- Urine bone biomarkers were decreased (i.e. deoxypyridinoline/creatinine ratio in HD rats).

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- A decrease in DXA scans of L5 lumbar, whole femur, distal femur, midshaft femur, and proximal femur, bone mineral density, bone area and bone mineral content was noted in the HD animals.
- Slight increase in L5 lumbar bone area was also seen in LD and MD female.

Report Title: 6-Month Repeated Dose Oral Toxicity Study in the Rat							Test Article: JNJ-28431754-ZAE	
BONE BIOMARKERS & PATHOLOGY								
Daily Dose During Dosing Period (mg eq./kg/day)	**0 (Vehicle)**		**4**		**20**		**100**	
No. of Animals	M: 19	F: 19	M: 18	F: 18	M: 19	F: 19	M: 18	F: 19
Densitometry[a]								
DXA Scan at the L5 Lumbar Vertebral Body								
Bone Mineral Content (g)	0.284	0.205	-	-	-	-	-	0.92*
Bone Area (cm^2)	1.396	1.070	-	1.20*	-	1.15*	0.9*	-
Bone Mineral Density (g/cm^2)	0.204	0.191	-	0.92*	-	0.91*	-	-
DXA Scan of the Whole Femur								
Bone Mineral Content (g)	0.713	0.469	-	-	-	-	0.90*	-
Bone Area (cm^2)	2.758	1.976	-	-	-	-	0.88*	0.94*
Bone Mineral Density (g/cm^2)	0.258	0.236	-	-	-	-	-	-
DXA Scan at the Distal Femur								
Bone Mineral Content (g)	0.201	0.145	-	-	-	-	-	-
Bone Area (cm^2)	0.764	0.565	-	-	-	-	0.90*	-
Bone Mineral Density (g/cm^2)	0.261	0.252	-	-	-	-	1.06*	-
DXA Scan at the Midshaft Femur								
Bone Mineral Content (g)	0.326	0.196	-	-	-	-	0.85***	-
Bone Area (cm^2)	1.274	0.884	-	-	-	-	0.87***	0.93*
Bone Mineral Density (g/cm^2)	0.256	0.221	-	-	-	-	-	-
DXA Scan at the Proximal Femur								
Bone Mineral Content (g)	0.189	0.129	-	-	-	-	-	0.91*
Bone Area (cm^2)	0.727	0.532	-	-	0.94*	-	0.89***	0.93*
Bone Mineral Density (g/cm^2)	0.258	0.242	-	-	-	-	-	-

[a] For controls, group means are shown. For treated groups, multiples of control are shown. Statistical significance is based on actual data (not on the multiples of control).
* - p<0.05, *** - p<0.001 , - No noteworthy findings

- Analysis of bone mechanical strength found a decline in L5 lumbar vertebral compression energy at all doses in males and HD females. The L5 lumber ultimate strength was increased in MD and HD males while elasticity was increased in MD males.
- Three point bend performed on femoral shaft, found a significant decrease in maximal load (~ 15%) and energy (~ 25%) in HD rats.
- Histomorphometry analysis found an increase in trabecular bone volume and number in HD males. The thickness and separation of trabecular bone was reduced in the same rats. The total cortical bone area and thickness was increased in HD females but not in males.
- These findings suggest canagliflozin related remodeling of the bones resulting in lower bone area and bone density in rats, especially at 100 mg/kg. Both trabecular hyperostosis and dense bone remodeling are linked to hypercalcium absorption. However, decrease in bone density and strength may have been further impacted by decrease in BW and chronic suppression of bone turnover by Ca imbalance.

6-Month Repeated Dose Oral Toxicity Study in the Rat

BONE BIOMARKERS & PATHOLOGY

Daily Dose During Dosing Period (mg eq./kg/day)	0		4		20		100	
Mechanical Testing[a]								
No. of Animals	M: 19	F: 19	M:18	F: 19	M:19	F: 19	M: 18	F: 19
Vertebral Compression at the L5 Lumbar Vertebral Body								
Energy (mJ)	74.97	66.20	0.65*	0.65**	0.59*	0.68x**	-	0.78*
Ultimate Strength (N/mm²)	21.21	27.91	-	-	1.25*	-	1.24*	-
Elastic Modulus (MPa)	447.98	666.40	-	-	1.73***	-	-	-
No. of Animals	M: 19	F: 19	M: 18	F: 19	M: 19	F: 19	M: 18	F: 19
Femoral Shaft - Three Point Bend								
Maximum Load (N)	283.44	198.54	-	-	-	-	0.84***	0.87***
Energy (mJ)	132.01	79.72	-	-	-	-	0.72*	0.74*
Histomorphometry[a]								
No. of Animals	M: 10	F: 10	M: 10	F: 10	M:10	F: 10	M: 10	F: 10
Trabecular bone volume (%)	16.473	28.717	-	-	-	-	1.48*	-
Trabecular thickness (µm)	61.083	52.770	-	-	-	-	0.94	-
Trabecular number (mm⁻¹)	2.667	5.352	-	-	-	-	1.55**	-
Trabecular separation (µm)	338.664	150.564	0.69*	-	-	-	0.58*	-
Total cortical bone area (mm²)	1.979	1.727	-	-	-	-	-	1.12*
Total cortical bone thickness (µm)	340.483	296.971	-	-	-	-	-	1.13*

[a] For controls, group means are shown. For treated groups, multiples of control are shown. Statistical significance is based on actual data (not on the multiples of control)
* - p<0.05, ** - p<0.01, *** - p<0.001 , - No noteworthy findings

Toxicokinetics:

- Parent drug and glucuronidate metabolites (M7 = JNJ-41488525 and M5 = JNJ-41980874) were also assessed in rats. Canagliflozin was absorbed slowly with Tmax of 0.5 to 7 hrs in rats.

- Elimination of canagliflozin was slow to moderate, with $t^1/_2$ ranging from 6.92 to 16.7 hours in rats.

- Systemic exposure increased in a dose related manner in both genders after single dose. However, after multiple dosing on Day 90 and 179, the exposure in the HD rats were lower by 30 to 40%.

- Canagliflozin exposure in females tended to be higher than males.

- The exposure to parent drug was substantially higher than M5 and M7 metabolites (Parent >>M7>M5). Although exposure to both M5 and M7 were significantly less than the parent drug and there was inadequate coverage, there was no safety concern as both M5 and M7 are highly water-soluble inactive glucuronide metabolites. In a recent mass balance study, higher levels of M7 (12% of total) and M5 (4% of total) was detected suggesting significantly higher concentrations of the two metabolites before hydrolysis.

- The NOAEL was ≤ 4 mg/ (AUC of 14.1 and 21.6µg.h/ml and Cmax of 1.1 and 1.56 µg/ml in male and female rats, respectively). The NAOEL exposure multiples was less than clinical exposure (300 mg QD, AUC_{0-24} of 26.1 µg.h/ml).

JNJ-28431754 Plasma Toxicokinetic Parameters in Female and Male Rats Following a Single or Multiple Oral Doses (4, 20 or 100 mg/kg/day) of JNJ-28431754-ZAE (TOX8574)

Gender	Dose (mg/kg)	Day	C_{max} (ng/mL)	t_{max} (h)	t1/2 (h)	AUC $_{(0-\infty)}$ (hr*ng/mL)	AUC $_{(0-24 h)}$ (hr*ng/mL)	AUC $_{(0-t h)}$ (hr*ng/mL)
Female	4	0	828	4.00	8.72	13700		11600
		90	1670	1.00	8.95		18700	18700
		179	1560	0.500	7.61		21600	21600
	20	0	4190	7.00	7.75	73900		65700
		90	6530	1.00	7.78		95600	95600
		179	5510	4.00	8.27		88300	88300
	100	0	25000	4.00	16.7	636000		385000
		90	29700	4.00	11.5		441000	441000
		179	29800	4.00	12.9		379000	379000
Male	4	0	927	4.00	6.92	12900		11700
		90	1020	4.00	7.75		14700	14700
		179	1100	4.00	7.90		14100	14100
	20	0	4360	4.00	8.69	72300		61000
		90	4070	4.00	9.48		66300	66300
		179	4020	7.00	11.6		68500	68500
	100	0	25700	4.00	10.1	479000		384000
		90	17500	1.00	14.2		307000	307000
		179	19300	7.00	10.4		321000	321000

All parameters were calculated using the mean plasma concentrations for each dose group/gender

JNJ-41488525 (M7) and JNJ-41980855 (M5) Plasma Toxicokinetic Parameters in Female and Male Rats Following 180 Oral Doses (4, 20 or 100 mg/kg/day) of JNJ-28431754-ZAE (TOX8574)

Analyte	Dose (mg/kg)	Gender	C_{max} (ng/mL)	t_{max} (h)	AUC $_{0-24h}$ (h*ng/mL)
JNJ-41488525	4	Female	30.5	0.500	115
		Male	17.4	1.00	116
	20	Female	74.3	0.500	606
		Male	87.1	0.500	559
	100	Female	595	1.00	5480
		Male	432	1.00	4020
JNJ-41980855	4	Female	NA	NA	NA
		Male	NA	NA	NA
	20	Female	8.24	0.500	9.12
		Male	13.1	0.500	48.3
	100	Female	55.3	4.00	446
		Male	53.3	1.00	460

Note: All parameters were calculated using the mean plasma concentrations for each dose group/gender   NA: Not Applicable

Dosing Formulation Analysis: The purity of canagliflozin was 97.6%. The canagliflozin solution concentrations were within the specified target (± 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.

Study title: 12-Month Oral Toxicity Study of JNJ-28431754-ZAE in Dogs

Study #	**TOX8446** (b) (4)
Study report location	JNJ Research Pharmaceutical Research , Raritan, NJ
CRO/Laboratory name and location	(b) (4)
Date of study initiation	Aug 14, 2007
GLP compliance statement	Yes,
GLP issues identified	None
QA statement	Yes
Drug, lot #, and % purity	11173N, 97.6%

Key Study Findings

- Dogs at ≥ 4 mg/kg appeared thin (males more than females), with frequent emesis and watery feces which is consistent with intestinal SGLT1 inhibition.
- Canagliflozin decreased BW gain at all doses in males (-19 to -28%) but not in females.
- Significant drug-related increase in urinary glucose excretion in both sexes at ≥4 mg/kg produced significant polyuria in both sexes.
- HD canagliflozin elevated serum K in both sexes.
- There were minimal elevations in urine GGT, NAG (renal tubular damage), Ca (up to 3x), Na per creatinine in MD and HD females but not in males.
- VitD and urinary deoxypyridinoline were decreased at all doses in both sexes at WK 17. There was no change in osteocalcin, crosslinked c-telopeptide, deoxypyridinoline at WK17. None of the above Ca/bone biomarkers were significantly decreased at WK52. However, there was an increase in serum bone alkaline phosphatase in HD females by up to 1.9x fold.
- Renal tubular regeneration and degeneration was observed in 1 C, MD and HD male. This finding was inconsistent with slight elevation in NAG noted in females but not in males.
- DXA scan of the femur found significant decrease in whole femur mineral density at all doses in males (but none in females) which is consistent with the decrease in BW gain in males.
- Unlike rats, there was no apparent evidence of trabecular bone hyperostosis (volume, number or trabecular thickness, trabecular separation, osteoid thickness, surface area volume) dogs at WK 52.
- Canagliflozin absorption in dogs was variable (slow in some and fast in others).
- Tmax ranged from 0.87 to 6 hrs with elimination half-life ranging from 9.72 hr to 25.9 hr in dogs.
- Canagliflozin exposure increased in less than dose-proportional manner in genders.
- Due to long half-life, the $AUC_{0-\infty}$ was significantly greater than AUC_{0-24} in both male and female dogs.
- The reviewer chose 100 mg/kg (F: 503 µg.h/ml) as NOAEL for females and 30 mg/kg (AUC: M: 264 µg.h/ml) as NAOEL for males due to bone changes.

Reviewer Comments:

As expected canagliflozin resulted in a dose-related glucosuria and associated polyuria. There was a dose-dependent decrease in BW gain in males (up to 28%) but not in females. The net

loss of glucose was likely responsible for the weight loss and hyperphagia. The increased food intake however was unable to compensate for glucose related caloric loss.

There was no persistent change in bone biomarkers at WK 52. The significant increase urinary Ca is consistent with the effect on Ca homeostasis seen in rats suggesting that it is likely to be relevant to humans although changes might be much less pronounced in humans. Since canagliflozin reduced bone mass density in males only, they were attributed to decrease in BW and Ca homeostasis. There was no weight loss or change in bone density in females. There was no evidence of renal tubule, pelvic or ureters dilatation signal in dogs as there were in rats in part due to lower response in dogs.

Based on decrease in bone mineral density and renal tubule regeneration and degeneration, the NOAEL in male and female dogs were 30 mg/kg dose (12x MRHD of 300 mf based on AUC) and 100 mg/kg (24x the MRHD of 300 mg, based on AUC), respectively.

Methods

Doses	**0, 4, 30 and 100 mg/kg**
Frequency of dosing	Single dose daily for 12 months
Route of administration	Oral
Dose volume	1 ml/kg
Formulation/Vehicle	0.5% Hypromellose solution
Species/Strain	Beagle dogs
Number/Sex/Group	4/sex/dose
Age	5 months old the time of treatment
Weight	6.6 to 8.6 kg males and 5.8 to 7.3 kg females
Satellite groups	No (TK was performed in the same animals on Day 1, WK 26 and Week 52. Samples were collected at 0, 0.5, 1, 2, 4, 8 and 24 hrs)
Unique study design	Not unique but the study included evaluation of bone biomarkers, hormones affecting Ca homeostasis, bone histomorphology which are not standard protocol for diabetes drugs.
Deviation from study protocol	Minimal

Study Design and Dosage

Group	Daily Dose[a]			Number of Animals									
				Total		Toxicokinetics		Clinical Pathology		Necropsy		Microscopic Pathology	
	Dose (mg/kg)	Volume (mL/kg)	Conc. (mg/mL)			Day 1, Week 26 & 52		Pretest, Week 26 and 52		Week 52			
				M	F	M	F	M	F	M	F	M	F
1	0	1	0	4	4	4	4	4	4	4	4	4	4
2	4	1	4	4	4	4	4	4	4	4	4	4	4
3	30	1	30	4	4	4	4	4	4	4	4	4	4
4	100	1	100	4	4	4	4	4	4	4	4	4	4

[a] Doses represent active ingredient. Doses and concentrations were corrected for free drug content and purity (correction factor = 1.025).

Dose selection was based on the 13-week study (TOX8214) in which dogs were treated with 4, 30 and 200/100 mg/kg. Doses up to 30 mg/kg were well tolerated but higher doses resulted in polyuria-induced dehydration. Clinical signs overt toxicity (hemorrhagic faces, erythema, decreased activity) resulting in lowering the dose to 100 mg/kg on Day 8. At 200/100 mg/kg, there was an increase in ALT (1.39×) and total bilirubin (1.69×), ALP (2.08×), BUN (1.65×), and CREAT (1.86×) relative to control.   Serum Ca (0.91×) and glucose (0.89×) were slightly lower in HD males. During the first week, females treated with 200 mg/kg had elevated creatinine (1.59×) and slight decrease in serum Ca (0.96×) values. The weight loss at 100 mg/kg in females was considered tolerable thus used as top dose.

Observational endpoints/timing

Clinical Findings	Twice daily, before and after dose
Body weights	Weekly
Food consumption	Daily
Ophthalmoscopy	Week 52
ECG	Predose, WK 52 at anticipated Tmax of 0.67 to 3.33 hrs
Hematology	Predose, WK 26 and 52 in overnight fasted animals
Clinical chemistry	Predose, WK 26 and 52 in overnight fasted animals
Urinalysis	Predose, WK 26 and 52 in 16-hr fasted animals into chilled containers. Fractional excretion (%) was calculated by the following formula.

$$\text{Fractional excretion (\%)} = \frac{[(\text{urine analyte conc.}) \times (\text{serum creatinine conc.})]}{[(\text{serum analyte conc.}) \times (\text{urine creatinine conc.})]}$$

Gross pathology	WK 52
Organ weights	WK 52
Histopathology	Adequate Battery: yes (x), no ( ) Peer review: yes (x), no ( )
Other	A complete urinalysis included specific gravity, fractional clearance of Cl, Ca, K, P, Na, N-Acetyl glucosaminidase to creatinine and gamma glutamyltransferase to creatinine was carried out. The study also included analysis of bone biomarkers during WK 17 and 52 after an overnight fast (serum bone specific ALP, Osteocalcin 1,25 dihydroxyvitamin D, Cross-linked C-telopeptide of type 1 collagen, Urine deoxypyridinoline and creatinine). Left femur, a section of lumbar vertebral column (L4 to L6) were collected for bone mass and bone strength analysis. Bone mass was measured using DXA scan (mineral content, area and density). Bone strength was measured by a three-point bending test for maximum load, stiffness and energy. Left tibia was collected and non-decalcified bone was shipped to ___ (b) (4) (Efficacy Pharmacology, ___ (b) (4) for histomorphology. The proximal tibia was decalcified. They were stained with Goldner's trichrome stain. Histomorphological parameters included trabecular volume, mineralized bone volume, trabecular thickness, number and separation, unmineralized bone (osteoid) volume, thickness and surface.

Observations and Results

Mortality:
One LD male (#2112) displayed clinical signs of neurological changes such as irregular gate, ataxia tremor and convolution (euthanized on Day 116). The histopathological findings were consistent with granulomatous meningoencephalomyelitis. There was no apparent relationship to the drug since there were no such findings in dogs even at higher doses.

Clinical Signs
- All groups had watery feces. It was more frequent in treated animals.
- Emesis was noted throughout the study in both sexes in all groups but more frequent in the HD males.
- Several dogs appeared thin.

12-Month Oral Toxicity Study of JNJ-28431754-ZAE in Dogs (TOX8446)

Daily Dose During Dosing Period (mg/kg)	0 (Control)		4		30		100	
	M	F	M	F	M	F	M	F
No. of Animals	M:4	F:4	M:4/3[e]	F:4	M:4	F:4	M:4	F:4
Noteworthy Findings								
Clinical Observations								
Emesis	15/4	27/4	23/4	24/4	15/4	41/4	36/4	25/4
Thin appearance	0/0	5/1	1/1	-	6/2	7/1	1/1	4/1
Unformed stool	88/4	12/4	332/4	74/4	395/4	453/4	933/4	591/4
Watery stool	13/3	1/1	24/4	10/4	48/4	72/4	273/4	132/4
Mucus in stool	38/4	37/4	24/4	20/4	38/4	91/4	107/4	121/4

Body Weights
- There was a drug-related decrease in BW gain (19 to 28%) in males at doses ≥ 4 mg/kg. BW in males was decreased slightly (5 to 9%, P> 0.05).
- There was no change in BW and BW gain in females.

Feed Consumption: There was no apparent change in food intake.
Ophthalmoscopy: There were no ophthalmic findings in dogs.
ECG: There were no abnormal ECG findings in dogs evaluated at Tmax 0.5 to 4 hrs post dose.
Hematology: There were no drug-related hematological or coagulation changes in dogs.

Clinical Chemistry
- Canagliflozin reduced blood glucose by 15% in LD and significantly at MD and HD (17%) in females at WK 52 relative to control. Blood glucose at WK 26 to WK 52 tended to be lower than basal (pretreatment) glucose levels in female at all doses and in males at HD.
- Serum K was decreased in HD males (9%) and females (15%) at WK 52, likely related to increased urinary excretion of electrolytes.
- Serum triglycerides (Trig) were increased in HD male and female at WK 52 relative to control.
- Bone specific ALK was increased in HD females relative to control. There was no notable change in males.

12-Month Oral Toxicity Study of JNJ-28431754-ZAE in Dogs (TOX8446)

Daily Dose During Dosing Period (mg/kg)	0 (Control)		4		30		100	
	M	F	M	F	M	F	M	F
No. of Animals	M:4	F:4	M:4/3[e]	F:4	M:4	F:4	M:4	F:4
Serum Chemistry [a]								
Glucose – Week 52	-	94	-	0.85x	-	0.83x*	-	0.83x*
Potassium (mEq/L) – Week 52	4.4	4.6	-	-	-	-	0.91x**	0.85x**
Alkaline phosphatase (U/L) – Week52	-	75	-	-	-	-	-	2.17x*
Triglycerides (mg/dL) – Week 52	44	47	-	-	-	-	1.51x	1.38x*

- No noteworthy findings
* or **: absolute values statistically significant from controls:* - $p < 0.05$, ** - $p < 0.01$

Urinalysis

- A dose-dependent and persistent increase in glucosuria and associated polyuria (osmotic diuresis) was noted at ≥ 4 mg/kg in both male and female dogs.
- Urine glucose in the LD and MD in both male and female dogs appeared to be greater than the HD groups. The reviewer speculates that higher doses may have inhibited intestinal SGLT1 receptors, thus preventing glucose/carbohydrate absorption/availability at higher doses.
- Urine Ca to creatinine was increased at 30 and 100 mg/kg at WK 26 but diminished by WK 52 in females. The changes appeared to parallel glucose excretion.
- Na/creatinine was minimally increased at all doses at WK 26 but at MD and HD at WK 52 in females suggesting glucosuria and diuresis were key factors. The increase was less than ">1%" threshold considered a marker to renal tubular dysfunction or medullary washout.
- There was a small increase in excretion of Ca, Cl, K, or P relative to creatinine.
- There were small increases in urinary GGT and NAG at WK 26 and 52 in both sexes at canagliflozin doses ≥ 4 mg/kg. Since urine volume was large, correction for creatinine exertion resulted in no or minimal fractional change in GGT and NAG. Fractional GGT and NAG were increased in MD and HD females at WK 26 but only fractional GGT in the HD females was increased at WK 52. Since there was no notable related histopathology, one has to conclude that renal function did not deteriorate to a point detectable histologically.

Mean urine volume and glucose absolute and fractional excretions in animals treated with JNJ-28431754-ZAE.

Week	Dose	Urine Volume (ml)		Gluc. Abs. Excr. (mg/16hr)		Gluc. Fract. Excr. (%)	
		M	F	M	F	M	F
26	0 mg/kg/day	113	77.0	7.37	7.05	0.15	0.05
	4 mg/kg/day	221	196	18,502	18,811	71.5	73.5
	30 mg/kg/day	236	187	18,020	12,622	77.7	73.0
	100 mg/kg/day	133	139	12,711	11,773	77.3	76.5
52	0 mg/kg/day	45	59.0	1.35	210	0.04	0.79
	4 mg/kg/day	220	216	10,810	15,361	63.0	57.1
	30 mg/kg/day	209	245	15,801	22,126	60.8	67.6
	100 mg/kg/day	90	158	8,220	15,352	66.7	54.1

M: male; F: female; Gluc. Abs. Excr.: Glucose absolute excretion;
Gluc. Fract. Excr.: Glucose fractional excretion

12-Month Oral Toxicity Study of JNJ-28431754-ZAE in Dogs (TOX8446)

Daily Dose During Dosing Period (mg/kg)	0 (Control)		4		30		100	
	M	F	M	F	M	F	M	F
No. of Animals	M:4	F:4	M:4/3[e]	F:4	M:4	F:4	M:4	F:4
Urinalysis								
Volume [a] (mL) - Week 26	113	77.0	1.96x	2.55x	2.09x	2.43x	1.18x	1.81x
Volume [a] (mL) - Week 52	45.0	59.0	4.89x	3.66x	4.64x	4.15x	2.0x	2.68x
Glucose [a] - Week 26	8	11	983x[c]	844x[c]	790x[c]	582x[c]	1,005x[d]	741x[b]**
Glucose [a] - Week 52	8[c]	267	505x[b]	10x[d]	1,068x	31x[c]	1,051x[c]	34x[d]
Glucose Absolute Excretion (mg/16 hr) [a] - Week 26	7.37	7.05	2,510x	2,668x	2,445x	1,790x	1,725x	1,670x
Glucose Absolute Excretion (mg/16 hr) [a] - Week 52	1.35	210	8,007x	73x	11,704x	105x	6,089x	73x
Glucose Fractional Excretion (%) [a] - Week 26	0.15	0.05	477x	1,470x	518x	1,460x	515x	1,530x
Glucose Fractional Excretion (%) [a] - Week 52	0.04	0.79	1,575x	72x	1,520x	86x	1,668x	68x
GGT Absolute Excretion (mg/16 hr) [a] - Week 26	4.398	1.583	1.73x	2.60x*	2.31x	3.73x*	1.44x	2.07x*
GGT/Creatinine (U/mg) – Week 26	-	2.280	-	-	-	2.78x	-	2.25x
GGT Absolute Excretion (mg/16 hr) [a] - Week 52	2.975	2.604	2.12x	1.14x	3.85x	2.18x	2.49x	2.23x
GGT/Creatinine (U/mg) – Week52	-	2.141	-	-	-	-	-	2.51x

[a] For controls, group means are shown. For treated groups, multiples of control are shown. Statistical significance is based on actual data (not on the multiples of control).
[b] n = 3   [c] n = 2   [d] n = 1
[e] Dog 2112 was euthanized on Day 116 (Week 16) for human reasons. The animal number for Week 26 and Week 52 was 3.
- No noteworthy findings
* or **: absolute values statistically significant from controls:* - p<0.05, ** - p<0.01
GGT Gamma glutamyltransferase

Daily Dose During Dosing Period (mg/kg)	0 (Control)		4		30		100	
	M	F	M	F	M	F	M	F
No. of Animals	M:4	F:4	M:4/3[b]	F:4	M:4	F:4	M:4	F:4
Urinalysis								
NAG Absolute Excretion (mg/16 hr) [a] - Week 26	0.542	0.186	1.89x	2.32x	2.46x	2.97x	1.68x	1.60x
NAG/Creatinine (U/mg) – Week 26	-	0.267	-	-	-	2.63x	-	1.99x
NAG Absolute Excretion (mg/16 hr) [a] - Week 52	0.226	0.264	3.62x	1.45x	6.01x	2.09x	2.84x	1.81x
NAG/Creatinine (U/mg) – Week 52	-	-	-	-	-	-	-	-
Calcium Absolute Excretion (mg/16 hr) [a] - Week 26	10.526	10.292	4.68x	3.10x	3.56x	2.83x	1.54x	2.71x
Calcium/Creatinine (mg/mg) – Week 26	-	0.138	-	-	-	2.33x*	-	2.94x**
Calcium Absolute Excretion (mg/16 hr) [a] - Week 52	2.833	8.015	8.64x*	1.97x	4.41x*	2.07x	2.86x*	1.88x
Chloride Absolute Excretion (mEq/16 hr) [a] - Week 26	13.692	11.020	2.35x	2.67x	1.97x	1.91x	1.29x	1.41x
Chloride Absolute Excretion (mEq/16 hr) [a] - Week 52	2.470	11.499	6.78x	1.41x	7.13x	2.04x	2.86x	1.09x
Phosphorus Absolute Excretion (mg/16 hr) [a] - Week 26	202.300	181.656	1.92x	1.99x	2.17x	1.77x	1.37x	1.35x
Phosphorus Absolute Excretion (mg/16 hr) [a] - Week 52	67.897	267.778	3.75x	1.30x	5.54x	1.80x	2.95x	1.20x
Potassium Absolute Excretion (mEq/16 hr) [a] - Week 26	12.013	12.352	2.15x	1.86x	1.91x	1.43x	1.25x	1.12x
Potassium Absolute Excretion (mEq/16 hr) [a] - Week 52	4.378	15.898	4.19x	1.60x	5.87x	2.02x	2.74x	1.52x
Sodium Absolute Excretion (mEq/16 hr) [a] - Week 26	4.291	3.366	2.05x	3.79x	2.52x	2.67x	1.51x	2.01x
Sodium Absolute Excretion (mEq/16 hr) [a] - Week 52	0.854	2.393	8.50x*	1.82x	12.00x*	5.23x*	3.70x*	3.69x*
Sodium /Creatinine (mEq/mg) [a] – Week 52	5.508	2.470	-	-	-	3.74x*	-	3.22x*

[a] For controls, group means are shown. For treated groups, multiples of control are shown.
[b] Dog 2112 was euthanized on Day 116 (Week 16) for human reasons. The animal number for Week 26 and Week 52 was 3.
NAG N-acetyl-β-D-glucosaminidase   - No noteworthy findings
* or **: absolute values statistically significant from controls:* - p<0.05, ** - p<0.01

Gross Pathology: There were no notable gross macroscopic observations in dogs.

Organ Weights: There was a significant non-dose dependent increase in pituitary weight in females but not in males.

12-Month Oral Toxicity Study of JNJ-28431754-ZAE in Dogs (TOX8446)

Daily Dose During Dosing Period (mg/kg)		0 (Control)		4		30		100	
		M	F	M	F	M	F	M	F
No. of Animals		M:4	F:4	M:4/3[b]	F:4	M:4	F:4	M:4	F:4
Histomorphometry		-	-	-	-	-	-	-	-
Organ Weights [a]									
Pituitary gland	Absolute (g)	-	0.050	-	1.32x**	-	1.46x**	-	1.30x*
	Relative to BW	-	0.001	-	-	-	-	-	-
	Relative to BrW	-	0.070	-	1.24**	-	1.37x*	-	1.27x
Histopathology		-	-	-	-	-	-	-	-

[a] For controls, group means are shown. For treated groups, multiples of control are shown. Statistical significance is based on actual data (not on the multiples of control).
- No noteworthy findings
* or **: absolute values statistically significant from controls:* - p<0.05, ** - p<0.01
BW Body weight; BrW Brain weight

55

Histopathology
Pear review: Yes
Adequate: Yes

There were no notable soft tissue histological findings in dogs.   The incidence of tubular regeneration/ degeneration treated males was similar to control. Standard evaluation of femur and sternum found no histopathology. Bone samples were evaluated in more detail under Special Evaluation.

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Incidence Summary of Microscopic Findings with Severity Levels Terminal Sacrifice
----------------------------------------------------------------------------------
                                                    -- A n i m a l s   A f f e c t e d --
Controls from group(s): 1                 Animal sex:    -- M a l e s --  |   -- F e m a l e s --
                                        Dosage group:  Ctls   2   3   4   |  Ctls   2   3   4
T i s s u e s   W i t h   D i a g n o s e s   No. in group:   4   3   4   4   |    4   4   4   4
Kidney ................................Number examined:   4   3   4   4   |    4   4   4   4
  SUBACUTE/CHRONIC INFLAMMATORY CELL INFILTRATE
                                              ->     3   3   3   3   |    4   4   4   3
                                              1>     1   0   1   1   |    0   0   0   1
..........................Total Incidence of Finding Observed:   1   0   1   1   |    0   0   0   1

  TUBULAR REGENERATION/DEGENERATION
                                              ->     3   3   3   3   |    4   4   4   4
                                              1>     1   0   1   1   |    0   0   0   0
..........................Total Incidence of Finding Observed:   1   0   1   1   |    0   0   0   0

  FIBROSIS WITH TUBULAR DILATION, TUBULAR LOSS AND MINERAL
  -DEPOSITS
                                              ->     4   2   4   4   |    4   4   4   4
                                              2>     0   1   0   0   |    0   0   0   0
..........................Total Incidence of Finding Observed:   0   1   0   0   |    0   0   0   0

  MINERAL DEPOSITS
                                              1>     4   3   4   4   |    4   4   4   4
..........................Total Incidence of Finding Observed:   4   3   4   4   |    4   4   4   4
----------------------------------------------------------------------------------
All Diagnoses; Subgroups: 1; Phases: P3; Death types: Scheduled FS; Date of death range: 28-Aug-08 To 29-Aug-08
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Special Evaluation: Bone Biomarker

- All doses of canagliflozin resulted in decreased urinary deoxypyridinoline (bone resorption marker) in both male and female dogs (p< 0.05 in females) at WK 17 but not at WK52.
- There were no statistically significant changes in crosslinked C-telopeptide of type 1 collagen (bone resorption marker) at WK 17 or WK 52
- There were no statistically significant changes in serum osteocalcin (bone formation marker) in dogs at WK 52.
- Bone specific ALK was increased at 100 mg/kg in females (P<0.05) but not statistically in HD males (P> 0.05) at WK 52.
- Canagliflozin dose-dependently decreased serum 1,25 dihydroxyvitamin D levels in both male and female dogs. The decreases were statistically significant in the HD animals at WK17. Although there was an increase in urinary excretion of Ca, the serum Ca levels were not significantly different from control dogs.
- In males, canagliflozin reduced bone mineral density of whole femur and proximal femur at all doses, distal femur at MD. The slight decrease in midshaft femur bone mineral density was not significant. There was no notable change in bone area.
- In females, canagliflozin did not show any significant change in bone mineral density.
- Three point strength test of femoral shaft found small (p>0.05) reduction in maximum load and stiffness in males but in females.

- Canagliflozin treated males had a decrease in non-declassified trabecular bone volume (secondary spongiosa of the proximal tibia). The trabecular bone volume was not decreased in females. In fact, there was a slight increase in the trabecular bone volume in LD females.
- There was no change in unmineralized bone tissue (osteoid) in either male or female dogs.

Bone turnover markers at WK 17 and WK52 in dogs treated with 0, 4, 30 and 100 mg/kg of can are shown in tables below:

12 Month Oral Toxicity Study of JNJ-28431754-ZAE in Dogs (TOX8446)
- Bone Turnover Marker Analysis -

DOSING PERIOD

Daily Dose During Dosing Period (mg/kg/day)	0 (Vehicle)		4		30		100	
No. of Animals	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4
Calcium Homeostasis Biomarker[a] (At Week 17)								
1,25 Dihydroxyvitamin D (pmol/L)	272.86	311.92	0.97x	0.84x	0.61x	0.74x	0.55x*	0.40x**
No. of Animals	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4
Bone Turnover Biomarkers[a] (At Week 17)								
Osteocalcin (ng/mL)	13.33	16.03	0.75x	1.08x	0.99x	0.86x	0.72x	0.95x
Crosslinked C-telopeptide of Type 1 Collagen (ng/mL)	0.98	0.99	1.09x	1.04x	1.04x	0.94x	1.05x	1.05x
Bone Specific Alkaline Phosphatase (U/L)	35.83	42.76	1.18x	1.09x	1.11x	1.23x	1.29x	1.33x
No. of Animals	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4
Urine Biomarker[a] (At Week 17)								
Deoxypyridinoline/creatinine Ratio (nM/mM)	9.41	8.71	0.77x	0.72x*	0.81x	0.71x*	0.86x	0.67x*

[a] For controls, group means are shown. For treated groups, multiples of control are shown. Statistical significance is based on actual data (not on the multiples of control)   * - p<0.05, ** - p<0.01

12 Month Oral Toxicity Study of JNJ-28431754-ZAE in Dogs (TOX8446)
- Bone Turnover Marker Analysis -

DOSING PERIOD

Daily Dose During Dosing Period (mg/kg/day)	0 (Vehicle)		4		30		100	
No. of Animals	M: 4	F: 4	M: 3	F: 4	M: 4	F: 4	M: 4	F: 4
Calcium Homeostasis Biomarker[a] (At Week 52)								
1,25 Dihydroxyvitamin D (pmol/L)	320.82	272.81	0.81x	1.14x	0.70x	0.87x	0.67x	0.76x
No. of Animals	M: 4	F: 4	M: 3	F: 4	M: 4	F: 4	M: 4	F: 4
Bone Turnover Biomarkers[a] (At Week 52)								
Osteocalcin (ng/mL)	7.87	3.66	0.60x	1.12x	0.90x	1.61x	0.64x	1.65x
Crosslinked C-telopeptide of Type 1 Collagen (ng/mL)	0.75	0.57	1.31x	0.89x	1.03x	0.83x	1.14x	1.07x
Bone Specific Alkaline Phosphatase (U/L)	22.80	25.22	1.53x	1.05x	0.81x	1.23x	1.33x	1.93x*
No. of Animals	M: 4	F: 4	M: 3	F: 4	M: 4	F: 4	M: 4	F: 4
Urine Biomarker[a] (At Week 52)								
Deoxypyridinoline/creatinine Ratio (nM/mM)	5.29	6.69	0.76x	0.71x	0.88x	0.82x	0.90x	0.75x

[a] For controls, group means are shown. For treated groups, multiples of control are shown. Statistical significance is based on actual data (not on the multiples of control)   * - p<0.05, ** - p<0.01

12 Month Oral Toxicity Study of JNJ-28431754-ZAE in Dogs (TOX8446)
- Bone Turnover Marker Analysis -

DOSING PERIOD

Daily Dose During Dosing Period (mg/kg/day)	0 (Control)		4		30		100	
No. of Animals	M: 4	F: 4	M: 3	F: 4	M: 4	F: 4	M: 4	F: 4
Densitometry (Week 52)[a]								
DXA Scan of the Whole Femur								
Bone Mineral Content (g)	12.019	9.499	0.94x	1.07x	0.96x	1.00x	0.92x	0.98x
Bone Area (cm^2)	22.594	20.427	1.04x	1.01x	1.04x	1.01x	0.99x	0.99x
Bone Mineral Density (g/cm^2)	0.531	0.465	0.91x**	1.05x	0.92x**	0.98x	0.93x*	0.99x
DXA Scan at the Distal Femur								
Bone Mineral Content (g)	3.787	2.979	0.96x	1.06x	0.94x	1.00x	0.91x	0.98x
Bone Area (cm^2)	7.019	6.418	1.05x	0.99x	1.06x	1.01x	0.97x	0.97x
Bone Mineral Density (g/cm^2)	0.539	0.464	0.91x	1.07x	0.89x*	0.99x	0.93x	1.01x
DXA Scan at the Midshaft Femur								
Bone Mineral Content (g)	4.659	3.638	0.91x	1.06x	0.98x	0.99x	0.92x	0.97x
Bone Area (cm^2)	8.799	7.729	1.00x	1.01x	1.02x	1.00x	0.97x	1.00x
Bone Mineral Density (g/cm^2)	0.529	0.471	0.92x	1.05x	0.96x	0.98x	0.95x	0.97x
DXA Scan at the Proximal Femur								
Bone Mineral Content (g)	3.506	2.797	0.97x	1.08x	0.95x	1.01x	0.93x	0.99x
Bone Area (cm^2)	6.660	6.111	1.08x	1.03x	1.05x	1.03x	1.03x	1.01x
Bone Mineral Density (g/cm^2)	0.526	0.458	0.90x**	1.04x	0.91x**	0.98x	0.90x**	0.99x

[a] For controls, group means are shown. For treated groups, multiples of control are shown. Statistical significance is based on actual data (not on the multiples of control)   * - $p<0.05$, ** - $p<0.01$

12 Month Oral Toxicity Study of JNJ-28431754-ZAE in Dogs (TOX8446)
- Bone Turnover Marker Analysis -

DOSING PERIOD

Daily Dose During Dosing Period (mg/kg/day)	0 (Control)		4		30		100	
Mechanical Testing[a]								
No. of Animals	M: 4	F: 4	M: 3	F: 4	M: 4	F: 4	M: 4	F: 4
Femoral Shaft - Three Point Bend (Week 52)								
Maximum Load (N)	1954.718	1409.038	0.84x	1.15x	0.89x	1.01x	0.91x	1.03x
Stiffness (N/mm)	1638.645	1208.802	0.72x	1.10x	0.88x	0.93x	0.87x	0.98x
Energy (mJ)	2716.868	1698.527	1.08x	1.43x	0.84x	1.28x	1.03x	1.38x

[a] For controls, group means are shown. For treated groups, multiples of control are shown. Statistical significance is based on actual data (not on the multiples of control).   * - $p<0.05$, ** - $p<0.01$

12 Month Oral Toxicity Study of JNJ-28431754-ZAE in Dogs (TOX8446)
- Bone Turnover Marker Analysis -

DOSING PERIOD								
Daily Dose During Dosing Period (mg/kg/day)	0 (Control)		4		30		100	
No. of Animals	M: 4	F: 4	M: 3	F: 4	M: 4	F: 4	M: 4	F: 4
Histomorphometry (Week 52)[a]								
Secondary Parameters								
Trabecular bone volume (%)	21.442	16.583	0.82x	1.30x*	0.93x	0.94x	0.83x	0.96x
Trabecular number (#/mm)	2.387	2.127	0.89x	1.15x	0.97x	1.05x	0.92x	0.98x
Trabecular thickness (µm)	89.734	78.039	0.93x	1.13x	0.96x	0.89x	0.90x	0.97x
Trabecular separation (µm)	331.244	394.499	1.17x	0.82x	1.05x	0.96x	1.13x	1.04x
Osteoid thickness (µm)	7.472	6.851	0.92x	1.06x	0.98x	1.02x	0.96x	1.17x
Osteoid surface (%)	3.936	3.227	0.84x	0.85x	0.97x	0.99x	0.96x	1.95x
Osteoid volume, tissue referent (%)	0.133	0.094	0.72x	1.03x	0.95x	1.09x	0.88x	2.17x
Mineralized bone volume (%)	21.309	16.489	0.82x	1.30x*	0.93x	0.94x	0.83x	0.95x

[a] For controls, group means are shown. For treated groups, multiples of control are shown. Statistical significance is based on actual data (not on the multiples of control).

[b] Statistics not performed on Bone surface per bone volume   * - p<0.05, ** - p<0.01

Toxicokinetics

- Canagliflozin absorption was variable from slow to fast.
- The Tmax in dogs ranged from 0.875 to 6 hrs with $t^1/_2$ ranging from 10 to 26 hrs. The sponsor had estimated the Tmax to be between 0.67 and 3.3 hr to carry out cardiovascular evaluations. It is not clear if the CV evaluations were extended beyond 3.3 hrs but since the Tmax in majority of MD and HD animals fell between 1 and 4 hrs with no CV abnormalities, there is minimal concern.
- AUC increased in less than dose-proportional manner in both male and females
- Repeated dosing increased Cmax in LD females but decreased Cmax and AUC at 30 and 100 mg/kg in both genders by 35 to 70%.
- There was slightly greater exposure in female dogs than males.
- Although dogs produced both M5 and M7, their exposure was less than those in humans. However, as inactive, water soluble glucuronide metabolites, neither pose any safety risk.
- The NOAEL was 30 and 100 mg/kg in male and female dogs. Exposure multiples in males and females were 10 and 19x the MRHD based on AUC (300 mg QD, AUC of 26.1 µg.h/ml),

DOSING PERIOD						
Daily Dose During Dosing Period (mg/kg)	4		30		100	
	M	F	M	F	M	F
No. of Animals	M:4/3[a]	F:4	M:4	F:4	M:4	F:4
Toxicokinetics:						
Day 1 C$_{max}$ (ng/mL)	3,200	3,050	15,200	17,300	26,800	23,400
Week 26 C$_{max}$ (ng/mL)	4,520	5,180	16,700	20,900	41,900	30,600
Week 52 C$_{max}$ (ng/mL)	4,190	5,670	21,400	20,200	39,300	38,600
Day 1 AUC$_{0-\infty}$	54,500	50,600	444,000*	437,000*	533,000*	901000*
Week 26 AUC$_{0-24h}$ (ng·h/mL)	54,100	67,100	234,000	262,000	570,000	445,000
Week 52 AUC$_{0-24h}$ (ng·h/mL)	52,200	67,000	262,000	264,000	529,000	503,000

[a] Dog 2112 was euthanized on Day 116 (Week 16) for human reasons. The animal number for Week 26 and Week 52 was 3.

*: AUC$_{(0-\infty)}$ extrapolated value was greater than 25%

Mean JNJ-41488525 (M7) Plasma Toxicokinetic Parameters in Male and Female (N = 4) Dogs Following a Single or Multiple Oral Doses (100 mg/kg/day) of JNJ-28431754-ZAE (TOX8446)

Gender	Dose (mg/kg)	Day	Subject	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	$AUC_{(0-\infty)}$ (h*ng/mL)	$AUC_{(0-24 h)}$ (h*ng/mL)
Female	100	Day 1	Mean	207	2.75	40.1	9060	3250
			SD	(89.9)	(1.50)	(23.7)	(3560)	(1800)
		Week 26	Mean	586	1.50	14.7	10900	7020
			SD	(56.2)	(0.577)	(2.81)	(3310)	(1050)
		Week 52	Mean	541	1.00	18.5	9980	5860
			SD	(115)	(0.00)	(12.5)	(4220)	(735)
Male	100	Day 1	Mean	295	1.50	14.1	4620	3240
			SD	(94.1)	(0.577)	(5.63)	(1510)	(1130)
		Week 26	Mean	783	2.25	11.8	11900	9050
			SD	(288)	(1.26)	(2.47)	(3540)	(3300)
		Week 52	Mean	515	1.50	11.3	7410	5670
			SD	(202)	(0.577)	(1.16)	(5270)	(3840)

Mean JNJ-41980874 (M5) Plasma Toxicokinetic Parameters in Male and Female (N= 4) Dogs a Single or Following Multiple Oral Doses (100 mg/kg/day) of JNJ-28431754-ZAE (TOX8446)

Gender	Dose (mg/kg)	Day	Subject	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	$AUC_{(0-\infty)}$ (h*ng/mL)	$AUC_{(0-24 h)}$ (h*ng/mL)
Female	100	Day 1	Mean	20.9	2.00	5.49	*417	284
			SD	(4.41)	(0.00)	(1.62)	(168)	(87.5)
		Week 26	Mean	45.0	1.75	18.3	913	499
			SD	(10.4)	(0.500)	(17.7)	(412)	(138)
		Week 52	Mean	52.3	1.25	7.36	635	483
			SD	(12.6)	(0.500)	(9.64)	(209)	(78.3)
Male	100	Day 1	Mean	32.4	1.75	7.69	*333	239
			SD	(8.73)	(0.500)	(9.36)	(196)	(109)
		Week 26	Mean	64.5	1.75	8.22	889	653
			SD	(12.7)	(0.500)	(6.42)	(198)	(159)
		Week 52	Mean	50.4	1.50	15.2	663	440
			SD	(15.1)	(0.577)	(13.8)	(286)	(235)

*= $AUC_{(0-\infty)}$ extrapolated value was greater than 25%

Dosing Formulation Analysis
Canagliflozin concentrations in the solutions were within the target concentrations (± 10%).

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

The genotoxicity of JNJ-28431754 was evaluated using TA98, TA100, TA1535 and TA1537 and WP2 *uvr*A. Precipitation occurred at ≥ 500 µg/plate. JNJ-28431754 was cytotoxic at the highest dose levels with or without S9 in the preliminary study. In the confirmatory assay, similar findings were observed. There was no notable increase in the number of revertants in the confirmatory assay. The positive controls significant increased the number of revertant colonies suggesting that the assay was working appropriately.

7.2 *In Vitro* Chromosomal Aberration Assays in Mammalian Cells

Genotoxicity of JNJ-28431754 was evaluated using mouse lymphoma cells (L5178Y/TK+/-) line. In the dose ranging test, JNJ-28431754 levels of 2.0, 3.9, 7.8, 15.6, 31.3, 62.5, 125, 250, 500 and 1000 µg/mL were evaluated. JNJ-28431754 was extremely cytotoxic in the 3-hr and 24 hr treatment without S9 and to lesser extent with S9. Precipitation was noted at ≥ 125 µg/ml with 3-hr treatment (± S9) and at ≥ 31.3 µg/ml with 24-hr treatment (-S9). In the definitive study with 3-hr treatment (-S9), JNJ-28431754 levels up to 60.0 µg/mL were tested and precipitation (≥ 20 µg/ml) and cytotoxicity (≥ 30 µg/ml) were noted. When cells were treated for 24 hrs (-S9) at concentrations of up to 40 µg/ml, precipitation (≥ 15 µg/ml) and cytotoxicity (≥20 µg/ml) was observed with 24-hr treatment (-S9). There was no evidence of an increase in the mutation frequency in the 3-hr and 24-hr treatment without S9. However due to limited number high enough JNJ-28431754 concentrations in the 24-h treatment test, the assay was repeated with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18 and 20 µg/mL. Again precipitation (≥ 16µg/ml) and toxicity (≥18 µg/ml) was observed with 24-hr repeat test. JNJ-28431754 was found negative for mutagenicity with 24-hr treatment.

With 3-hr treatment with S9, JNJ-28431754 concentrations up to 60 µg/ml were tested. Precipitation (≥32.5µg/ml) and cytotoxicity (≥ 52.5 µg/ml) was noted with S9. JNJ-28431754 increased mutation frequency in a dose-dependent manner but less than 2 fold of the vehicle control. The 3-hr test with S9 was repeated using 10, 20, 30, 32, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 47 and 50 µg/ml. As before, precipitation (≥ 30 µg/ml) and cytotoxicity was (≥ 44 µg/ml) was noted. JNJ-28431754 significantly increased the mutation frequency by 2.1 to 5.3 fold relative to vehicle control. The increase in mutation frequency was in both small and large colonies. Under the test condition, JNJ-28431754 was considered mutagenic in the 3-hr treatment with S9. Since mutagenicity did not occur with 3- and 24-h treatment without S9, suggests that addition of S9 is introducing a new metabolite that might be mutagenic. It is highly possible that S9 metabolizing the parent to a mutagenic metabolite. It should be noted that JNJ-28431754 was more toxic and tend to precipitate at lower concentrations without S9 and adding S9 extended the concentration range to be tested. Whether adding S9 allowed higher concentrations to be tested is unknown. Two issues that may have an impact on the results are, 1) although the metabolic profile of JNJ-28431754 appeared to be similar in humans and animals, they are quantitatively different, 2) JNJ-28431754 is a substrate and a weak inhibitor of efflux transporter proteins, MDR1 (IC_{50} 8.5 µg/ml) and MRP2 (9.5 µg/ml).

7.3   *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

In the micronucleus assay, single doses of JNJ-28431754 were given to the male and female rat (500, 1000 and 2000 mg/kg). There were no deaths after single dose of JNJ-28431754 up to 2000 mg/kg. Clinical signs in female and males rats were stained ano-genital area, diarrhea, loose stool, and decreased feces at all dose levels. Ptosis, red discolored nasal and eye discharge, and decreased spontaneous motor activity were noted at ≥ 1000 mg/kg in both male and female rats. Additional clinical signs noted in female treated with 2000 mg/kg included labored breathing, ataxia, and alopecia on the left ventral side. Vehicle and positive controls were within the acceptable range suggesting that the study was acceptable. JNJ-28431754 was not cytotoxic to the bone marrow at doses up to 2000 mg/kg in males at 24 or 48 hr harvest time, or in females at 24-h harvest. JNJ-28431754 was not cytotoxic to the bone marrow at doses up to 2000 mg/kg in female at 24; however at 48 harvest time, a statistically significant trend in decreasing ratios of PCE:NCEs suggesting cytotoxicity to the bone marrow in females at doses of 500-2000 mg/kg. JNJ-28431754 did not induce any statistically significant increases in the frequency of micronucleated PCEs. Under the assay conditions, JNJ-28431754 was not considered a genotoxic.

7.4   Other Genetic Toxicity Studies

Comet Assay

In comet assay single dose of JNJ-28431754 was administered to SD rats (500, 100 and 2000 mg/k of JNJ-28431754. Drug exposure was also evaluated at 2000 mg/kg.
Liver samples collected at 3 and 24 hrs after treatment were assessed for DNA damage expressed by as Comet % tail intensity, Olive tail moment and tail migration. There was no dose response effect or significant increase in DNA damage at any dose level in liver cells. The statistically significant increase in the % Comet tail intensity at 2000 mg/kg in males at 3 hours post dose and at 500 mg/kg in females at 24 post dose were within the historical vehicle control range and not considered biologically significant. The positive control induced a statistically significant increase in the Comet % tail intensity as compared to that of the saline vehicle control. Under the assay condition, JNJ-28431754 was considered negative in the rat *in vivo* Comet assay.

The sponsor had carried out several special toxicology studies to assess the potential drug effect on cornea and skin (phototoxicity and skin sensitization).   In the *in-vitro* bovine corneal opacity-permeability assay, 20% suspension solution of JNJ-28431754 did not increase corneal opacity; however, a small increase in permeability was noted. With an *in-vitro* score of 3.2, the 20% suspension solution of JNJ-28431754 was considered as a potentially but very mild eye irritant under the assay conditions.

In the *in-vitro* cyto and photo-toxicity, JNJ-28431754 was added to Balbc/3T3 mouse fibroblast culture and exposed to ultraviolet A (UVA). The IC_{50} in the presence and absence of UVA were 17.7 and 78.1 mg/l, respectively. The mean photo-effect was 0.28. Since any value greater

than 0.15 is considered phototoxic, JNJ-28431754 was considered phototoxic in this *in-vitro* study.

In the *in-vivo*, phototoxicity study, single dose of JNJ-28431754 (5, 50 and 500 mg/kg), administered to Long Evans pigmented rats, no phototoxicity was noted at 5 mg/kg (NOAEL). However, phototoxicity was noted at 50 mg/kg (mild erythema 1 F) and at 500 mg/kg (erythema and mild to moderate edema 3/5 F & 1/5 M) on day after dose. There were no microscopic changes. There were no ocular changes when rats were exposed to UVA and UVB radiation. The Cmax and AUC at NOAEL dose (5 mkd) were 1.5 µg/ml and 20 µg.h/ml, respectively, approximately 14-fold greater than clinical dose of 10 mg, based on AUC (1.4 µg.h/ml)

Skin sensitization was also assessed using JNJ-28431754 induced proliferation of auricular lymph node lymphocytes of CBA/J mice. Topical application of JNJ-28431754 in N,N-dimethylformamide at 2.5, 15 and 30 mg/100 g for 3 days over the ear skin did not cause redness or swelling of the ear. H^3-thymidine injection of Day 6 to evaluate lymph node incorporation of radioactivity did not find any notable radioactivity in the ear lymph node. The stimulation index was les than 3 suggesting JNJ-28431754 is not a contact sensitized under the assay condition. In the clinical study carried out in Netherlands, some of the adverse events were erythema, pruritus, rash, skin irritation and burning. The animal studies do provide some indication that JNJ-28431754 may cause skin rash and irritation and possibly edema and swelling.

In summary, canagliflozin (JNJ-28431754) was negative in Ames and rat micronucleus assay. However, in the in vitro mouse lymphoma assay (MLA), 3-hr treatment with S9 mix was positive. Although the 3- and 24-h treatment without S9, were negative, the positive finding with S9 suggests introduction of a <u>potential metabolite</u> that might be mutagenic. When the test was repeated, the frequency of mutation was 2 to 4 fold the control levels at concentration > 30 µg/ml but significantly less (half) than the positive control. Since the sponsor had already repeated the assay and found the same results, the potential DNA damage caused by JNJ-28431754 or its metabolite was evaluated using the in vivo Comet's assay. The comet assay results did not support the positive mouse lymphoma assay with S9 mix suggesting that perhaps the metabolites produced by S9 may not occur in vivo or the mouse lymphoma test was giving false positive response. Since Ames test with S9 did not find a positive response and Comet assay was also negative, canagliflozin overall was considered nongenotoxic. The results were further supported by the negative mouse carcinogenicity, a model with a metabolic profile similar to humans with sufficient M5 and M7 exposure. The carcinogenicity safety report suggesting dose-dependent increase in the incidence of renal tubular, adrenal and testicular tumors in rats had slightly different metabolic profile than those in mice and humans.

Study title: *In Vitro* Bacterial Reverse Mutation Test with ⬛⬛⬛⬛⬛⬛⬛ (b) (4) in *Salmonella typhimurium(TOX10446)*

Key findings: The genotoxicity of ⬛⬛⬛⬛⬛⬛ (b) (4), a ⬛⬛⬛ (b) (4) degradant identified during the 18-month stability study was tested in triplicates using five strains of *Salmonella typhimurium*, TA98, TA1537, TA100, TA1535 and TA102, in the absence and in the presence of rat S9-mix. ⬛⬛⬛⬛⬛⬛ (b) (4) was considered mutagenic in *S. typhimurium* strains TA98 and TA100 in the absence and in the presence of S9-mix.

Study no.: TOX10446
Volume #, and page #: Electronic eCTD
Conducting laboratory and location: Drug Safety Sciences, Beerse site, Belgium
Date of study initiation: Aug 20, 2012
GLP compliance: Performed according to GLP but not considered GLP without QA
QA reports: yes ( ) no (x)
Drug, lot #, and % purity: Lot #2079-025-701, 92.3%, MW: 476.5, MF: C24H25FO7S

Methods
Strains/species/cell line:

Experiment	without S9	with S9
Mutation	TA98, TA1537, TA100, TA1535, TA102	TA98, TA1537, TA100, TA1535, TA102

Doses used in definitive study:

Experiment	without S9	with S9
Mutation	9.77, 19.53, 39.06, 78.13, 156.25, 312.5, 625, 1250, 2500, 5000	9.77, 19.53, 39.06, 78.13, 156.25, 312.5, 625, 1250, 2500, 5000

Negative controls: ethanol
Positive controls:

Positive control	CAS No.	Vehicle	S9	Conc. (µg/plate)	Strains
2-nitrofluorene	607-57-8	DMSO	without	5	TA98
sodium azide	26628-22-8	Water	without	1	TA1535, TA100
9-aminoacridine	90-45-9	DMSO	without	50	TA1537
2-aminoanthracene	613-13-8	DMSO	with	2.5	TA98, TA100, TA1535, TA1537
2-aminoanthracene	613-13-8	DMSO	with	7.5	TA102
4-nitroquinoline-N-oxide	56-57-5	DMSO	without	5	TA102

Incubation and sampling times: Approximately 0.1 ml of the test drug, plus 0.1 ml of bacteria culture and 0.5ml of S9-mix or 0.1 M sodium-potassium phosphate buffer (pH 7.4) were transferred to a sterile tube. Two ml of 0.6% top agar was added, the mixture was overlaid

onto plates containing minimum glucose agar. The plates were incubated at 37°C for 48 to 72 hrs and revertant colonies were counted by automated colony counter.

Study validity:

Test validity was examined by use of several positive controls and a negative control (vehicle). The sterility of the material and the genotypes of bacterial strains were tested by applying the highest concentration of vehicle used in the test with S9 mix onto a plate containing minimum glucose agar and incubated at 37°C for 2 days. The tester strain characteristics were checked for genotype (requirement of amino acid, ability of DNA repair and ampicillin resistance).
A 2-fold increase in the mean number of revertant with one of the stains TA98, TA102 or TA100 or 3-fold increase in the TA1535 and TA1537 at one or more concentrations relative to control was considered positive.

Results: _____ (b) (4) was considered mutagenic based on 2 fold increased number of revertants by more than 2 fold in bacterial strains TA98 and TA100 in the absence and in the presence of S9-mix.

In Vitro Bacterial Reverse Mutation Test with ____ (b) (4) in *Salmonella typhimurium*		Test Article: ____ (b) (4)					
Test for Induction of: gene mutations		**No. of Independent Assays:** 1			**GLP Compliance:** no		
Strains: S. Typhimurium strains TA98, TA1537, TA100, TA1535 and TA102		**No. of Replicates:** 3			**Date of Treatment:** Aug 2012		
Metabolizing System: Rat S9-mix		**Approximate No. of Bacteria Assayed/Dose:** 10^9			**Study No:** TOX10446		
Vehicles:		**Cytotoxic Effects:** yes			**Location in CTD:**		
For Test Article: Ethanol		**Genotoxic Effects:** yes					
For Positive Controls: DMSO and water		**Treatment:** In Vitro					

			Assay 1 (Plate Incorporation) Revertant Colony Counts (Mean ± SD)				
Metabolic Activation	Test Article	Concentration Level (µg/plate)	TA98	TA100	TA1535	TA1537	TA102
Without Activation	Ethanol (b) (4)	0	18.0±4.6	94.3±6.7	9.3±3.2	6.0±1.0	275.3±15.3
		9.77	23.0±4.6	81.0±12.0	8.7±2.1	3.3±2.5	196.0±16.5
		19.53	17.7±5.5	79.7±7.5	9.0±3.6	4.0±1.7	204.3±25.7
		39.06	24.7±3.8	92.7±7.2	10.7±1.2	8.7±3.1	215.3±10.0
		78.13	22.7±3.8	125.7±12.3	8.3±1.2	10.0±5.6	198.0±14.5
		156.25	49.7±14.0 *	214.3±12.9 *	11.0±4.4	6.0±1.7	206.7±20.4
		312.5	51.0±13.9 *	97.3±23.2 #	21.0±6.2	6.3±4.5	144.7±15.3
		625	17.0±5.6 #	31.7±3.1 #	11.7±1.5 #	2.0±1.0 #	110.3±4.0 #
		1250	8.7±2.5 #	8.0±7.9 @	4.3±1.5 #	1.0±1.0 @	41.0±2.0 #
		2500	11.7±4.9 @	0.0±0.0 @	1.0±1.0 @	0.0±0.0 @	2.3±2.5 @
		5000	0.0±0.0 @	0.0±0.0 @	0.0±0.0 T	0.0±0.0 T	0.0±0.0 @
	2-Nitrofluorene	5.00	385.0±53.0 *	-	-	-	-
	SodiumAzide	1.00	-	406.0±27.6 *	316.7±20.2 *	-	-
	9-Amino-Acridine	50.00	-	-	-	50.0±17.4 *	-
	4-Nitroquinoline-N-Oxide	5.00	-	-	-	-	3504.3±136.6 *
With Activation	Ethanol (b) (4)	0	19.3±1.5	101.0±11.8	11.7±2.1	5.0±1.0	315.3±29.7
		9.77	18.3±3.2	61.0±7.9	6.0±1.7	3.0±1.0	233.3±33.2
		19.53	13.3±3.1	71.0±11.0	7.0±1.7	5.3±1.2	182.3±13.3
		39.06	18.7±9.0	75.7±5.7	5.7±2.5	7.0±1.0	232.0±32.1
		78.13	31.3±2.1	77.7±8.1	6.7±3.2	7.0±1.0	251.3±41.7
		156.25	35.3±6.4	93.3±4.2	9.3±4.0	7.3±0.6	288.3±44.1
		312.5	43.0±6.6 *	132.3±15.0	8.0±0.0	6.3±2.5	172.3±18.8
		625	91.7±14.4 *	254.7±18.6 *	13.7±4.9	7.0±1.7	159.3±11.1
		1250	33.0±1.7 #	101.0±16.0 #	8.7±4.5 #	6.0±2.0 #	121.3±9.5 #
		2500	10.7±3.1 #	20.7±6.4 @	1.3±0.6 @	2.3±1.2 @	88.7±7.8 #
		5000	3.0±2.6 @	2.0±2.0 @	0.0±0.0 @	0.0±0.0 T	37.7±8.1 @
	2-Amino-anthracene	2.5	870.0±422.0 *	686.3±50.8 *	55.3±0.6 *	72.0±14.0 *	-
	2-Amino-anthracene	7.5	-	-	-	-	1397.0±206.6 *

* more than 2-fold increase with TA 98, TA100 and TA102 compared to the vehicle control, more than 3-fold increase with TA1535 and TA1537 compared to the vehicle control

@: Pinpoints   #: Thinning   T: Toxic   - : Not Applicable

8   Carcinogenicity

Study title: 2-Year Oral Gavage Carcinogenicity Study of JNJ-28431754-ZAE in CD-1 Mice

Study no.:	TOX8799 (b) (4) study # 886-190)
Study report location:	(b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 12, 2008 (terminated June 7, 2010)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ZR600348PFA111, 98%
CAC concurrence:	Yes

Key Study Findings

- Daily oral administration of 10, 30 and 100 mg/kg canagliflozin for two years did not increase the incidence of any type of tumor in CD-1 mice. Exposure at 100mg/kg in male and female mice was 7 and 14x the AUC of 300 mg clinical dose.

- End-of-study survival was adequate for statistical evaluation of all dose groups for both sexes; however, Kaplan-Meier curves suggest an effect on survival in males at all doses, but notably the MD and HD. Urogenital inflammation/obstruction was cited more often as the cause of death in dosed males, notably at the HD.

- Pharmacodynamic activity is modest in mice and apparent primarily in the HD males (increased food intake, slight decrease in BW)

- Non-neoplastic findings in females was limited to increased kidney weight and an increased incidence of bilateral nephrosis in the MD/HD. Note, hydronephrosis was also observed in the control group, albeit at a lower incidence.

- Non-neoplastic findings in males included increased kidney weight, hydronephrosis, and dilation of the bladder, ureter, and renal pelvis. Findings were most evident at the MD/HD.

- Calcification of tissues was not observed in tissues of male or female mice.

- Maximum exposure to M5 and M7 glucuronide metabolites were ≤ 1x (0.04x and 0.5x, respectively), relative to human exposure at the 300mg clinical dose.

Adequacy of Carcinogenicity Study: There were sufficient number of animals with adequate exposure reaching the terminal sacrifce.

Evaluation of Tumor Findings : There was no significant canagliflozin related increase in tumor incidence in mice at doses up to 100 mg/kg. The top dose was approximately 7 to 14x the 300 mg clinical dose with AUC of 26.1 µg.h/ml. Exposure (AUC) to the glucuronide metabolites (M5 and M7) in mice was less than in humans; however, there is no safety concern as these metabolites are pharmacologically inactive and water soluble glucuronide metabolites.

Methods

Doses:	0, 10, 30 and 100 mg/kg/day
Frequency of dosing:	Daily
Dose volume:	10 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% hypromellose in deionized water
Basis of dose selection:	MTD (death occurred at ≥ 250 mg/kg)
Species/Strain:	Crl:CD-1 (Icr) mice
Number/Sex/Group:	65/sex/group plus 39 for TK (20 for controls)
Age:	6 weeks (4 weeks at the time of arrival)
Animal housing:	3-4 per metal wire mesh cages
Paradigm for dietary restriction:	Not restriction (Certified Diet #5002)
Dual control employed:	Single control group
Interim sacrifice:	No
Satellite groups:	TK mice 36 /sex/group except for Control (18)
Deviation from study protocol:	Mid dose females were dosed for 101 WKs, rather than 104 WKs. Minor other deviations (missing adrenal gland, pituitary gland, TK blood sample collection time error in few animals....) were unlikely to have had any impact on the study outcome. Ophthalmoscopy was performed due to eye irritant properties of canagliflozin (positive in vitro bovine corneal opacity-permeability assay).

Observations and Results:

Mortality

- Drug-related deaths in HD male mice appeared to be due to increased urogenital inflammation/obstruction, although there were no drug-related renal histological changes or calculi.
- Survival of C, LD, MD and HD males were 31, 26, 29 and 20 out of 65 starting animals per group, respectively. Survival of females at the same doses was 25, 25, 18 and 33 out of 65, respectively.
- Slightly different survival rate by the FDA statistician were attributed to different dates of starting the terminal killing.

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(b) (4) Study Number 886-190
2-Year Oral Gavage Carcinogenicity Study of JNJ-28431754-ZAE in CD-1 Mice (Study TOX8799)

Summary of Probable Cause of Death - MALE

Cause of Death	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
Number of Animals	65	65	66	66
Summary of Animal Disposition				
died after dosing	0	0	1	0
died prior to euthanasia	2	0	1	3
euthanized *in extremis*	19	16	19	21
found dead	13	23	16	22
terminal necropsy	31	26	29	20
Cause of Death				
accidental injury	0	0	1	0
amyloidosis	0	1	2	0
brain tumor	1	0	0	0
chronic progressive nephropathy/uremia	4	0	0	0
dosing error	2	0	2	1
dosing injury	0	0	0	1
eyes inflammation/necrosis	1	0	0	0
harderian glands adenoma; undetermined	0	0	1	0
heart failure/atrial thrombus	1	0	0	0
hemangiosarcoma/hemangioma	1	3	1	1
histiocytic sarcoma	1	0	0	1
leukemia	0	0	1	0
liver cyst, biliary	0	0	2	0
liver tumor	1	2	0	0
lung tumor	2	1	0	0
lymphoid tumor	2	4	1	1
mammary tumor	1	0	0	0
obstruction/impaction, gastrointestinal tract	0	0	0	2
polyarteritis	1	0	0	0
schwannoma	0	0	1	0
skin inflammation/necrosis	2	5	1	2
skin tumor	0	0	1	0
undetermined	1	1	3	7
urogenital inflammation/obstruction	13	22	20	30

(b) (4) Study Number 886-190
2-Year Oral Gavage Carcinogenicity Study of JNJ-28431754-ZAE in CD-1 Mice (Study TOX8799)

Summary of Probable Cause of Death - FEMALE

Cause of Death	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
Number of Animals	65	65	64	64
Summary of Animal Disposition				
accidental death	0	0	1	0
died after dosing	1	0	0	0
euthanized *in extremis*	22	18	23	15
found dead	17	22	22	16
terminal necropsy	25	25	18	33
Cause of Death				
accidental injury	0	3	2	0
amyloidosis	0	1	2	0
bone tumor	0	1	0	0
chronic progressive nephropathy/uremia	3	1	4	1
dosing error	2	2	1	2
dosing injury	0	3	0	0
fibrosarcoma/fibroma	1	0	0	0
heart inflammation/necrosis	0	0	1	0
hemangiosarcoma/hemangioma	2	0	3	2
histiocytic sarcoma	2	1	2	0
inflammation/septicemia	0	1	1	0
kidney inflammation/necrosis	1	0	0	0
large intestine, rectum prolapse	0	0	0	1
leukemia	1	0	0	0
lipoma/liposarcoma	0	1	0	0
lung tumor	0	2	1	0
lymphoid tumor	6	11	9	5
mammary tumor	2	0	0	0
ovarian cyst/hemorrhage	9	5	2	8
ovaries abscess	0	0	0	1
ovary tumor	1	0	0	0
pituitary tumor	0	1	0	0
polyarteritis	1	3	0	0
skin inflammation/necrosis	1	1	4	1
skin tumor	1	0	1	2
undetermined	1	1	5	3
urogenital inflammation/obstruction	0	0	2	0
uterus hemorrhage	2	1	0	1
uterus inflammation/necrosis	1	0	0	0
uterus tumor	3	1	6	3
vagina prolapse	0	0	0	1

Kaplan-Meier plot of survival estiamtes in male and female mice treated with canagliflozin for up to 2-years.

Summary of Survival Estimates - MALE

Summary of Survival Estimates - FEMALE

2-Year Oral Gavage Carcinogenicity Study of JNJ-28431754-ZAE in CD-1 Mice (Study TOX8799)   Test Article: JNJ-28431754-ZAE

Daily Dose (mg/kg/day)	0 (Control)		10		30		100	
Gender	M: 65	F: 65	M: 65	F: 65	M: 66	F: 64	M: 66	F: 64
No. of Animals								
Died or Sacrificed Moribund	34	40	39	40	37	46[b]	46[c]	31
Terminal Sacrifice	31	25	26	25	29	18	20	33
Survival (%)	47.7	38.5	40.0	38.5	43.9	28.1	30.3	51.6

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Clinical Signs

- Abdominal distention (C:13, LD:11, MD:14, HD:26) was more common in the 100 mg/kg treated males suggesting a small degree of carbohydrate malabsorption. Abdominal distensions were not common in treated female mice. Abdominal distention and soft feces are considered signs of SGLT1 inhibition.

Body Weights

- Statistically significant changes in body weight were not observed in either males or females. However, a clear trend toward lower weight gain was seen at all doses in males starting at week 20. Drug exposure was lower in males than females.
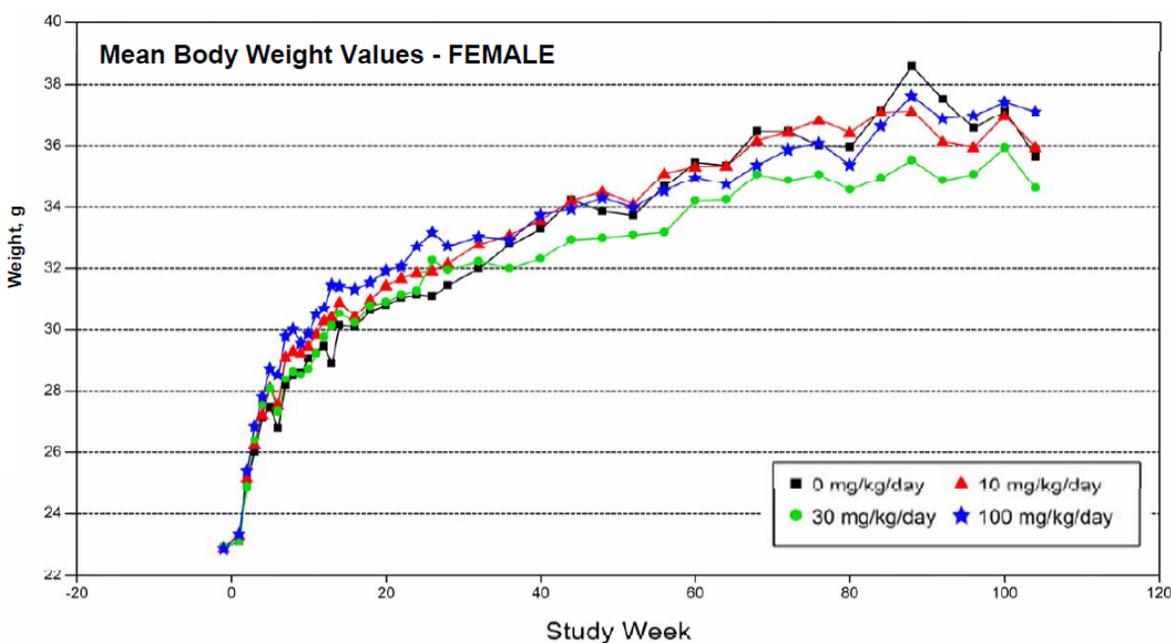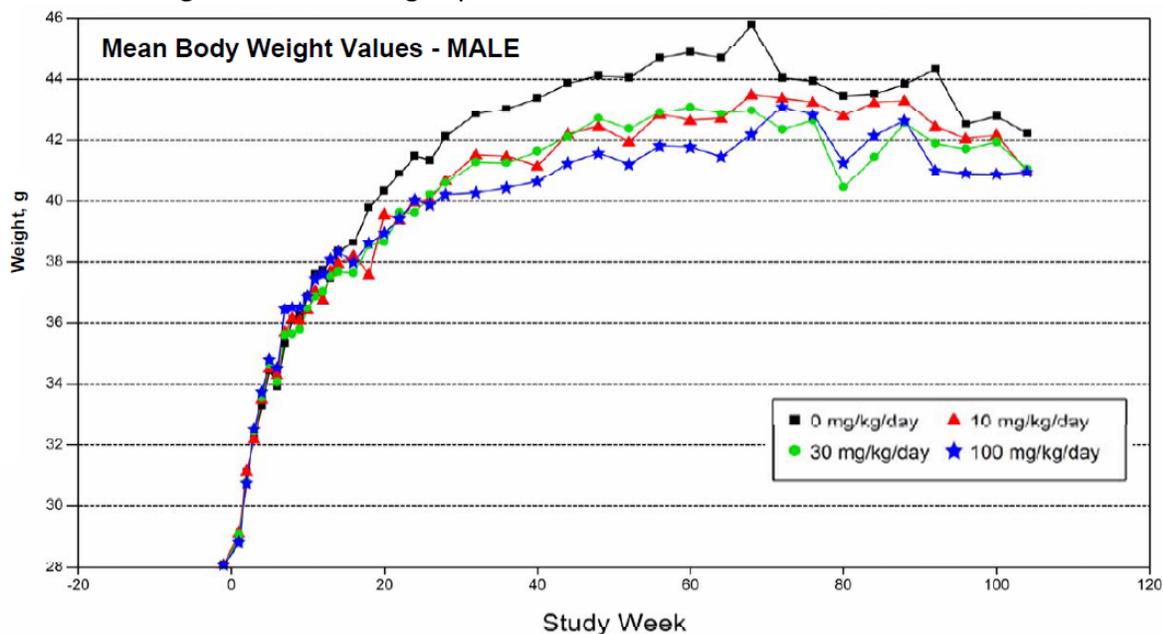
Feed Consumption

- Food intake increased at ≥30 mg/kg in males and 100 mg/kg females, likely due to urinary loss of glucose (not measured).

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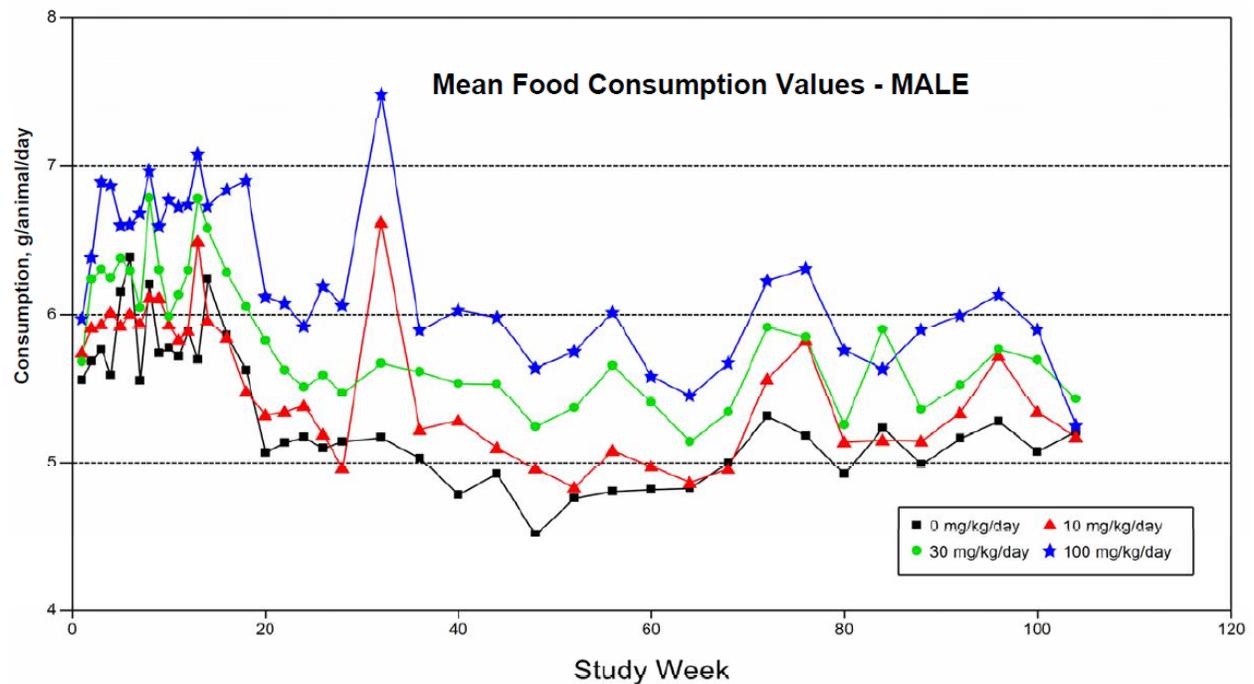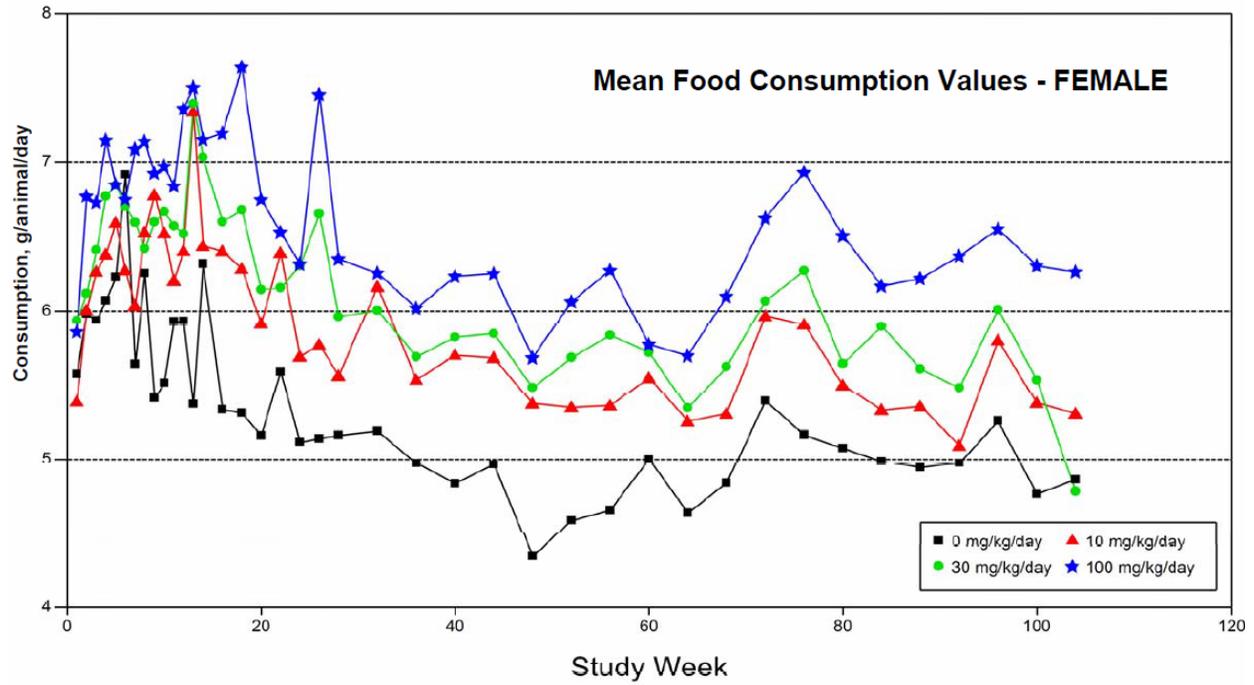
Ophthalmoscopy

- There were no drug-related microscopic findings in eyes at 12- or 24-month observations. However, all dose groups exhibited corneal opacities which may have reduced the sensitivity of the exam in detecting drug-related changes.

Clinical Chemistry

- Mean ALP and ALT were increased slightly in MD male and female mice. Since there were no consistent and similar findings in the HD animals, the rise in ALP and ALT in MD mice may have been coincidental.
- Some individual animals displayed signs of liver injury (increase in ALT, ALP, AST, bilirubin and cholesterol) while others has an elevation in renal biomarkers (BUN and creatinine and phosphorus) but since they were also seen in controls, the elevation in most animals was considered unrelated to the treatment.

Summary of Clinical Chemistry Values - MALE

Endpoint	Study Interval	0 mg/kg/day			10 mg/kg/day			30 mg/kg/day			100 mg/kg/day		
		Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
AST U/L	Terminal	52.0	18.31	16	95.4	174.32	13	108.7	96.73	15	54.2	23.26	10
ALT U/L	Terminal	45.7	50.00	16	50.4	94.18	13	91.2	128.79	15	33.8	15.22	10
Alkaline Phosphatase U/L	Terminal	49.5	33.82	16	40.8	10.42	13	89.8[b]	55.27	15	48.6	11.16	10

N - Number of measures used to calculate mean
SD - Standard Deviation
NA - Not Applicable/Not Available

[#] No statistics performed due to lack of variability or sample size

Summary of Clinical Chemistry Values - FEMALE

Endpoint	Study Interval	0 mg/kg/day			10 mg/kg/day			30 mg/kg/day			100 mg/kg/day		
		Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Alkaline Phosphatase U/L	Terminal	82.0	35.19	13	93.2	36.58	13	156.9[b]	68.60	9	91.2	48.92	17

N - Number of measures used to calculate mean
SD - Standard Deviation

[b] Significantly different from control; (p<0.01)

Organ Weight:

- Kidney weights increased in MD and HD females; this was not associated with renal dilatation.
- Kidney weight did not increase in male mice despite a higher incidence of renal pelvic dilatation.

Changes in Kidney Weight in Male and Female Mice

Summary of Organ Weight Values - MALE

	0 mg/kg/day			10 mg/kg/day			30 mg/kg/day			100 mg/kg/day		
Endpoint	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Kidneys												
g	0.972	0.132	31	0.971	0.146	26	1.042	0.171	29	0.965	0.118	20
Kidneys/BWt %	2.3693	0.2287	31	2.4514	0.2747	26	2.6564[b]	0.4583	29	2.4321	0.2504	20

Summary of Organ Weight Values - FEMALE

	0 mg/kg/day			10 mg/kg/day			30 mg/kg/day			100 mg/kg/day		
Endpoint	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Kidneys												
g	0.630	0.080	25	0.686	0.082	24	0.726[b]	0.107	18	0.800[b]	0.143	33
Kidneys/BWt %	1.8422	0.2429	25	1.9883	0.2716	24	2.1689[b]	0.3017	18	2.2467[b]	0.4069	33
Kidneys/BrWt ratio	1.2324	0.1595	25	1.4104[b]	0.1688	24	1.4772[b]	0.2691	18	1.6712[b]	0.3042	33

N - Number of measures used to calculate mean   [b] Significantly different from control; (p<0.01)   SD - Standard Deviation

Gross Pathology

- The incidence and severity of renal pelvic dilatation increased in a dose-dependent manner in male mice, affecting all dose groups, with a related increase in renal cysts and distended ureters. Similar renal changes were not seen in females.
- The pharmacological response to canagliflozin in mice is poor compared to the robust response observed in rats, relative to inhibition of both SGLT1 and SGLT2. This likely explains the minimal renal findings in the mice.
- There were no drug-related increases in palpable masses. In general, the incidence of palpable tumors was low in both males and females.

Reference ID: 3254315

(b) (4) Study Number 886-190
2-Year Oral Gavage Carcinogenicity Study of JNJ-28431754-ZAE in CD-1 Mice (Study TOX8799)
Summary of Macroscopic Observations - MALE
Terminal

Tissue / Observation	Severity	0 mg/kg/day DOS	0 mg/kg/day SNC	10 mg/kg/day DOS	10 mg/kg/day SNC	30 mg/kg/day DOS	30 mg/kg/day SNC	100 mg/kg/day DOS	100 mg/kg/day SNC
Number of Animals Examined		34	31	39	26	37	29	46	20
kidneys									
cyst		3	8	2	7	7	11	9	9
	- minimal	0	1	0	1	0	1	0	3
	- mild	3	7	1	5	3	10	2	6
	- moderate	0	0	0	0	4	0	7	0
	- severe	0	0	1	1	0	0	0	0
dilatation, pelvic		2	0	9	0	9	0	15	0
	- minimal	0	0	1	0	0	0	0	0
	- mild	2	0	5	0	1	0	5	0
	- moderate	0	0	0	0	4	0	9	0
	- severe	0	0	3	0	4	0	1	0
enlarged		0	0	1	0	1	0	1	0
	- mild	0	0	1	0	1	0	0	0
	- moderate	0	0	0	0	0	0	1	0
large intestine, cecum									
distended with fluid	- moderate	0	0	1	0	0	0	0	0
distended with gas	- mild	0	0	1	0	0	0	0	0
enlarged	- moderate	0	0	0	0	0	0	1	0
impacted	- moderate	0	0	0	0	0	0	2	0
large intestine, colon									
impacted		0	0	0	0	0	0	3	0
	- moderate	0	0	0	0	0	0	2	0
	- severe	0	0	0	0	0	0	1	0
penis and surrounding tissue									
abrasion/scab	- mild	1	0	1	0	0	0	0	0
extended	- no grade	0	2	0	0	0	0	0	0
thymus gland									
enlarged	- severe	1	0	0	0	0	0	0	0
focus/foci, red	- mild	1	0	0	0	0	0	0	0
not identified	- no grade	1	0	0	0	0	0	0	0
small		3	2	3	1	6	1	12	3
	- mild	1	1	0	1	2	0	0	1
	- moderate	1	1	1	0	0	1	1	0
	- severe	1	0	2	0	4	0	11	2
ureters									
distended with urine		2	0	8	0	7	1	13	0
	- mild	2	0	4	0	3	1	7	0
	- moderate	0	0	4	0	3	0	6	0
	- severe	0	0	0	0	1	0	0	0
not identified	- no grade	0	0	0	0	0	0	1	0
urinary bladder									
distended with urine		14	4	20	3	18	6	25	2
	- mild	3	1	3	1	1	3	1	2
	- moderate	6	3	9	2	6	1	14	0
	- severe	5	0	8	0	11	2	10	0
swollen/thickened	- moderate	0	0	1	0	0	0	0	0

DOS - Died or euthanized on study
SNC - Scheduled necropsy

Study Number 886-190
2-Year Oral Gavage Carcinogenicity Study of JNJ-28431754-ZAE in CD-1 Mice (Study TOX8799)
Summary of Macroscopic Observations - FEMALE
Terminal

Tissue Observation	Severity	0 mg/kg/day DOS	0 mg/kg/day SNC	10 mg/kg/day DOS	10 mg/kg/day SNC	30 mg/kg/day DOS	30 mg/kg/day SNC	100 mg/kg/day DOS	100 mg/kg/day SNC
Number of Animals Examined		40	25	40	25	46	18	31	33
kidneys									
cyst		0	0	2	2	3	1	1	2
	- minimal	0	0	0	1	1	1	0	0
	- mild	0	0	2	1	2	0	1	2
dilatation, pelvic		1	1	2	0	4	0	1	1
	- mild	0	1	2	0	2	0	1	1
	- moderate	1	0	0	0	2	0	0	0
discoloration, tan		0	0	1	0	1	1	1	0
	- mild	0	0	1	0	1	1	0	0
	- severe	0	0	0	0	0	0	1	0
ovaries									
cyst		30	19	27	17	36	14	23	23
	- minimal	0	1	1	0	0	0	0	0
	- mild	18	8	11	9	31	12	12	12
	- moderate	9	10	13	8	4	2	5	10
	- severe	3	0	2	0	1	0	6	1
enlarged		0	1	0	1	1	1	0	1
	- mild	0	1	0	0	0	1	0	0
	- moderate	0	0	0	1	1	0	0	0
	- severe	0	0	0	0	0	0	0	1
ureters									
discoloration, yellow	- moderate	0	0	0	0	1	0	0	0
distended with urine	- mild	1	0	1	1	2	0	0	0
enlarged	- severe	0	1	0	0	0	0	0	0
nodule	- present	0	0	1	0	0	0	0	0
not identified	- no grade	0	0	0	0	1	0	0	0
urinary bladder									
distended with urine		0	0	0	0	2	0	1	0
	- moderate	0	0	0	0	1	0	1	0
	- severe	0	0	0	0	1	0	0	0
swollen/thickened	- moderate	0	0	1	0	1	0	0	0

DOS - Died or euthanized on study
SNC - Scheduled necropsy

Histopathology

Histopathology evaluations were carried out for control and HD mice (100 mg/kg). For the LD and MD groups, only the tumors and targeted tissues were examined (kidney, bladder and penis tissues from males). The p values for common (> 1%) and rare tumors (<1%) in the positive trend analysis were 0.005 and 0.025, respectively. The survival adjusted test was conducted in accordance to Peto et al (prevalence/mortality adjusted method) by the sponsor. It should be noted that the FDA Office of Biostatistics uses the Poly-K method where mortality is not considered in tumor analysis. The statistical significance in the pair wise comparison for common and rare tumors was set at 0.01 and 0.05, respectively.

Peer Review

The histopath slides were reviewed by the original and a peer review pathologist. The study pathologists were [(b) (4)] (review pathologist) and [(b) (4)] (Study Pathologist). The pathologists concluded that the quality of the slides and tissue accountability were acceptable.

All tissues were examined microscopically from the following mice:
Control males (0 mg/kg/day)
        Sacrificed males (1003, 1015, and 1037)
        Dead or Euthanized males (1023, 1033, 1041, and 1054)
High Dose males (100 mg/kg/day)
        Sacrificed males (4019, 4054, 4056, 4061, and 4064)
        Dead or Euthanized males (4004, 4007, 4030, 4047, and 4065)
Control females (0 mg/kg/day)
        Sacrificed females (1536, 1545, 1549, and 1560)
        Dead or Euthanized females (1525, 1540, and 1565)
High Dose females (100 mg/kg/day)
        Sacrificed females (4503, 4517, 4536, and 4546)
        Dead or Euthanized females (4505, 4511, 4530, and 4563)
In addition, all tumors were examined from all mice; and from all males kidneys, urinary bladder and penis sections were examined.

Neoplastic Findings:

- There were no statistically significant drug-related increases in tumor incidence in male or female CD-1 mice.

Non Neoplastic

- Non neoplastic lesions were limited to urinary tract distension, kidney hydronephrosis, dilatation of ureters and bladder in males.
- High dose males had higher incidence of dilatation of kidney (cysts and pelvic), ureters and urinary balder. It is not clear if the incidence of renal dilatory findings were due to obstruction of urethra which is common in male CD-1 mice or due to increase urinary output due to drug pharmacology. Since there was evidence of urinary distension in control male mice as well, the renal system findings in males may have been combination of both. Urinary obstruction in mouse referred to as mouse urological syndrome can occur spontaneously. Since there were no drug-related histological findings of calculi and tumors, the clinical relevance of the renal findings therefore is relatively limited.

2-Year Oral Gavage Carcinogenicity Study of JNJ-28431754-ZAE in CD-1 Mice (Study TOX8799)   Test Article: JNJ-28431754-ZAE

Daily Dose (mg/kg/day)	0 (Control)		10		30		100	
Gender	M: 65	F: 65	M: 65	F: 65	M: 66	F: 64	M: 66	F: 64
Histopathology - Neoplastic Lesions	–	–	–	–	–	–	–	–
Histopathology								
Number Evaluated	65	65	65	65	66	64	66	64
Kidneys								
hydronephrosis, bilateral	8	1	17	5	19	8	24	6
hydronephrosis, unilateral	1	2	3	2	3	1	2	5
Ureters								
dilatation	9	2	21	1	18	3	26	1
Urinary Bladder								
dilatation	18	0	24	0	25	2	29	0

Most Notable Histopath Findings (target tissues) in Mice (for Reference).

Summary of Microscopic Observations - MALE									
		0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
Tissue Observation	Severity	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		34	31	39	26	37	29	46	20
eyes		(34)	(31)	(39)	(26)	(37)	(29)	(46)	(20)
cataract	- mild	0	0	0	1	0	0	0	0
degeneration/atrophy, retina, bilateral	- mild	0	0	0	0	0	1	0	0
degeneration/atrophy, retina, unilateral		1	1	1	1	1	1	3	2
	- minimal	0	0	0	0	0	1	0	1
	- mild	1	1	0	1	1	0	3	1
	- moderate	0	0	1	0	0	0	0	0
joint, tibiofemoral		(34)	(31)	(39)	(26)	(37)	(29)	(46)	(20)
inflammation, chronic	- minimal	0	0	1	0	0	0	0	0
inflammation, granulomatous	- mild	0	0	0	0	0	0	1	0
inflammation, subacute/chronic	- minimal	0	0	0	3	0	0	0	0
leukemia, granulocytic, malignant, multicentric		0	0	0	0	1	0	0	0
metaplasia, cartilaginous/osseous		4	13	4	8	9	17	9	11
	- minimal	0	3	0	0	0	3	0	3
	- mild	4	9	3	4	7	8	6	6
	- moderate	0	1	1	4	2	5	3	2
	- severe	0	0	0	0	0	1	0	0
within normal limits		30	18	34	17	27	12	36	9
kidneys		(34)	(31)	(39)	(26)	(37)	(29)	(46)	(20)
adenoma, tubular cell, benign, primary		0	0	0	0	0	1	0	0
amyloid	- moderate	0	0	1	0	2	0	0	0
anomaly, developmental	- severe	0	0	0	1	0	0	0	0
bacterial colonies	- mild	0	0	1	0	0	0	0	0
cyst		3	12	5	12	5	13	2	13
	- minimal	0	6	1	6	1	3	0	4
	- mild	3	6	4	4	4	10	1	9
	- moderate	0	0	0	2	0	0	1	0
hemorrhage	- severe	0	0	0	0	0	0	1	0
hydronephrosis, bilateral		8	0	16	1	17	2	24	0
	- minimal	1	0	6	1	2	2	3	0
	- mild	6	0	7	0	6	0	10	0
	- moderate	1	0	1	0	6	0	11	0
	- severe	0	0	2	0	3	0	0	0
hydronephrosis, unilateral		1	0	2	1	0	3	2	0
	- minimal	1	0	2	1	0	1	1	0
	- mild	0	0	0	0	0	2	1	0
hyperplasia, tubular		0	0	1	0	0	0	1	0
	- minimal	0	0	1	0	0	0	0	0
	- mild	0	0	0	0	0	0	1	0
metaplasia, osseous	- minimal	1	0	0	1	0	0	0	0
mineralization	- minimal	9	16	4	10	2	7	6	3
nephropathy, chronic progressive		30	31	33	26	28	27	39	20
	- minimal	17	14	15	8	17	14	26	10
	- mild	6	17	17	18	7	13	9	10
	- moderate	3	0	1	0	4	0	3	0
	- severe	4	0	0	0	0	0	1	0
polyarteritis		2	3	2	4	0	1	0	2
	- minimal	1	0	0	4	0	1	0	0
	- mild	1	3	2	0	0	0	0	2
pyelonephritis, bilateral	- severe	0	0	1	0	0	0	0	0
pyelonephritis, unilateral	- mild	0	0	1	0	0	0	2	0
within normal limits		2	0	2	0	2	1	3	0

Summary of Microscopic Observations - MALE

Tissue Observation	Severity	0 mg/kg/day DOS	0 mg/kg/day SNC	10 mg/kg/day DOS	10 mg/kg/day SNC	30 mg/kg/day DOS	30 mg/kg/day SNC	100 mg/kg/day DOS	100 mg/kg/day SNC
Number of Animals Examined		34	31	39	26	37	29	46	20
penis and surrounding tissue		(34)	(31)	(39)	(26)	(37)	(29)	(46)	(20)
bacterial colonies		7	0	4	0	5	0	13	1
	- minimal	0	0	0	0	1	0	4	1
	- mild	4	0	2	0	4	0	8	0
	- moderate	3	0	1	0	0	0	1	0
	- severe	0	0	1	0	0	0	0	0
erosion/ulcer		10	2	9	1	11	0	11	0
	- minimal	1	0	1	0	1	0	1	0
	- mild	3	2	3	1	6	0	4	0
	- moderate	3	0	3	0	4	0	5	0
	- severe	3	0	2	0	0	0	1	0
hemangioma, benign, multicentric		0	0	0	0	0	1	0	0
inflammation, chronic-active		15	3	14	4	18	1	23	2
	- minimal	4	1	3	3	7	1	10	2
	- mild	4	2	4	1	5	0	6	0
	- moderate	4	0	6	0	6	0	7	0
	- severe	3	0	1	0	0	0	0	0
testes		(34)	(31)	(39)	(26)	(37)	(29)	(46)	(20)
adenoma, interstitial cell, benign, primary		0	0	2	2	0	0	1	1
degeneration/atrophy, seminiferous tubules, bilateral		6	3	4	5	7	3	4	5
	- minimal	2	2	2	4	3	1	3	1
	- mild	3	0	2	0	2	2	1	2
	- moderate	1	1	0	0	2	0	0	2
	- severe	0	0	0	1	0	0	0	0
ureters		(34)	(31)	(39)	(26)	(36)	(29)	(46)	(20)
dilatation		8	1	20	1	16	2	25	1
	- minimal	0	0	0	0	0	1	1	0
	- mild	5	1	10	1	5	1	15	1
	- moderate	3	0	9	0	5	0	5	0
	- severe	0	0	1	0	6	0	4	0
lymphoma, malignant, multicentric		1	0	0	0	0	0	0	0
within normal limits		25	30	19	25	20	27	21	19
urinary bladder		(34)	(31)	(39)	(26)	(37)	(29)	(46)	(20)
dilatation		14	4	21	3	20	5	27	2
	- mild	2	2	1	0	0	1	4	1
	- moderate	7	2	13	2	11	3	11	1
	- severe	5	0	7	1	9	1	12	0
edema	- minimal	0	0	1	0	0	0	0	0
hemorrhage	- minimal	2	0	4	0	0	1	0	0
hyperplasia	- mild	0	0	0	1	1	0	0	0

DOS - Died or euthanized on study   SNC - Scheduled necropsy   ( ) - Number observed

Summary of Microscopic Observations - FEMALE

Tissue Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		40	25	40	25	46	18	31	33
eyes		(40)	(25)	(40)	(25)	(46)	(18)	(31)	(33)
cataract	- moderate	0	0	0	0	0	0	0	1
degeneration/atrophy, retina, bilateral		1	0	2	3	1	4	4	3
	- minimal	0	0	0	0	0	2	0	0
	- mild	1	0	1	2	1	2	4	2
	- moderate	0	0	1	1	0	0	0	1
degeneration/atrophy, retina, unilateral		2	4	4	4	2	2	3	2
	- minimal	0	1	2	1	0	1	0	0
	- mild	1	3	2	3	1	1	3	1
	- moderate	1	0	0	0	0	0	0	1
	- severe	0	0	0	0	1	0	0	0
keratopathy		1	0	0	1	0	0	1	2
	- minimal	1	0	0	0	0	0	0	2
	- mild	0	0	0	1	0	0	1	0
kidneys		(40)	(25)	(40)	(25)	(46)	(18)	(31)	(33)
infiltration, mononuclear cell		0	2	0	0	1	0	1	0
	- minimal	0	1	0	0	1	0	1	0
	- mild	0	1	0	0	0	0	0	0
leukemia, granulocytic, malignant, multicentric		1	0	0	0	0	0	0	0
lymphoma, malignant, multicentric		3	5	10	2	8	2	4	3
metaplasia, osseous	- minimal	0	0	2	0	0	0	0	0
mineralization	- minimal	5	1	1	0	0	1	0	1
nephropathy, chronic progressive		30	23	27	22	29	15	22	27
	- minimal	12	18	15	15	12	10	11	19
	- mild	13	4	7	7	8	3	7	7
	- moderate	2	1	2	0	5	2	3	1
	- severe	3	0	3	0	4	0	1	0
cyst		0	0	2	2	5	1	1	3
	- minimal	0	0	2	0	0	1	0	2
	- mild	0	0	0	2	5	0	1	1
hydronephrosis, bilateral		0	1	5	0	7	1	2	4
	- minimal	0	0	3	0	2	1	0	3
	- mild	0	1	2	0	3	0	2	1
	- moderate	0	0	0	0	2	0	0	0
hydronephrosis, unilateral		2	0	1	1	0	1	1	4
	- minimal	1	0	1	1	0	1	1	2
	- mild	1	0	0	0	0	0	0	1
	- moderate	0	0	0	0	0	0	0	1

Summary of Microscopic Observations - FEMALE

Tissue Observation	Severity	0 mg/kg/day		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day	
		DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Number of Animals Examined		40	25	40	25	46	18	31	33
mammary gland		(40)	(25)	(40)	(25)	(46)	(18)	(31)	(33)
adenocarcinoma, malignant, primary		0	0	0	0	0	0	0	1
adenoma, benign, primary		0	0	0	1	0	0	0	0
amyloid	- mild	0	0	0	0	1	0	0	0
dilatation, gland/lumen		0	2	3	3	5	2	0	0
	- minimal	0	0	0	1	2	0	0	0
	- mild	0	2	3	1	3	2	0	0
	- moderate	0	0	0	1	0	0	0	0
hyperplasia, lobular		0	2	0	3	1	2	1	1
	- minimal	0	2	0	3	1	1	0	1
	- mild	0	0	0	0	0	1	0	0
	- moderate	0	0	0	0	0	0	1	0
within normal limits		35	20	31	18	34	15	28	31
ureters		(39)	(25)	(39)	(25)	(45)	(18)	(31)	(33)
dilatation		2	0	1	0	3	0	0	1
	- moderate	2	0	0	0	1	0	0	1
	- severe	0	0	1	0	2	0	0	0
within normal limits		37	25	38	24	42	18	31	32
urinary bladder		(40)	(25)	(40)	(25)	(44)	(18)	(31)	(33)
adenocarcinoma, malignant, secondary		0	0	1	0	0	0	0	0
dilatation	- severe	0	0	0	0	2	0	0	0
inflammation, subacute/chronic	- mild	0	0	0	1	1	0	0	0
lymphoma, malignant, multicentric		3	2	8	2	4	0	3	2
mesenchymal tumor, benign, primary		0	0	1	0	0	0	0	1

DOS - Died or euthanized on study   SNC - Scheduled necropsy   ( ) - Number observed

2-Year Oral Gavage Carcinogenicity Study of JNJ-28431754-ZAE in CD-1 Mice (Study TOX8799)   Test Article: JNJ-28431754-ZAE

Daily Dose (mg/kg/day)	0 (Control)		10		30		100	
Gender	M: 65	F: 65	M: 65	F: 65	M: 66	F: 64	M: 66	F: 64
No. of Animals								
Died or Sacrificed Moribund	34	40	39	40	37	46[b]	46[c]	31
Terminal Sacrifice	31	25	26	25	29	18	20	33
Survival (%)	47.7	38.5	40.0	38.5	43.9	28.1	30.3	51.6
Noteworthy Findings								
Body Weight	–	–	–	–	–	–	–	–
Food Consumption (g/animal/day)	–	–	–	↑	↑	↑	↑	↑
Clinical Observations[a]								
abdomen distended	70/13	124/15	–	–	–	–	162/26	–
Mass Findings	–	–	–	–	–	–	–	–
Ophthalmoscopy	–	–	–	–	–	–	–	–
Clinical Chemistry	–	–	–	–	–	–	–	–
Organ Weights	–	–	–	–	–	–	–	–
Gross Pathology [d]								
Number Evaluated	65	65	65	65	66	64	66	64
Ureters								
distended with urine	2	1	8	2	8	2	13	0
Urinary Bladder								
distended with urine	18	0	23	0	24	2	27	1
Histopathology - Neoplastic Lesions	–	–	–	–	–	–	–	–
Histopathology[d]								
Number Evaluated	65	65	65	65	66	64	66	64
Kidneys								
hydronephrosis, bilateral	8	1	17	5	19	8	24	6
hydronephrosis, unilateral	1	2	3	2	3	1	2	5
Ureters								
dilatation	9	2	21	1	18	3	26	1
Urinary Bladder								
dilatation	18	0	24	0	25	2	29	0

[a] Weeks 1 to 104. Reported as Number of Times Observed/Total Number of Animals Affected.   – No Noteworthy Findings
[b] Last animal was euthanized *in extremis* during Week 103 (animal number 3528).   ↑ increase
[c] Last animal was euthanized *in extremis* during Week 101 (animal number 4026).
[d] Reported as Number Observed

Toxicokinetics

- Canagliflozin was rapidly absorbed after single dose with Tmax ranging from 1 to 2 hrs with $t_{1/2}$ of 3 to 6.17 hrs.
- The increase in systemic exposure was less than dose-proportional. Exposure increased with repeated dosing at LD and MD levels. Exposure tended to decrease with multiple dosing at 100 mg/kg (HD).
- Canagliflozin exposure tended to be higher in female mice than in males.

Metabolites
- Canagliflozin was rapidly metabolized to M7 (JNJ-41488525) and M5 (JNJ-41980874) The Tmax of 1 to 2 hrs except in females where M7 and M5 Tmax was 8 hrs at 10 mg/kg. M7 exposure in males was dose proportional at 10 and 30 mg/kg but greater than dose-proportional at 100 mg/kg.
- Exposure to JNJ-28431754 was much higher than to either metabolite. The rank order of exposure in mouse was canagliflozin >> M7 > M5.

Plasma canagliflozin was measured by ███████████████████ (b) (4) with a lower limit of quantification of 50 ng/ml. Following the completion of the analysis for canagliflozin, plasma samples were sent to JNJ Pharmaceutical Research & Development in Raritan, NJ and then to ██████████████ (b) (4) for analysis of plasma metabolites (M5 and

M7). The collected raw data was electronically sent to NJ site for PK analysis with WinNonlin PK software (Version 5.2.1, Pharsight).

TK patameters after single or 182 daily oral doses of canagliflozin in male and female mice

Day	Dose (mg/kg)	Gender	C_{max} (ng/mL)	t_{max} (h)	AUC $_{(0-\infty)}$[a] (ng·h/mL)	$t_{1/2}$ (h)	CL/F (mL/h/kg)
0	10	Female	1340	2.00	12800	4.97	784
		Male	1180	1.00	8170	4.78	1220
	30	Female	12300	1.00	93200	4.37	322
		Male	8840	1.00	62000	3.09	484
	100	Female	47600	1.00	344000	6.17	290
		Male	57200	1.00	324000	3.75	309
181	10	Female	3720	2.00	36400	8.13	237
		Male	2380	2.00	11300	2.91	726
	30	Female	13100	1.00	102000	4.93	284
		Male	10300	1.00	47700	3.47	623
	100	Female	37300	2.00	353000	6.31	261
		Male	27600	1.00	194000	4.74	499

[a] AUC $_{(0-24 h)}$ on Day 181.

Analyte	Dose (mg/kg)	Gender	C_{max} (ng/mL)	t_{max} (h)	AUC $_{(0-24 h)}$ (ng·h/mL)	$t_{1/2}$ (h)
JNJ-41488525 (M7)	10	Female	581	8.00	1950	NC
		Male	170	1.00	695	27.2
	30	Female	1740	2.00	6250	2.58
		Male	400	1.00	1590	3.93
	100	Female	1570	2.00	11100	6.83
		Male	2160	1.00	9530	3.14
JNJ-41980874 (M5)	10	Female	21.3	8.00	87.9	NC
		Male	NC	NC	NC	NC
	30	Female	37.4	1.00	166	5.33
		Male	15.8	1.00	71.2	7.73
	100	Female	121	1.00	909	9.66
		Male	67.7	1.00	261	4.43

NC: Not Calculated due to insufficient data, or late t_{max}.

Stability and Homogeneity:

Drug levels were sampled at several time points. The solution concentrations were within -±10% of the target concentrations.

Impurities seen in the batches used in early toxicology studies were removed from the latter batches. Any remaining impurity was below the qualification threshold of ▮ (b) (4)

Study title: 24-Month Repeated Dose Oral Carcinogenicity Study of JNJ-28431754-ZAE in the Rat.

Study no.:	TOX8986
Study report location:	Janssen Research & Development, Division of Janssen Pharmaceutica N.V. B-2340 Beerse, Belgium
Conducting laboratory and location:	Drug Safety Sciences, Beerse site Turnhoutseweg 30, B-2340 Beerse, Belgium
Date of study initiation:	July 16, 2008 (last day of dosing, July 17, 2010)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ZR600348PFA111, purity of 98%
CAC concurrence:	Yes

Key Study Findings

Doses of 10, 30 and 100 mg/kg canagliflozin to rats (n=65/sex) had minimal impact on survival. Slightly higher survival may have been related to treatment related decrease in BW. Slightly higher survival in the treated rats had no apparent effect on incidence of tumors. Exposure margins achieved were 1-2x, 5-7x, and 12-21x, respectively, in males and females.

Consistent with glucosuria/polyuria, body weight decreased and food intake increased with dose. Also, serum glucose and calcium decreased and triglycerides/BUN increased in dosed groups.

Extensive mineralization was observed in kidneys (cortex, medulla, papilla, pelvis and vascular), aorta, eyes, stomach, heart vessels, lungs, sciatic nerve vessels, mesenteric lymph and tongue vessels in both sexes.

Hyperostosis of sternum and stifle bone was noted at ≥ 30 mg/kg in both male and female rats.

Neoplastic Findings

Renal Tubular Tumors
Males: Renal tubular adenoma + carcinoma: 0, 0, 2, 12
Females: Renal tubular adenoma + carcinoma: 0, 0, 0, 8
Renal tubular tumors (adenoma & carcinoma combined) increased numerically in MD males and statistically in HD male and female rats. A PWG concluded that the two renal tumors in the MD and one in HD males were spontaneous and not drug-related based on amphophilic histological features.

Testicular Tumors
Testicular Leydig cell tumors increased at all doses: 1, 8, 20, 24
Terminal serum testosterone was significantly lower (0.5x controls) and LH levels were undetectable. The sponsor attributes these tumors to elevated LH in male rats.

<u>Adrenal Pheochromocytoma Tumors</u>
Males: 4, 4, 7, 28
Females: 2, 1, 3, 7
Adrenal medullary neoplasia (pheochromocytoma) increased at 100 mg/kg statistically in males and numerically in females.

Liver
There was a statistically significant positive trend for liver tumors (adenoma and carcinoma combined) with canagliflozin in males. The incidence in HD males fell above the site historical control data (9/65 vs. 7/65) but within overall historical range (different sites). The significance of the positive trend is not clear.

Bladder
Numerically higher incidence of bladder tumors in HD males (n=2) and females (n=3) which reached statistical significance in trend analysis. There was also a bladder tumor in 1 LD female.

Adequacy of Carcinogenicity Study: The rat study protocol was reviewed and concurred with
        eCAC. The choice of animal model, carcinogenicity doses and tumor data analysis (Peto's
        method) is acceptable. There were sufficent number of rats exposed to the treatmet at
        the end of the study to performe appropriate statistical analysis.

Appropriateness of Test Models: SD rat is the standard rat strain that had similar
        pharmacological response to canagliflozin as humans.

Evaluation of Tumor Findings:

Renal Tumors
        Renal tumors in the HD male and female rats were considered treatment related. The
        two renal tumors in the MD males were considered spontaneous and unrelated to the
        treatment by the PWG.   The high fructose diet study provided sufficient evidence for
        carbohydrate malabsorption as the mode of action. The potential GI effect of
        canagliflozin is unlikely to be tolerated by humans thus likely to be self-limiting. Since
        renal tumors were increased at 12 to 22x the clinical dose, the safety margin for renal
        tumor was 5 to 7x the MRHD.

Adrenal Cell Tumors
        Adrenal tumors were considered treatment related. Similar to renal tumors,
        carbohydrate malabsorption caused by canagliflozin induced inhibition of intestinal
        SGLT1 was shown to be the mode of action. The safety margin for adrenal tumors was 5
        to 7x the MRHD.

Leydig Cell Tumors
        Leydig cell tumors were considered treatment related. They occurred at all doses with
        no safety margin. Since rat Leydig cells are known to have high density of LH receptors

than humans and there was significant increase in LH in rats but not in humans, Leydig cell tumors were accepted as clinically irrelevant.

Methods

Doses:	10, 30 and 100 mg/kg/day
Frequency of dosing:	Daily, seven days a week
Dose volume:	5 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% Methocel (hydroxypropyl methylcellulose)
Basis of dose selection:	3- and 6-month rat studies
Species/Strain:	Sprague Dawley (SD) rats from [redacted] (b) (4)
Number/Sex/Group:	65 per sex/group
Age:	6 weeks at the start of dosing (day 0)
Animal housing:	Polysulphone cages with wire mesh top (5 rats/sex per cage)
Paradigm for dietary restriction:	Add lib access to food and water. Food was restricted before bleeding.
Dual control employed:	Single vehicle control
Interim sacrifice:	Blood samples were collected at WK 52 in fed state
Satellite groups:	Toxicokinetic (3 male and 3 females/group)
Deviation from study protocol:	Blood and urine samples were collected at interim 12-months (M: Day 363, F: Day 364) from the first 20 fasted rats/sex/group and all surviving rats at 24-months (Day 729 through737). Additional nonstandard parameters measured in the study were hormonal analysis (LH and Testosterone) and urine NAG analysis at 1 and 2 years. Additional examination included ophthalmoscopy due observation of borderline non to mild eye irritant properties of canagliflozin in the in vitro bovine corneal opacity-permeability assay.

	Identity number (computer number) of rats	
Dosage groups (colour code)	Males	Females

V: Vehicle (blue)
   Dosage: 0 mg eq./kg/day
   Concentration: 0 mg eq./ml
   Volume: 5 ml/kg/day
	1 - 65	401 - 465

L : Low (red)
   Dosage: 10 mg eq./kg/day
   Concentration: 2 mg eq./ml
	101 - 165	501 - 565

M : Medium (yellow)
   Dosage: 30 mg eq./kg/day
   Concentration: 6 mg eq./ml
	201 - 265	601 - 665

H : High (green)
   Dosage: 100 mg eq./kg/day
   Concentration: 20 mg eq./ml
	301 - 365	701 - 765

Observations and Results

Mortality
- Canagliflozin doses up to 100 mg/kg had no apparent effect on mortality in rats.
- Slightly higher survival rate in the treated rats was attributed to lower body weight.
- The FDA statistician's analysis generally agreed with the sponsor's analysis. The FDA statistician attributed small differences in the mortality rate to the different termination times.

Kaplan-Meier plot of Survival
Males

Kaplan-Meier plot of Survival
Females

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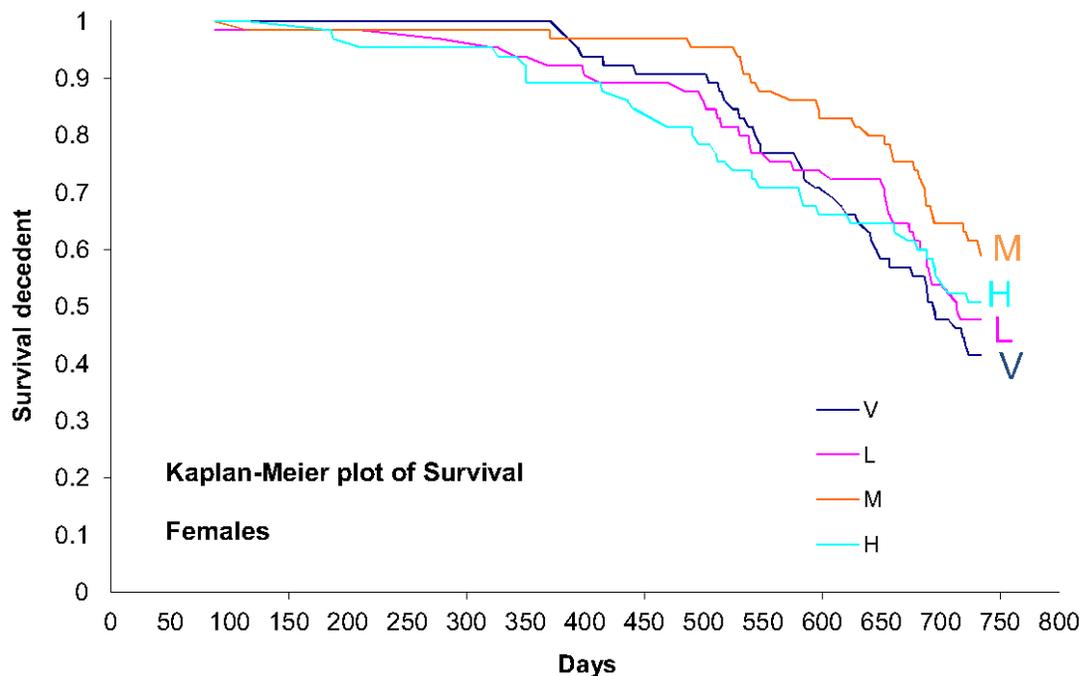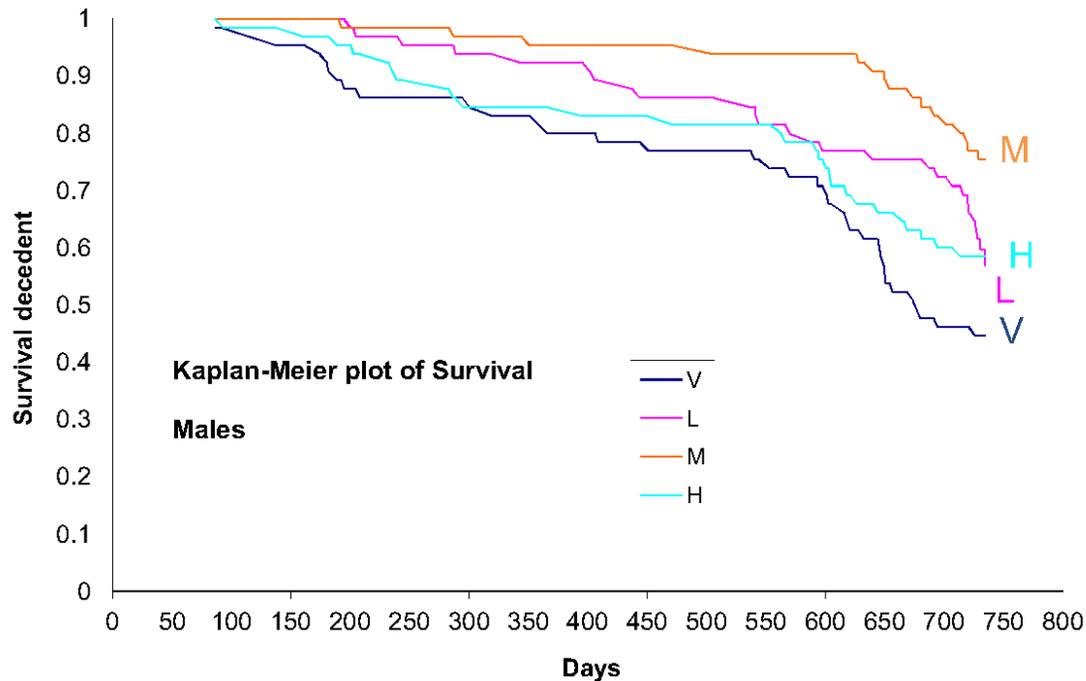
MORTALITY   Incidence per dosage group (cumulative)

	Males Dosage Group (mg eq./ kg)											
	Vehicle			Low:10			Med.:30			High:100		
Week	X / N	S	%	X / N	S	%	X / N	S	%	X / N	S	%
106	36/65		55.4	27/65		41.5	16/65	***	24.6	27/65		41.5

	Females Dosage Group (mg eq./ kg)											
106	38/65		58.5	34/65		52.3	26/65		40.0	32/65		49.2

Significance level computed with Fisher Exact probability test (two-tailed): * p < .05 ** p < .01 *** p < .001
(Significance computed versus the Vehicle dosage group)
X: Number of animals dead or sacrificed at stated period   N: Total number of animals   S: Significance

Clinical Signs

- Wet bedding was frequently observed in canagliflozin treated rats. They were seen at all doses in both sexes (males>females), evident after the first dose. The duration and severity increased in a dose-dependent manner.
- Sustained high sensitivity of rats to canagliflozin was marked by severe polyuria produced by excess glucose in urine. Rats appeared to be the most sensitive rodent model.
- Dehydration and emaciated appearance was noted in some (< 10%) HD male and female rats, likely due to excessive urination that was not compensated by increased water intake.

Common Clinical Signs in Male and Female SD rats in the 2-Year Study:

Males				
Dosage Group (mg eq./ kg):	Vehicle X / N	Low:10 X / N	Med.:30 X / N	High:100 X / N
Poor condition	5 / 65	6 / 65	3 / 65	9 / 65
Emaciated Animal	0 / 65	3 / 65	3 / 65	5 / 65
Dyspnea	2 / 65	4 / 65	7 / 65	7 / 65
Feces, soft	0 / 65	4 / 65	4 / 65	25 / 65 ***
Trimmed/cut abnormal incisors	0 / 65	1 / 65	0 / 65	0 / 65
Abdominal distension	3 / 65	1 / 65	3 / 65	5 / 65
Bradypnea	0 / 65	0 / 65	0 / 65	2 / 65
Waste of food	3 / 65	0 / 65	0 / 65	0 / 65
Malformed incisors	1 / 65	0 / 65	0 / 65	1 / 65
Wet bedding	0 / 65	40 / 65 ***	60 / 65 ***	65 / 65 ***

Significance level computed with Fisher Exact probability test (two-tailed): * p < .05   ** p < .01   *** p < .001
(Significance computed versus the Vehicle dosage group)

Females				
Dosage Group (mg eq./ kg):	Vehicle X / N	Low:10 X / N	Med.:30 X / N	High:100 X / N
Poor condition	4 / 65	3 / 65	2 / 65	9 / 65
Emaciated Animal	1 / 65	2 / 65	3 / 65	7 / 65
Waste of food	24 / 65	38 / 65 *	39 / 65 *	40 / 65 **
Decreased general activity	15 / 65	18 / 65	16 / 65	23 / 65
Wet bedding	0 / 65	10 / 65 **	25 / 65 ***	25 / 65 ***
Urogenital discharge, red/brown	5 / 65	5 / 65	0 / 65	8 / 65

Significance level computed with Fisher Exact probability test (two-tailed): * p < .05 ** p < .01 *** p < .001
(Significance computed versus the Vehicle dosage group)
X: Number of positive animals N: Total number of animals

Body Weight

- Significant decrease in body weight and body weight gain was observed at all dose levels of canagliflozin at nearly every time point throughout the study in both male and female rats. The decrease in BW was attributed to excessive sustained renal glucose excretion in rats. Increased food intake was unable to compensate for weight loss due to loss of glucose.

- Males -

- Females -
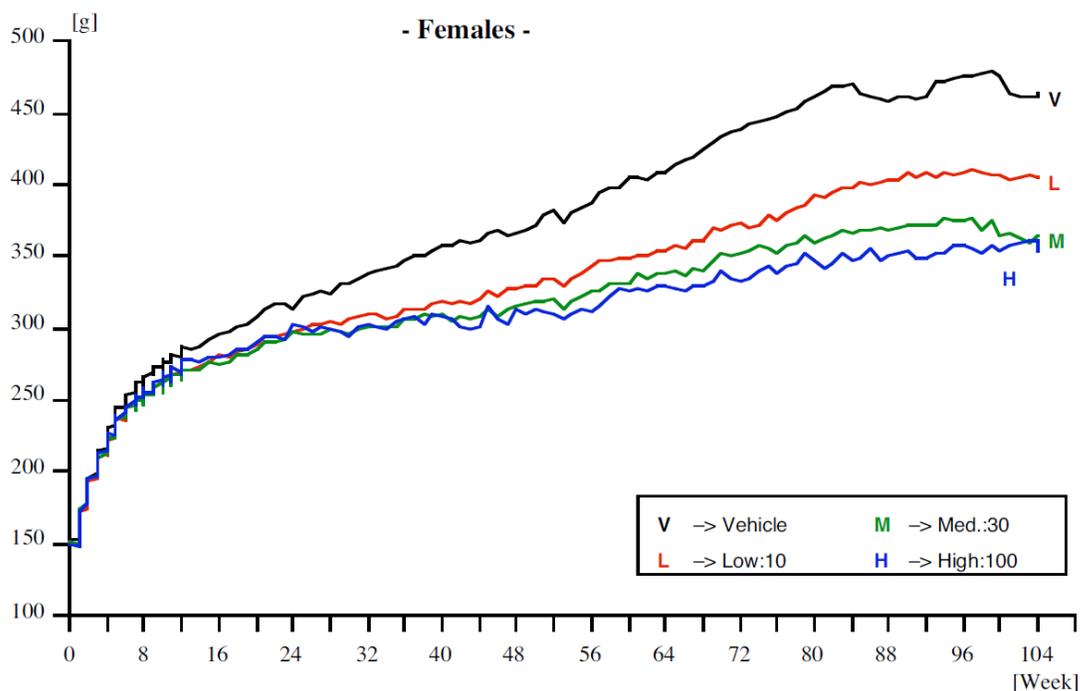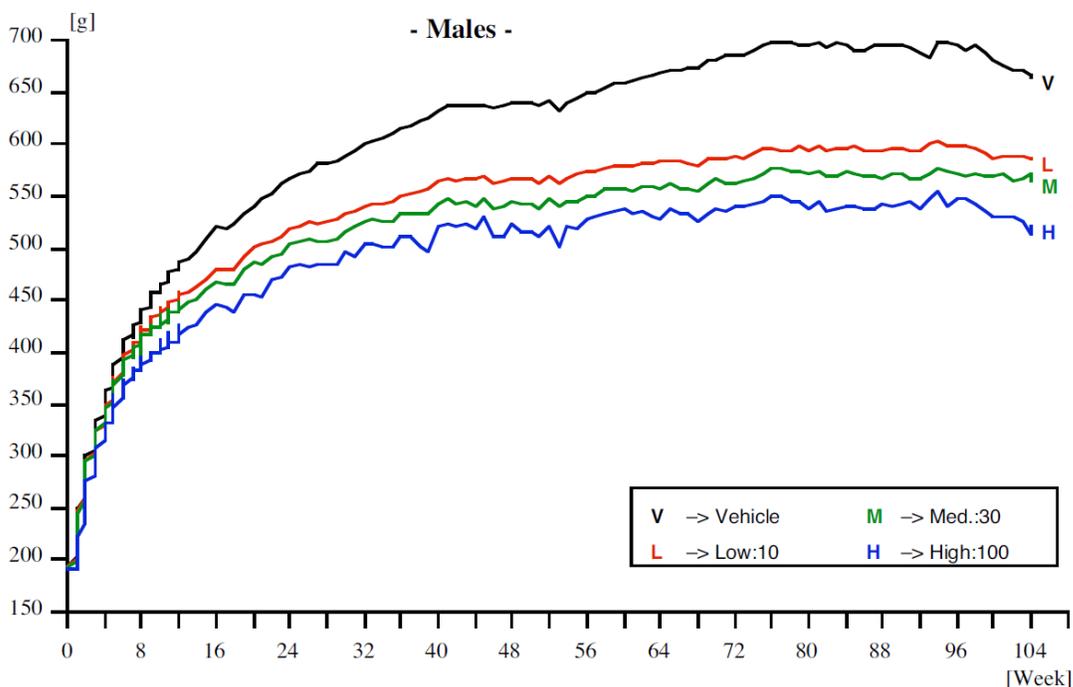
Canagliflozin Related Changes in BW, BW Gain and Food Intake at WK 106 in Rat

Daily Dose (mg eq./kg)		0(Vehicle)		10		30		100	
No. of Animals	M:65	F:65	M:65	F:65	M:65	F:65	M:65	F:65	
Noteworthy Findings									
Died or Sacrificed Moribund	36 / 65	38 / 65	27 / 65	34 / 65	16 / 65***	26 / 65	27 / 65	32 / 65	
Body Weight[a, b]	663	463	0.885 ***	0.875 **	0.852 ***	0.788 ***	0.787 ***	0.767 ***	
Body Weight Gain[a, b]	469	311	0.840 ***	0.817 **	0.789 ***	0.688 ***	0.704 ***	0.666 ***	
Food Consumption[a, b]	189	141	1.238	1.248	1.307	1.255	1.418	1.390	

[a] At end of dosing period.
[b] For vehicle controls, group means are shown. For treated groups, multiples of control/baseline are shown.

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Feed Consumption

- Dose-related increase in food intake was noted in LD, MD and HD males (1.34x, 1.34x and 1.48x) and females (1.49x, 1.34 and 1.59x) relative to the corresponding controls. The increased food intake was in response to caloric loss caused by excessive urinary glucose excretion.

Ophthalmoscopy

- Evaluation of the eye/fundus was not possible in all animals due to spontaneous age associated corneal/lens changes. However, the degree of reflectivity tended to be greater in HD females than control females.
- There were no drug related ocular findings in male rats at doses up to 100 mg/kg. The relevance and validity of the eye findings is not certain (see histopath table).

Hematology

- Total WBC at 1 year was reduced in males at all doses (0.77x) and HD females (0.79x) compared to control rats. The decrease in WBC persisted to study end, especially in the HD rats (up to 0.72x the control).
- RBC were slightly reduced in MD and HD females at 1 year but were comparable to control at the end of the 2-year study.

Experiment TOX8986
24-Month Repeated Dose Oral Carcinogenicity Study in the Rat
JNJ-28431754-ZAE - OR/GAV - RAT

HAEMATOLOGY
Mean values per dosage group
Periodical, recorded in week 52

| | | Dosage Groups (mg eq./kg) | | | | | | | |
| | | MALES | | | | FEMALES | | | |
Parameter	Unit	Vehicle	Low:10	Med.:30	High:100	Vehicle	Low:10	Med.:30	High:100
Act. Part. Thromb. Time	sec	18.1 (0.3)	17.9 (0.2)	17.5 (0.2)	17.0 (0.2) **	18.2 (0.2)	17.7 (0.3)	17.4 (0.2) **	16.2 (0.3) ***
Prothrombin Time	sec	13.3 (0.2)	14.0 (0.2) *	14.4 (0.2) ***	14.4 (0.2) ***	11.1 (0.1)	11.8 (0.1) ***	11.8 (0.1) ***	11.6 (0.2)
White blood cells	10^3/µl	9.9 (0.4)	9.2 (1.2) *	7.6 (0.5) **	7.7 (0.5) **	6.5 (0.3)	6.4 (0.3)	6.7 (0.4)	5.1 (0.3) ***
Red blood cells	10^6/µl	9.02 (0.09)	8.85 (0.12)	9.06 (0.10)	8.94 (0.13)	7.72 (0.08)	7.71 (0.10)	7.44 (0.09) *	7.05 (0.13) ***
Haemoglobin	g/dl	15.8 (0.1)	15.1 (0.2) *	15.4 (0.2)	15.4 (0.3)	14.5 (0.1)	14.5 (0.2)	13.9 (0.2) *	13.4 (0.2) ***
Haematocrit	%	47.0 (0.4)	45.0 (0.5) *	46.2 (0.6)	46.8 (0.9)	42.7 (0.4)	42.6 (0.5)	41.1 (0.6) *	39.6 (0.6) ***
Reticulocytes	%	1.7 (0.0)	1.9 (0.1)	2.0 (0.1) *	1.9 (0.1) *	2.0 (0.1)	1.6 (0.1)	1.8 (0.1) *	2.4 (0.3)
Reticulocytes	10^3/µl	154.7 (4.4)	163.3 (8.3)	180.4 (9.0) *	170.3 (6.2)	153.0 (8.1)	127.6 (6.4) *	134.9 (6.6)	165.8 (15.7)
Lymphocytes	10^3/µl	7.24 (0.31)	6.24 (0.61) **	4.49 (0.37) ***	4.53 (0.23) ***	4.76 (0.25)	4.80 (0.28)	4.51 (0.24)	3.23 (0.19) ***
Eosinophils	10^3/µl	0.14 (0.01)	0.12 (0.03) *	0.19 (0.09) *	0.06 (0.01) ***	0.14 (0.01)	0.10 (0.01)	0.11 (0.01)	0.07 (0.01) ***
Basophils	10^3/µl	0.10 (0.01)	0.06 (0.01) **	0.06 (0.01) **	0.06 (0.00) ***	0.05 (0.01)	0.05 (0.00)	0.05 (0.01)	0.03 (0.00) ***

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

Mean values per dosage group   Terminal, recorded in week 105 till 106

Parameter	Unit	Dosage Groups (mg eq./ kg) MALES				FEMALES			
		Vehicle	Low:10	Med.:30	High:100	Vehicle	Low:10	Med.:30	High:100
Act. Part. Thromb. Time	sec	15.3 (0.3)	15.4 (0.2)	15.5 (0.2)	15.1 (0.2)	15.6 (0.2)	15.7 (0.2)	15.3 (0.2)	14.5 (0.4) *
White blood cells	10^3/µl	9.6 (0.8)	7.9 (0.5) *	9.5 (3.0) ***	9.5 (1.9) **	7.8 (0.7)	6.8 (0.4)	5.6 (0.3) **	5.6 (0.3) **
Lymphocytes	10^3/µl	5.50 (0.23)	4.44 (0.26) **	4.05 (0.55) ***	3.53 (0.41) ***	4.17 (0.24)	3.52 (0.21) *	2.94 (0.15) ***	2.50 (0.11) ***
Monocytes	10^3/µl	0.29 (0.02)	0.31 (0.03)	0.32 (0.07) *	0.35 (0.05)	0.33 (0.06)	0.24 (0.02)	0.22 (0.02) *	0.25 (0.02)
Eosinophils	10^3/µl	0.11 (0.01)	0.08 (0.01) **	0.07 (0.01) ***	0.07 (0.02) ***	0.12 (0.01)	0.07 (0.01) **	0.07 (0.01) **	0.06 (0.01) ***
Basophils	10^3/µl	0.06 (0.01)	0.06 (0.01)	0.05 (0.00) ***	0.06 (0.01) *	0.04 (0.01)	0.04 (0.00)	0.03 (0.00)	0.03 (0.00) *

Clinical Chemistry

- Canagliflozin decreased interim (up to 0.52x control) and final (up to 0.48x the control) serum glucose levels in both sexes.
- Serum calcium decreased slightly (up to 0.94x) at WK 52 and WK 106.
- Serum triglycerides increased up to 4x and BUN up to 2.5x in both sexes and at all doses of canagliflozin.
- ALP (up to 2x) and ALT (1.78x) increased at the interim point in males. Slightly higher terminal ALP suggests increased bone turnover in males marked by hyperostosis.
- Interim and final plasma albumin levels were slightly lower in the MD and HD female rats.
- Although some of the interim changes noted above persisted until the end of the study, they were lower in magnitude at the end of the study.
- Terminal creatinine and cholesterol levels were unchanged.

Changes in Prominent Serum Chemistry Parameters as Percent of the Corresponding Controls at WK 52 (interim) and WK 106

Daily Dose (mg eq./kg)	0(Vehicle)		10		30		100	
No. of Animals	M:65	F:65	M:65	F:65	M:65	F:65	M:65	F:65
Week 52								
Calcium (mg/dl)	9.9	10.3	0.960 ***	0.951 ***	0.960 ***	0.951 ***	0.960 ***	0.942 ***
Inorg. phosphorus (mg/dl)	5.9	-	-	-	-	-	1.102 **	-
Albumin (g/dl)	-	5.1	-	-	-	0.941 **	-	0.922 ***
Glucose (mg/dl)	110	109	0.636 ***	0.706 ***	0.527 ***	0.596 ***	0.518 ***	0.532 ***
Triglycerides (mg/dl)	63	47	1.238 *	1.532 *	1.603 ***	1.851 ***	1.540 ***	3.596 ***
Urea nitrogen (mg/dl)	14.0	15.8	1.636 ***	1.405 ***	2.014 ***	1.804 ***	2.143 ***	1.930 ***
Total bilirubin (mg/dl)	0.09	0.15	-	-	0.778 **	0.733 **	0.556 ***	0.733 ***
Alk. phosphatase (U/l)	53	-	1.491 ***	-	1.642 ***	-	2.057 ***	-
Alanine aminotransferase (U/I)	37	-	1.541 ***	-	1.649 ***	-	1.784 ***	-
Week 105-106								
Chloride (mmol/l)	-	101	-	-	-	-	-	1.040 **
Calcium (mg/dl)	9.8	10.4	0.990 **	0.962 ***	0.990 **	0.942 ***	0.990 **	0.962 **
Inorg. phosphorus (mg/dl)	5.5	-	-	-	-	-	1.055 *	-
Albumin (g/dl)	-	4.6	-	-	-	0.935 **	-	0.935 *
Glucose (mg/dl)	89	94	0.640 ***	0.723 ***	0.584 ***	0.649 ***	0.483 ***	0.564 ***
Triglycerides (mg/dl)	69	57	1.464 **	1.614 **	1.768 ***	2.368 ***	2.000 ***	4.123 ***
Urea nitrogen (mg/dl)	14.9	14.5	1.403 ***	1.434 ***	1.805 ***	1.793 ***	2.060 ***	2.428 ***
Total bilirubin (mg/dl)	-	0.12	-	-	-	-	-	0.583 ***
Alk. phosphatase (U/l)	48	-	1.375 ***	-	1.354 ***	-	2.021 ***	-
Alanine aminotransferase (U/I)	53	-	1.170 ***	-	1.340 ***	-	1.302 ***	-

Urinalysis

- Significant dose-dependent increase in mean interim and terminal urine volume in male and female rats up to 3.4x in control in males and 2.8x the control in females. The increase in urine volume correlated to wet bedding on Day 1 until the end of the study suggesting that SD rats were very sensitivity to pharmacological effect of canagliflozin and sensitivity was maintained until the end of the study.
- Significant increase in interim and terminal urine glucose by up to 4x the control levels in both sexes. The persistent osmotic effect of glucosuria resulted in enhanced urine output in both sexes.
- Drug-related increase in urine specific gravity was noted in treated rats.
- Urine pH was reduced in both sexes at WK 52 and 106, corresponding to higher urinary ketones
- There was more positive occult blood in urine in treated rats than controls suggesting leaky dilated renal, ureter and bladder tissues.

Daily Dose (mg eq./kg)	0(Vehicle)		10		30		100	
No. of Animals	M:65	F:65	M:65	F:65	M:65	F:65	M:65	F:65
Urinalysis[b]								
Week 52								
Specific gravity	1.026	1.027	1.020 ***	1.018 ***	1.020 ***	1.018 ***	1.018 ***	1.016 ***
pH	7.5	6.9	0.867 ***	0.913 **	0.840 ***	0.884 ***	0.827 ***	0.870 ***
Volume (ml)	14.1	9.9	1.851 ***	1.717 ***	2.390 ***	2.000 ***	2.879 ***	2.616 ***
Glucose (Score) [c]	0.00	0.00	3.80 ***	4.00 ***	3.85 ***	3.95 ***	3.90 ***	4.00 ***
Ketones (Score)	1.30	0.90	2.192 ***	1.167	2.769 ***	2.000 **	3.038 ***	3.444 ***
Occult Blood (Score)	-	0.25	-	3.000 **	-	2.800 **	-	3.200
Color (Score)	1.40	1.40	0.714 **	0.750 **	0.750 **	0.750 **	0.714 **	0.714 **
Week 105 - 106								
Specific gravity	1.028	1.023	1.016 ***	1.016 ***	1.015 ***	1.019 ***	1.009 ***	1.012 ***
pH	6.9	6.6	0.942 *	0.970	0.928 **	0.924 ***	0.899 ***	0.894 ***
Volume (ml)	14.6	14.7	2.130 ***	1.830 ***	2.479 ***	1.796 ***	3.432 ***	2.755 ***
Glucose (Score) [c]	0.00	0.00	3.61 ***	3.94 ***	3.57 ***	3.92 ***	3.71 ***	3.97 ***
Ketones (Score)	1.10	0.89	1.391	0.831	2.100 ***	2.360 ***	2.655 ***	2.933 ***
Occult Blood (Score)	-	1.07	-	1.112	-	1.196	-	1.561
Color (Score)	1.38	-	0.688 **	-	0.529 ***	-	0.551 ***	-

[b] For vehicle controls, group means are shown. For treated groups, multiples of control/baseline are shown. Statistical significance is based on actual data.
[c] For glucose, the actual group means are shown (instead of multiples of control).

Hormone Analysis at the End of the 2-Year Study

- Terminal serum testosterone levels significantly decreased, whether measured by the sponsor or by [REDACTED] (b) (4) (see table).
- Terminal LH levels (sponsor and CSU) were low or undetectable, contradicting the "increase in LH hypothesis" as a cause of testicular tumors. It should be noted an elevation in LH may have occurred during the first few months of life to trigger Leydig cell hyperplasia. It is not unexpected for aged rats to have diminished testosterone and low or undetectable levels of LH. The sponsor attributed the low/undetectable LH in control and treated rats to old age (Lamano Carvalho et al, Histol Histopath 1988:413-417 and Desjardins, Biology of Reproduction, 1981:24, 1-21).

Terminal Serum Testosterone and LH Levels in Males Measured at the Sponsor's Facility and at [REDACTED] (b) (4)

Johnson & Johnson Pharmaceutical Research & Development,
a division of Janssen Pharmaceutica N.V.

| Parameter | Unit | Dosage Groups (mg eq./ kg) MALES | | | |
		Vehicle	Low:10	Med.:30	High:100
Testosterone		1.34	1.27	0.64	0.69
	nmol/l	(0.25)	(0.29)	(0.03)	(0.08)
				***	***
Luteinizing hormone		0.78	0.78	0.78	0.78
	ng/ml	(0.00)	(0.00)	(0.00)	(0.00)

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

Mean Serum Testosterone Levels in Male rats Determined by [REDACTED] (b) (4)

Dosage :	Vehicle	Dosage :	Low	Dosage :	Medium	Dosage :	High
			10 mg eq/kg		30 mg eq/kg		100 mg eq/kg
IDN	ng/ml	IDN	ng/ml	IDN	ng/ml	IDN	ng/ml
MEAN	0.58	MEAN	0.50	MEAN	0.25	MEAN	0.23
St error	0.08	St error	0.09	St error	0.02	St error	0.04
		Significance		Significance	***	Significance	***

Gross Pathology

Kidney:
Calculi and gravel was found in more animals in the dosed groups than in control. Findings were most frequent at the HD, including pale discoloration, an irregular surface, and swelling.

Adrenal:
Swelling was observed in HD males and in females at all doses; this finding was also seen in the control group at a lower incidence.

Male Reproductive Organs:
Swollen and discolored testicular tissue was observed at all doses. Small seminal vesicles and prostate were observed at the MD and HD.

Gross Pathology Findings in the 2-year SD Rat Carcinogenicity Study

Daily Dose (mg eq./kg)	0(Vehicle)		10		30		100	
No. of Animals	M:65	F:65	M:65	F:65	M:65	F:65	M:65	F:65
Gross Pathology								
Animals Examined	65	65	65	65	65	65	65	65
ADRENAL GLANDS								
- Swollen	6	13	-	21	-	26	24	34
COAGULATING GLAND(S)								
- Small	6		-		13		22	
GENERAL OBSERVATIONS			- No noteworthy findings					
- Dehydration	3	0	-	-	-	-	8	4
- Emaciation	3	3	-	-	-	-	11	17
KIDNEYS								
- Calculus in pelvis	0	0	1	5	2	7	1	6
- Contents: abnormal	0	0	-	-	1	-	-	3
- Discoloration: pale	5	1	9	7	14	9	24	34
- Focus/area depressed	0	0	3	-	4	-	-	4
- Focus/area discolored	3	1	-	-	-	-	10	-
- Granulated surface	0	0	-	-	-	-	1	-
- Gravel in pelvis	0	3	4	9	5	8	4	17
- Irregular surface	0	0	1	-	1	1	9	12
- Mass	0	0	-	-	-	-	8	3
- Pelvic dilatation	4	1	6	4	10	6	8	4
- Swollen	4	0	-	-	-	-	19	11
- Irregular shape	0	0	-	-	-	-	-	1
PROSTATE								
- Small	2		-		11		20	
SEMINAL VESICLES								
- Small	7		-		14		23	
TESTES								
- Focus/area discolored	1		6		14		24	
- Mass	0		-		1		1	
- Swollen	0		2		10		12	
URETER(S)								
- Dilatation	3	1	4	5	5	5	8	5
URINARY BLADDER								
- Thickened	0	0	-	1	2	2	2	2

- No noteworthy findings

Histopathology

Peer Review: Report states peer reviewed but <u>no name or data provided</u>.

Study pathologist: ████████████ (b) (4) (contributing pathologist)
Peer review pathologist: Not disclosed

After initial readings, JNJ convened a PWG to evaluate the renal tissues. The PWG assessment of renal slides is presented in Appendix A.

Neoplastic

- Renal tubular tumors (adenoma and carcinoma) increased numerically in MD (n=2) and significantly in the HD (n=12) males and HD (n=8) females in the pair wise comparison relative to control.
- Trend analysis for renal tumor found significant dose-response relationship (positive trend) between canagliflozin dose and rise in incidence of renal tubule tumors in rats.
- A dose-related increase in incidence of adrenal tumors reached statistical significances at HD male and female rats.
- Testicular tumors were increased at all doses of canagliflozin in males which correlated with atrophy of secondary sex organs.

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Neoplastic Tumors in Male Rats Treated with Canagliflozin (% change)

Tumors in male rats (n=65/sex/group)		Canagliflozin dose, mg/kg				Trend,
		0	10	30	100	P value
	Exposure multiples		1X	5X	12X	
Adrenal Gland	Benign pheochromocytoma	4	4 (6%)	7 (11%)	26 (40%) 0.000	0.000
	Malignant pheochromocytoma	0	0	1	2	
	Combined, number of rats	4	4	7	28 0.000	0.000
Kidney	Benign renal tubular adenoma, basophilic	0	0	0	8 (12%) 0.000	0.004
	Benign renal tubular adenoma, amphophilic			1		
	Malignant, renal tubular carcinoma, basophilic	0	0	0	4 (6.2%)	0.037
	Malignant, renal tubular carcinoma, amphophilic	0	0	1	1	
	Combined, number of rats	0	0	2	12[a] 0.000	0.000
Liver	adenoma+ carcinoma	3	3	2	10	0.003
Testes	Benign interstitial adenoma	1 (1.5%)	8 (12%) 0.03	20 (31%) 0.000	24 (37%) 0.000	0.000
Urinary Bladder	Benign, papilloma transitional cells	0	0	0	1	
	Carcinoma, transitional	0	0	0	1	
	Combined, number of rats	0	0	0	2	0.04

a= one animal had both adenoma and carcinoma, thus total was deducted by one. The total however included both the amphophilic (spontaneous?) and basophilic tumors (drug-related).
P values were from Dr. Min's analysis.

Neoplastic tumors in female rats treated with canagliflozin (n= 65/sex/group)

Tumors in female rats		Canagliflozin dose, mg/kg				Trend,
		0	10	30	100	p value
	Exposure multiples		2X	7X	21X	
Adrenal Gland	Benign pheochromocytoma	2	1	3	7	0.008
Kidney	Benign renal tubular adenoma, basophilic	0	0	0	7 0.003	0.000
	Malignant renal tubular carcinoma, basophilic	0	0	0	2 (3%)	
	Combined, number of rats	0		0	8[a]	0.000
Urinary Bladder	Benign, papilloma transitional cells	0	1	0	3	

a= one animal had both adenoma and carcinoma thus counted as one

There were 2 MD and 12 HD males and 8 HD females with renal tubular tumors. The PWG considered amphophilic/vacuolated renal tubule tumors in 2 MD (#222, #259) and 1 HD (#309) males as spontaneous (not related to treatment) thus reducing the total number of treatment-related tumors to 11 HD males and 8 HD females. One HD male and female had both renal tubule adenoma and carcinoma. They were counted as carcinoma, the severest form of the tumor.

Description of Spontaneous Renal Tumors

The individual animal histopath for the two MD (#222, #259) and one HD male (#309) with renal tubule tumors that were considered spontaneous by the PWG are shown below. The individual animal data was initialized by the contributing pathologist (b) (4) and appears to be the same as the PWG diagnosis.

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INDIVIDUAL ANIMAL DATA SHEETS                                    | 222 |

                                    CONT./FF. ANIMAL NO. :       222
.................................................................................

  SMALL INTESTINE, ILEUM
  - 01: Distention, moderate degree.          - Dilatation, grade 1.
  - 02: Contents: abnormal, frothy, yellow.   - No corresponding finding.
  - 03: Contents: gas, moderate degree.       - No corresponding finding.
  SMALL INTESTINE, JEJUNUM
  - 01: Distention, slight degree.            - No corresponding finding.
  - 02: Contents: abnormal, frothy, yellow.   - No corresponding finding.
  TESTES
  - 01: Bilateral: focus/area discolored, one, - Adenoma interstitial cell, single,
        between 3 and 6 mm, yellow.             unilateral (benign neoplasm, definitely
                                                incidental).
  THYMUS
  - 01: Small, marked degree.                 - Atrophy/involution, grade 4.
  NO OTHER NECROPSY OBSERVATIONS NOTED


MICROSCOPIC FINDINGS

  ADRENAL GLANDS (evaluated by (b) (4)
    No microscopic finding corresponding to necropsy observation no. 01.
   -Clear/swollen zona glomerulosa cells, (multi)focal, bilateral, grade 2
   -Hyperplasia cortical, multifocal, bilateral, grade 3
    one with cystic degeneration
   -Hyperplasia medullary, focal, unilateral, grade 3
  BONE MARROW, FEMUR (evaluated by (b)(4)
   -Amount of fat, grade 1
  COAGULATING GLAND(S) (evaluated by (b) (4)
   -Atrophy, bilateral, grade 2
    This finding corresponds to necropsy observation no: 01.
  EPIDIDYMIDES (evaluated by (b)(4)
   -Cellular debris, unilateral, grade 1
   -Inflammation, focal, fat, unilateral, grade 1

  KIDNEYS (evaluated by (b) (4):
   -Adenoma renal tubule, single, cystic, cortex, unilateral
    (benign neoplasm, definitely incidental)
    clear/vacuolated cells, focally amphophilic/vacuolated
    This finding corresponds to necropsy observation no: 01.
   -Chronic progressive nephropathy, bilateral, grade 3
   -Dilatation pelvic, bilateral, grade 2
    This finding corresponds to necropsy observation no: 02.
   -Dilatation tubule(s), bilateral, grade 1
   -Hypertrophy tubule(s), cortex, focal, unilateral, grade 1
  PROSTATE (evaluated by (b) (4)
   -Atrophy, grade 1
    This finding corresponds to necropsy observation no: 01.
   -Inflammation chronic, focal/multifocal, grade 2
   -Mineralization, secretory material, ventral, grade 1
   -Tubules containing granulocytes/cell debris, (multi)focal, grade 2
  SEMINAL VESICLES (evaluated by (b) (4)
   -Atrophy, bilateral, grade 2
    This finding corresponds to necropsy observation no: 01.
  TESTES (evaluated by (b) (4)
   -Adenoma interstitial cell, single, unilateral (benign neoplasm, definitely incidental)
    This finding corresponds to necropsy observation no: 01.
   -Atrophy, focal, unilateral, grade 2
   -Hyperplasia interstitial cell, multifocal, bilateral, grade 2
```

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INDIVIDUAL ANIMAL DATA SHEETS
_____

ANIMAL NUMBER: 259   SEX: Male   DOSE GROUP: M

    First day on test   : 16-Jul-08
    Last day on test   : 23-Jul-10
    Days on test   :   738
    Date of necropsy   : 23-Jul-10
    Sacrifice group   : Terminal sacrifice group
    Status at necropsy   : Terminal sacrifice group
....
PALPABLE MASSES

    NO PALPABLE MASSES NOTED

NECROPSY OBSERVATION   CORRESPONDING MICROSCOPIC FINDING

    ADRENAL GLANDS
    - 01: Bilateral: discoloration: pale, slight   - No corresponding finding.
          degree.
    KIDNEYS
    - 01: Cortex, left: mass, one, between 6 and   - Carcinoma renal tubule, single, well-
          10 mm, white-tan.   differentiated, unilateral (malignant
                                                       neoplasm).
    MICROSCOPIC FINDINGS

    ADRENAL GLANDS (evaluated by (b) (4)
      No microscopic finding corresponding to necropsy observation no. 01.
     -Hyperplasia medullary, focal, unilateral, grade 1
    KIDNEYS (evaluated by (b) (4)
     -Carcinoma renal tubule, single, well-differentiated, unilateral (malignant neoplasm)
      based on necrosis and size; amphophilic/vacuolated; cortex
      This finding corresponds to necropsy observation no: 01.
     -Chronic progressive nephropathy, bilateral, grade 2
     -Dilatation pelvic, unilateral, grade 2
     -Dilatation tubule(s), bilateral, grade 1
     -Hyperplasia renal tubule atypical, cortex, focal, unilateral, grade 1
     -Hyperplasia transitional cell, focal, unilateral, grade 1
     -Hypertrophy tubule(s), cortex, focal, unilateral, grade 1
    PROSTATE (evaluated by (b) (4)
     -Inflammation chronic, focal/multifocal, grade 1
     -Mineralization, secretory material, ventral, grade 1
     -Tubules containing granulocytes/cell debris, (multi)focal, grade 1
    STOMACH (evaluated by (b) (4)
     -Dilatation gastric gland(s), grade 1
     -Erosion(s), glandular region, focal, grade 1
    TESTES (evaluated by (b) (4)
     -Adenoma interstitial cell, single, unilateral (benign neoplasm)
     -Hyperplasia interstitial cell, multifocal, bilateral, grade 2

INDIVIDUAL ANIMAL DATA SHEETS
_____

ANIMAL NUMBER: 262   SEX: Male   DOSE GROUP: M

    First day on test   : 16-Jul-08
    Last day on test   : 23-Jul-10
    Days on test   :   738
    Date of necropsy   : 23-Jul-10
    Sacrifice group   : Terminal sacrifice group
    Status at necropsy   : Terminal sacrifice group
....
PALPABLE MASSES

    NO PALPABLE MASSES NOTED

NECROPSY OBSERVATION   CORRESPONDING MICROSCOPIC FINDING

    KIDNEYS
    - 01: Cortex, left: mass, one, between 3 and   - Hyperplasia mesenchymal, focal, unilateral
          6 mm, pale, slight degree.   grade 3.
    LIVER
    - 01: All lobes: swollen, slight degree.   - No corresponding finding.
    - 02: All lobes: prominent lobular pattern,   - Vacuolization hepatocellular, periportal,
          slight degree.   small, grade 1.
    - 03: Left lateral lobe: focus/area white-tan,   - Necrosis, focal/multifocal, grade 2.
          one, between 1 and 3 cm, fixed in a
          separate gauze pad.
    MICROSCOPIC FINDINGS

    ADRENAL GLANDS (evaluated by (b) (4)
     -Hyperplasia cortical, focal, unilateral, grade 3
     -Hyperplasia medullary, focal, unilateral, grade 3
    GENERAL OBSERVATIONS (evaluated by SDJ):
      Microscopic examination not applicable to necropsy observation no.01.
    KIDNEYS (evaluated by (b) (4)
     -Chronic progressive nephropathy, bilateral, grade 3
     -Dilatation tubule(s), bilateral, grade 2
     -Hyperplasia mesenchymal, focal, unilateral, grade 3
      This finding corresponds to necropsy observation no: 01.
     -Hyperplasia transitional cell, focal, unilateral, grade 1
     -Mineralization pelvis, unilateral, grade 4

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INDIVIDUAL ANIMAL DATA SHEETS

ANIMAL NUMBER:    309        SEX: Male           DOSE GROUP:  H


   First day on test      : 16-Jul-08
   Last  day on test      : 15-Jul-10
   Days on test           :     730
   Date of necropsy       : 15-Jul-10
   Sacrifice group        : Terminal sacrifice group
   Status at necropsy     : Terminal sacrifice group
.........................................................................

PALPABLE MASSES

   NO PALPABLE MASSES NOTED


NECROPSY OBSERVATION                     CORRESPONDING MICROSCOPIC FINDING
   KIDNEYS
   - 01: Right: mass, one, between 1 and 3 cm,   - Carcinoma renal tubule, unilateral
         white-tan.                                (malignant neoplasm).
   PROSTATE
   - 01: Small, marked degree.              - Atrophy, grade 4.
   SEMINAL VESICLES
   - 01: Bilateral: small, marked degree.   - Atrophy, bilateral, grade 4.

   TESTES
   - 01: Left: swollen, moderate degree.    - Adenoma interstitial cell, bilateral
                                              (benign neoplasm).
   - 02: Bilateral: foci/areas discolored,  - Adenoma interstitial cell, bilateral
         multiple, between 1 and 3 cm, yellow.    (benign neoplasm).
   - 03: Right: discoloration, red.         - Adenoma interstitial cell, bilateral
                                              (benign neoplasm).

MICROSCOPIC FINDINGS

   ADRENAL GLANDS (evaluated by  (b) (4) :
    -Ectasia, (multi)focal, unilateral, grade 1
    -Hyperplasia cortical, focal, unilateral, grade 1
   BONE, STERNUM (evaluated by  (b) (4)
    -Hyperosteosis, grade 2
   BONE, STIFLE (evaluated by  (b) (4)
    -Hyperosteosis, grade 1

   EPIDIDYMIDES (evaluated by  (b) (4)
    -Decreased spermatozoa, bilateral, grade 5
   EYES (evaluated by  (b) (4)
    -Atrophy retina, bilateral, grade 2
   KIDNEYS (evaluated by  (b) (4)
    -Carcinoma renal tubule, unilateral (malignant neoplasm)
    cortex/outer medulla
    eosinophilic/vacuolated; partially amphophilic
    This finding corresponds to necropsy observation no: 01.
    -Chronic progressive nephropathy, bilateral, grade 3
    -Dilatation tubule(s), bilateral, grade 3
    -Hyperplasia simple, (multi)focal, bilateral, grade 2
    -Hyperplasia transitional cell, multifocal, unilateral, grade 1
    -Hypertrophy tubule(s), cortex, multifocal, bilateral, grade 1
    -Inflammation pelvis, unilateral, grade 2
    -Mineralization cortex, bilateral, grade 2
    -Mineralization medulla, multifocal, bilateral, grade 2
   PROSTATE (evaluated by  (b) (4)
    -Atrophy, grade 4
    This finding corresponds to necropsy observation no: 01.
    -Inflammation chronic, focal/multifocal, grade 3
    -Mineralization, secretory material, ventral, grade 1
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Note:

(b) (4) analysis (examined the slides before the PWG) had only considered one MD male rat (#259) with renal tumor as spontaenous. He classified the second MD male (#222) to have a transition stage from amphophiphilic-vacuolar to basophilic tubule cell tumor and sugegsted examining DNA samples for possible genetic linkage them. Spontaneous amphophilic renal tumors have been known to occur in rats that are full or half sibling.   The sponsor headed his advise and analyzed liver samples from rats with "spontaneous" renal tumors for possible genetic link.   Liver DNA analysis found no geneitic relationship among them.

Neoplastic Findings

The three prominent neoplastic lesions in 11 HD male rats (renal tubule, adrenal and testicular tumors) and associated histopath changes in the same animals are shown below.

Animal No. (males)	301	306	310	314	321	322	332	333	351	355	364
Group Symbol	H	H	H	H	H	H	H	H	H	H	H
Days on test	T	T	T	T	T	T	604	T	T	680	T
KIDNEY											
Adenoma (B)/carcinoma (N)	B	B	B	N	B	B	B	B+N	B	N	N
Localization of tumor	c	c	cm	cm	cm	cm	cm	cm	cm	cm	cm
CPN	3	3	2	2	2	4	2	3	3	2	2
Dilated tubules	3	3	2	1	3	2	3	3	3	1	3
Hyperplasia renal tub.	1 S	1At	1 S					1 S			1S
Hypertrophy renal tub.	1	1	1		1	1	1	1	1		1
Hyperplasia transitional		2	1	1	2	2		3		3	2
Mineralization cortex	1	1			1	1	1	1	1		
Mineralization medulla	2	1	1		1		1	1		1	
Mineralization papilla	1	1			1	1	2				
Mineralization pelvis		3	4	1	4	2		4		4	3
Gravel in pelvis										P	P
MINERALIZATION	301	306	310	314	321	322	332	333	351	355	364
Mineralization vascular	P	P	P	P	P	P	P	P	P	P	P
Mineralization stomach	P				P			P			
Mineralization nose	2	2	2		2			2	1	2	1
URINARY BLADDER											
Inflammation chronic										2	
Inflammation acute								3			
Hyperplasia transitional cell								2			
URETER(S)											
Inflammation acute								2			
ADRENAL MEDULLA											
Hyperplasia				1		3		3		4	
Pheochromocytoma	B	B	B		B	B			B		B
TESTES – Leydig cell											
Hyperplasia			1	2	2	3	2	2			1
Adenoma (B)			B		B	B	B	B	B	B	
ACCESSORY SEX GLAND(S): Atrophy			P			P	P	P	P	P	
BONE: Hyperostosis	4	4	3		3	3	2	3	2	3	3

Legend:
T: terminal
c: cortex; cm: cortex + outer medulla   P: present or increased in one or more tissues
CPN: chronic progressive nephropathy   S: simple; At: atypical

The two prominent neoplastic lesions in eight female rats (renal tubule, adrenal tumors) and associated histopath changes in the same animals are shown below.

Animal No. (females)	709*	721	730	740	747	752	755	756
Group Symbol	H	H	H	H	H	H	H	H
Days on test	469	672	680	T	T	T	T	T
KIDNEY								
Adenoma (B)/carcinoma (N)	B	B	B	B+N	B	B	B	N
Localization of tumor	cm	m	m	cm	m	c	cm	cm
CPN		3	2	3	3	2	3	4
Dilated tubules		3	3	3	3	2	3	3
Hypertrophy renal tubule		1			1	1		
Hyperplasia transitional	3	3	3	3	3	3	3	1
Mineralization cortex		1	1		2	1		1
Mineralization medulla					1			1
Mineralization papilla		1	2	2		1		2
Mineralization pelvis	3	1	4	4	2	4	1	2
Gross: gravel in pelvis			P	P	P	P		P
MINERALIZATION								
Mineralization vascular		P		P	P	P	P	P
Mineralization stomach			P		P			
Mineralization nose		3	2	2		1	2	2
URINARY BLADDER								
Inflammation chronic		1						
Papilloma transitional cell			P					
ADRENAL MEDULLA								
Hyperplasia		2	3		3	3	2	4
Pheochromocytoma		B						
BONE: Hyperostosis	1	4	4	3	3	1		2

Legend:

* Cannibalized (several tissues missing; autolysis)

T: terminal

c: cortex; m: outer medulla; cm: cortex + outer medulla

CPN: chronic progressive nephropathy

P: present or above background in one or more tissues

The historical incidence of adrenal, renal tubular cell and testicular Leydig cell tumors from several laboratories including the sponsor's facility at Beerse are provided for reference. Although adrenal tumors are common, renal tubular tumors are rare (less than 1%).

Historical Control Data in Sprague-Dawley Rats for Relevant Tumors

Tumor Type	Study Site				
	J&JPRD Beerse				(b) (4)
Males					
Benign pheochromocytoma	54/540 (10%) max 13/65	327/3001 (10.90%)	233/2144 (10.87%)	69/589 (11.71%)	88/634 (13.88%)
Malignant pheochromocytoma	6/540 (1.11%) max 2/65	39/3001 (1.30%)	30/2144 (1.40%)	15/589 (2.55%)	31/634 (4.89%)
Renal tubular cell adenoma	1/540 (0.18%) max 1/70	8/3005 (0.27%)	9/2146 (0.42%)	2/590 (0.34%)	5/634 (0.79%)
Renal tubular cell carcinoma	0/540	6/3005 (0.20%)	8/2146 (0.37%)	0/590	0/634
Testicular interstitial adenoma	12/540 (2.22%) max 4/70	75/3003 (2.50%)	52/2145 (2.42%)	9/589 (1.53%)	25/634 (3.94%)

Historical Control Data in Sprague-Dawley Rats for Relevant Tumors

Tumor Type	Study Site				
	J&JPRD Beerse				(b) (4)
Females					
Benign pheochromocytoma	14/540 (2.59%) max 4/70	75/3214 (2.33%)	45/2344 (1.92%)	22/589 (3.74%)	31/633 (4.90%)
Malignant pheochromocytoma	6/540 (1.11%) max 3/65	14/3214) (0.44%)	13/2344 (0.55%)	1/589 (0.17%)	6/633 (0.95%)
Renal tubular cell adenoma	1/540 (0.18%) max 1/70	4/3209 (0.13%)	1/2344 (0.04%)	1/590 (0.17%)	0/634
Renal tubular cell carcinoma	0/540	6/3209 (0.19%)	2/2344 (0.09%)	0/590	0/634

Non-Neoplastic Findings

- Histopath findings were seen in adrenal gland, kidneys, bladder, bone, testes and ureters in canagliflozin treated rats.
- Significant increase in adrenal medullary hyperplasia in MD males and HD males and females.
- Drug-related increase in hyperostosis was seen at MD and HD in female and male rats. The severity and incidence increased with dose. Hyperostosis has been observed in other canagliflozin toxicology studies in rats. Hyperostosis is histologically recognizable within 2 weeks of canagliflozin treatment which appears to be related to Ca mobilization (increased GI absorption and renal excretion). Reduction in dietary Ca in the 6-month rat study reduced/prevented hyperostosis in rats.
- Dose-related increase in renal chronic progressive nephropathy (CPN) in both sexes. The severity and incidence was increased with dose. The sponsor had associated canagliflozin induced increase in nephropathy to increased incidence of renal tubule tumors. Since only severe CPN has been suggested by some to be associated with renal tumors, the agency rejected this hypothesis. PWG downgraded the severity of CPN by 1 to 2 grades and rejected CPN association with treatment related increase in renal tubule tumors in the HD male and female rats.
- Dose-dependent increase in renal pelvic and tubule dilatation, transitional cell hyperplasia, tubular hypertrophy and mineralization (cortex, medulla, papilla, and pelvis) was seen in both male and female rats.
- According to new histopath evaluations, the simple tubular hypertrophies appear to be random and only slightly increase in the HD males (6 vs. 3 in control). In the initial safety report, the data provided by the sponsor suggested a dose-dependent increase in renal tubule hyperplasia in C, LD MD and HD males (6, 8, 12 and 17, respectively) and females (5, 6, 6 and 12, respectively). The PWG had diagnosis had changes the incidence of hyperplasia to hypertrophy. By doing so, the PWG concluded that there were no dose-related increases in tubule hyperplasia to suggest preneoplastic lesions were leading to renal tubule tumors HD males and females.
- Mineralization was common with greater frequency in the HD animals, especially in female rats. The tissues with signs of mineralization included aorta, eyes, large intestine, larynx, lungs, nose, peyer's patch, stomach, and trachea and vasculatures in some organs (bone, eyes, heart, kidneys, lymph, peripheral sciatic nerve and tongue).
- Significant dose-related increase in incidence of testicular atrophy
- Significant drug-related increase in testicular interstitial hyperplasia
- Significant drug-related mineralization of aorta, brain, heart, epididymides, eyes, large intestine, lungs, nose, peripheral sciatic nerve, peyer's patch, prostate, skeletal muscle, stomach and vasculatures (eyes, heart, kidneys, lymph and tongue) in male rats.
- Increased dilatation of rectum in HD rats was likely due to carbohydrate malabsorption related and fermentation.
- The incidences of parathyroid hyperplasia were higher in HD male and female rats.

Treatment-related Histopathological and Corresponding Gross Changes

sex	MALES					FEMALES				
group symbol	V	L	M	H	hcd	V	L	M	H	hcd
ADRENAL GLANDS										
Gross:										
Swollen	6	5	7	24		13	21	26	34	
Histopathology	65	64	64	65		65	63	62	64	
Pheochromocytoma benign	4	4	7	**26**	13	2	1	3	**7**	3
Pheochromocytoma malignant	-	-	1	**2**	2	-	-	-	-	3
Hyperplasia medullary	15	17	**30**	29		8	6	8	**27**	
grade 1	7	8	12	9		5	3	3	5	
grade 2	4	6	7	6		3	3	4	11	
grade 3	3	3	9	10		-	-	1	10	
grade 4	1	-	2	4		-	-	-	1	
BONE, STERNUM										
Histopathology	64	65	65	65		64	65	64	64	
Hyperostosis	-	1	**6**	**50**		-	-	**3**	**41**	
grade 1	-	-	1	8		-	-	2	13	
grade 2	-	1	3	22		-	-	1	19	
grade 3	-	-	1	18		-	-	-	7	
grade 4	-	-	1	2		-	-	-	1	
grade 5	-	-	-	-		-	-	-	1	
BONE, STIFLE										
Histopathology	65	65	64	65		64	64	65	64	
Hyperostosis	1	2	**7**	**48**		2	4	**11**	**55**	
grade 1	-	1	5	10		1	4	8	15	
grade 2	1	1	2	**21**		-	-	2	17	
grade 3	-	-	-	**14**		1	-	1	**18**	
grade 4	-	-	-	**3**		-	-	-	**5**	
GENERAL OBSERVATIONS										
Gross: Dehydration	3	1	2	**8**		-	-	-	4	
Gross: Emaciation	3	3	3	**11**		3	6	7	17	
KIDNEYS										
Gross:										
Calculus in pelvis	-	1	2	1		-	5	7	6	
Content: abnormal	-	-	1	-		-	-	-	3	
Discoloration: pale	5	9	14	24		1	7	9	34	
Focus/area depressed	-	3	4	-		-	-	-	4	
Focus/area discolored	3	4	1	10		1	1	3	4	
Granulated surface	-	-	-	1		-	-	-	-	
Gravel in pelvis	-	4	5	4		3	9	8	17	
Irregular surface	-	1	1	9		-	-	1	12	
Mass	-	-	2	8		-	-	1	**3**	
Pelvic dilatation	4	6	10	8		1	4	6	4	
Swollen	4	5	6	19		-	1	2	11	
Irregular shape	-	-	-	-		-	-	-	1	
Histopathology	65	65	64	65		65	64	65	65	
RTT basophilic: incidence	-	-	-	11		-	-	-	8	
Adenoma renal tubule, basophilic	-	-	-	**8**	0	-	-	-	**7**	1
Carcinoma renal tub., basophilic	-	-	-	**4**	0	-	-	-	**2**	0
Adenoma renal tubule Amphophilic-Vacuolar (= spontaneous)			1		1#					0
Carcinoma renal tubule Amphophilic-Vacuolar (= spontaneous)			1	1	0					0

sex	MALES					FEMALES				
group symbol	V	L	M	H	hcd	V	L	M	H	hcd
Carcinoma transitional cell	-	-	-	*1*	0	-	-	-	-	0
Chronic progressive nephropathy	44	60	59	56		23	37	47	50	
grade 1	23	28	16	9		19	31	34	10	
grade 2	10	23	32	25		3	4	7	19	
grade 3	10	7	8	16		1	2	4	17	
grade 4	1	2	3	5		-	-	1	2	
grade 5	-	-	-	1		-	-	1	2	
Cyst (s)						1	3	3	9	
grade 1						1	2	-	8	
grade 2						-	1	-	1	
grade 3						-	-	3	-	
Dilatation pelvic	4	10	17	18		3	7	14	15	
grade 1	-	3	4	7		-	2	5	5	
grade 2	2	5	10	9		1	3	4	5	
grade 3	1	1	2	2		2	2	5	4	
grade 4	1	1	1	-		-	-	-	1	
Dilatation tubule(s)	13	47	49	53		14	40	46	50	
grade 1	11	39	33	10		13	36	28	9	
grade 2	1	8	14	27		1	4	18	20	
grade 3	1	-	2	15		-	-	-	21	
grade 4	-	-	-	1		-	-	-	-	
Hyperplasia renal tubule simple	3	1	3	6		3	2	-	2	
grade 1	3	1	3	5		3	2	-	1	
grade 2	-	-	-	1		-	-	-	1	
Hyperplasia transitional cell	13	17	17	36		24	23	27	54	
grade 1	7	14	11	13		17	13	12	12	
grade 2	6	2	5	13		7	9	12	18	
grade 3	-	-	1	10		-	1	2	22	
grade 4	-	1	-	-		-	-	1	2	
Hypertrophy tubule(s)	13	14	20	27		7	5	6	14	
grade 1	13	14	20	27		7	5	6	14	
Mineralization cortex	3	5	7	24		4	4	4	29	
grade 1	3	5	7	22		4	4	4	23	
grade 2	-	-	-	2		-	-	-	5	
grade 3	-	-	-	-		-	-	-	1	
Mineralization medulla	-	4	9	44		7	11	22	32	
grade 1	-	3	8	36		7	10	21	28	
grade 2	-	1	1	8		-	1	1	3	
grade 3	-	-	-	-		-	-	-	1	
Mineralization papilla	-	2	6	19		1	6	3	30	
grade 1	-	2	4	15		1	6	3	18	
grade 2	-	-	2	2		-	-	-	9	
grade 3	-	-	-	2		-	-	-	1	
grade 4	-	-	-	-		-	-	-	2	
Mineralization pelvis	12	15	22	27		56	38	39	56	
grade 1	11	6	7	9		28	19	15	15	
grade 2	1	3	4	6		21	5	11	20	
grade 3	-	3	5	6		6	8	4	9	
grade 4	-	3	5	6		1	5	7	11	
grade 5	-	-	1	-		-	1	2	1	

Legend: hcd: historical control data Beerse (maximum incidence in control or vehicle group)
Incidences/grades in **bold** considered treatment-related
Incidences in *italic*: considered equivocal
RTT: renal tubular tumor
#: the renal tubular tumor in one vehicle male of TOX6928 was considered an
amphophilic-vacuolated (= spontaneous tumor-type), based on the morphological criteria
by the PWG (Ref. 9). In TOX 6928 it was called "clear-cell type".

sex	MALES					FEMALES				
group symbol	V	L	M	H	hcd	V	L	M	H	hcd
LARGE INT. RECTUM										
Histopathology	62	63	64	65		65	63	65	65	
Dilatation	3	2	1	**8**		4	4	8	**20**	
grade 1	1	1	-	2		2	2	6	11	
grade 2	2	1	1	5		2	2	2	9	
grade 3	-	-	-	1		-	-	-	-	
PARATHYROID GLAND(S)										
Histopathology	64	63	64	60		59	63	62	60	
Hyperplasia	20	20	19	**11**		11	13	13	**2**	
grade 1	10	11	13	10		5	9	10	2	
grade 2	8	3	5	-		5	4	2	-	
grade 3	2	6	1	1		1	-	1	-	
URETER(S)										
Gross: dilatation	3	**4**	**5**	**8**		1	**5**	**5**	**5**	
Histopathology	64	64	64	65		63	63	65	65	
Hyperplasia transitional cell	-	-	-	**3**		-	-	1	1	
grade 1	-	-	-	2		-	-	-	1	
grade 2	-	-	-	1		-	-	-	-	
grade 3	-	-	-	-		-	-	1	-	
Inflammation acute	2	2	-	**4**		-	-	-	**1**	
grade 1	1	1	-	-		-	-	-	1	
grade 2	1	1	-	4		-	-	-	-	
Inflammation chronic	-	-	-	**2**		-	-	-	**2**	
grade 1	-	-	-	-		-	-	-	-	
grade 2	-	-	-	2		-	-	-	-	
grade 3	-	-	-	-		-	-	-	1	
grade 4	-	-	-	-		-	-	-	1	
Minerals/calculi in lumen	-	**1**	-	-		-	**1**	**2**	**1**	
grade 1	-	-	-	-		-	-	-	-	
grade 2	-	1	-	-		-	-	-	-	
grade 3	-	-	-	-		-	1	-	1	
grade 4	-	-	-	-		-	-	2	-	
URINARY BLADDER										
Gross: thickened	-	-	**2**	**2**		-	**1**	**2**	**2**	
Histopathology	64	65	64	65		64	63	64	65	
Carcinoma transitional cell	-	-	-	*1*	0	-	-	-	-	0
Papilloma transitional cell	-	-	-	*1*	1	-	*1*	-	*3*	1
Hyperplasia transitional cell	2	2	**4**	**12**		-	**3**	**3**	**5**	
grade 1	1	1	2	5		-	3	3	3	
grade 2	1	1	1	7		-	-	-	1	
grade 3	-	-	1	-		-	-	-	-	
grade 4	-	-	-	-		-	-	-	1	
Inflammation acute	2	5	3	**9**		1	1	1	1	
grade 1	1	1	-	5		1	1	-	1	
grade 2	1	3	2	2		-	-	1	-	
grade 3	-	1	1	2		-	-	-	-	
Inflammation chronic	4	-	**9**	**8**		1	2	2	7	
grade 1	3	-	3	2		1	2	2	4	
grade 2	1	-	5	3		-	-	-	2	
grade 3	-	-	1	3		-	-	-	-	
grade 4	-	-	-	-		-	-	-	1	
Metaplasia squamous cell	-	-	**1**	-		-	-	**1**	-	
grade 1	-	-	-	-		-	-	1	-	
grade 2	-	-	1	-		-	-	-	-	

sex	MALES					FEMALES				
group symbol	V	L	M	H	hcd	V	L	M	H	hcd
TESTES										
Gross										
Focus/area discolored	1	6	14	24						
Mass	-	-	1	1						
Swollen	-	2	10	12						
Histopathology	65	65	64	65						
Adenoma interstitial cell	1	8	20	24	4					
Hyperplasia interstitial cell	12	24	35	32						
grade 1	9	14	13	10						
grade 2	2	8	20	17						
grade 3	1	-	2	5						
grade 4	-	2	-	-						
PROSTATE										
Gross: small	2	3	11	20						
Histopathology	65	65	64	65						
Atrophy	2	3	10	18						
grade 1	-	1	2	-						
grade 2	1	-	-	5						
grade 3	-	2	7	8						
grade 4	1	-	1	5						
SEMINAL VESICLES										
Gross: small	7	4	14	23						
Histopathology	65	65	64	65						
Atrophy	10	5	11	25						
grade 1	3	3	1	-						
grade 2	2	-	1	7						
grade 3	4	1	7	10						
grade 4	1	1	2	8						
COAGULATNG GLAND(S)										
Gross: small	6	4	13	22						
Histopathology	65	65	64	65						
Atrophy	8	4	11	22						
grade 1	1	2	1	2						
grade 2	5	-	2	5						
grade 3	1	1	6	8						
grade 4	1	1	2	7						
MINERALIZATION (Histopathology)										
- STOMACH	-	1	1	11		-	-	-	5	
grade 1	-	-	1	3		-	-	-	2	
grade 2	-	1	-	7		-	-	-	1	
grade 3	-	-	-	1		-	-	-	2	
- NOSE	11	15	14	36		1	4	6	28	
grade 1	10	15	10	11		-	4	5	12	
grade 2	1	-	4	25		1	-	1	15	
grade 3	-	-	-	-		-	-	-	1	
- VASCULAR										
Aorta	-	1	4	20		-	-	-	6	
Bone, stifle	-	-	1	5		-	-	-	5	
Eyes	1	-	2	17		-	-	-	8	
Heart	-	2	1	9		-	-	1	9	
Kidneys (hilus)	4	3	15	20		1	-	2	9	
Lung	37	48	51	43		38	27	30	47	
grade 1	34	44	40	32		38	27	29	40	
grade 2	3	4	11	10		-	-	1	6	
grade 3				1		-	-	-	1	
Lymph n mesenteric	1	4	4	28		-	-	-	15	
Peripheral n sciatic	1	-	-	9		-	-	-	1	
Tongue	-	2	8	38		-	-	-	22	

Toxicokinetics

- Canagliflozin absorption was relatively slow in rats with Tmax ranging from 0.5 to 7 hrs.
- Exposure increased in a dose-proportional manner from 10 to 30 mg/kg and more than dose-proportional manner from 30 to 100 mg/kg after single dose administration in both male and female rats. The increase in exposure appeared to be dose-proportional in general.
- Single and multiple dose exposures in males were similar however, in females, multiple dose exposure was greater than single dose exposure. Repeat dose AUC in females was generally greater than those in males suggesting gender differences in rats similar to that observed in mice.

	Male			Female		
Dose (mg eq./kg/day)	10	30	100	10	30	100
	Day 0					
C_{max} (ng/ml)	1819	5298	21818	1365	4666	18696
T_{max} (h)	6.25	7.00	6.25	5.00	4.00	6.25
AUC_{0-inf} (ng.h/ml)	27684	83598	370792[1]	20812[1]	69834	408911[2]
	Day 90					
C_{max} (ng/ml)	2693	6281	20737	2151	9439	25098
T_{max} (h)	2.5	4.63	4.75	1.75	1.38	3.50
$AUC_{0-24\,h}$ (ng.h/ml)	33245	83517	260904	34566	124276	388771
	Day 180					
C_{max} (ng/ml)	3110	8283	23387	4707	14925	36873
T_{max} (h)	3.5	4.75	3.88	1.75	3.00	3.13
$AUC_{0-24\,h}$ (ng.h/ml)	38348	117724	315976	61955	188367[1]	559863

[1] n = 3   [2] n = 2

Stability and Homogeneity: Analysis of dosing solution concentration were within the ± 10% of the target concentrations.

9   Reproductive and Developmental Toxicology

Reproductive studies were reviewed by Dr. Mink.

10   Special Toxicology Studies

Title: 6-Month repeated dose oral and subcutaneous toxicity study of JNJ-28431754-ZAE in the male rat under standard diet and low calcium diet feeding conditions with a 1-month and 3-month interim kill

Study #	TOX10093
Study report location	Janssen, Belgium
CRO/Laboratory name and location	Janssen Research and Development, Beerse, Belgium
Date of study initiation	28 Jun 11
GLP compliance statement	Yes
GLP issues identified	None
QA statement	yes
Drug, lot #, and % purity	JNJ-28431754-ZAE; batch ZR600348PFA271; purity 99.8%

Key Study Findings:
- Yellow fecal discoloration observed in all groups fed the fructose diet, including control. Fructose control animals gained the most weight whereas all drug treated groups gained less weight than respective controls. The most significant reduction in weight gain was in the drug treated standard diet group. Feed consumption was increased in all drug treated groups, regardless of diet, with consumption generally highest in the drug treated standard diet group.
- In animals administered 100 mg/kg fed the standard diet, slight decreases in reticulocyte counts were noted at all-time points while slight decreases in RBC, Hb, and Hct counts occurred at the 6-month time-point.
- Increased BUN, ALP, and ALT occurred in all drug treated groups compared to controls. Ca and inorganic phosphorous levels were also increased in the drug treated standard diet group. BUN and ALP levels were higher while Ca, inorganic phosphorous and ALT levels were lower in the drug treated fructose diet group than in the drug treated standard diet group. Important to the Sponsor's hypothesis, the fructose diet prevented the elevation in Ca seen in the standard diet drug treated group. Vitamin D and PTH levels were lower than that of respective controls in the drug treated groups. The reductions in PTH levels in the drug treated fructose groups were of a smaller magnitude than that seen in the drug treated standard diet group, indicating that the fructose diet partially prevented the drug-induced decrease.
- Increased urine volume, glucose, Na, Cl, phosphate, NAG, and protein levels were noted in all drug treated groups. In animals fed the fructose diet, proteins, phosphate (adjusted for creatinine), and NAG levels were increased while Na, K, Cl, and Mg levels (adjusted for creatinine) were all decreased compared to animals fed the standard diet. The level of Ca was increased only in the group administered 100 mg/kg and fed the standard diet; there was no increase in Ca levels in treated animals maintained on the fructose diet. Thus,

feeding rats a fructose based diet prevented the excess calcium excretion induced by JNJ-28431754-ZAE when administered to rats maintained on a standard diet.

- Adrenal and kidney weights were increased in the 3 drug treated groups. Absolute adrenal and kidney weights were also increased in the fructose diet control group, but the increases did not attain statistical significance following adjustment for body weight. The increases in organ weights were slightly greater in the drug treated fructose diet groups than in the drug treated standard diet group at months 1 and 3, but not at 6 months. The increased kidney weights were associated with gross observations of swelling. Weights of the coagulating gland, prostate, and seminal vesicle were reduced in the drug treated standard diet group at each time point, indicating that the fructose diet may have protected against the induction of these effects. Microscopic examination revealed tubular and pelvic dilatation in the kidney in the drug treated groups, occurring at a minimally higher incidence in the standard diet group. KIM-1 and/or BrdU uptake were also slightly higher in kidney structures of the drug treated standard diet group indicating subtle tubular damage and increased cell proliferation. Hypertrophy was noted in the adrenal glands of all drug treated groups, occurring at a higher incidence in those fed the fructose diet while BrdU uptake, indicating proliferation, was increased in the drug treated standard diet group only. In bone, hyperostosis was limited to the drug treated standard diet group.
- Exposure (both C_{max} and AUC) increased in a slightly less than proportional manner in the drug treated fructose fed groups. Exposure to test article at 100 mg/kg in the standard diet was comparable to 65 mg/kg in the fructose diet.

Reviewer Comments: The administration of JNJ-28431754-ZAE to rats fed a fructose based diet prevented or minimized many, but not all, of the effects typically observed following the administration of test article to rats fed a standard diet. Notably, the fructose diet prevented the elevation in Ca and minimized the reduction in PTH levels in plasma while the urinalysis data showed that feeding rats a fructose based diet prevented the excess calcium excretion typically observed in JNJ-28431754-ZAE treated rats.

Postmortem examination revealed drug related effects on the kidney, adrenals, and/or bone of all drug treated groups. In the kidney, tubular and pelvic dilation occurred in all drug treated groups; feeding of a fructose based diet did not dramatically reduce the occurrence of these effects. However, the drug treated fructose diet group did not show the increased KIM-1 and/or BrdU staining seen with the drug treated standard diet group indicating the fructose diet prevented the proliferation seen in drug treated rats fed the standard diet. In the adrenal glands, hypertrophy was noted in all drug treated groups microscopically, occurring at a higher incidence in those fed the fructose diet while BrdU uptake, in contrast, was increased in the drug treated standard diet group only, indicating that the fructose diet may have prevented the proliferation of adrenal tissue though not the hypertrophy. In bone, hyperostosis was limited to the drug treated standard diet group indicating that this effect was prevented by the fructose diet. Additional effects on coagulating gland, prostate, and seminal vesicle weights were only

seen in the drug treated standard diet group, indicating the fructose diet prevented these effects.

Overall, the HF study provided sufficient data supporting the hypothesis that canagliflozin induced renal and adrenal cell proliferation was likely caused by canagliflozin induced inhibition of intestinal SGLT1 leading to carbohydrate malabsorption.

Methods

Doses	0 and 100 mg/kg/day with standard and fructose diets, 65 mg/kg/day with fructose diet
Frequency of dosing	daily
Route of administration	oral gavage
Dose volume	5 mL/kg
Formulation/Vehicle	aqueous suspension containing 0.5 % (w/v) Methocel (F4M Premium EP)
Species/Strain	SD(Crl: CD) rat
Number/Sex/Group	30 males/group (10/group killed at 1, 3, and 6 month time points)
Age	8 – 9 weeks at dosing initiation
Weight	299 – 411 g at dosing initiation
Satellite groups	15 (3/group for tk analysis)
Unique study design	yes – standard parameters + cell proliferation assessment (non-GLP) + bone biomarkers (non-GLP) + endocrinology
Deviation from study protocol	none specifically mentioned

Study Objective: It has been hypothesized that canagliflozin causes carbohydrate malabsorption at high doses by inhibiting intestinal SGLT1, leading to hypercalciuria and hyperostosis. The carbohydrate malabsorption and increased intestinal calcium absorption is also thought to play a role in the induction of the renal and adrenal tumors as was seen in the carcinogenicity study.

To test this hypothesis, rats were fed a glucose/galactose free 40% fructose diet. Since fructose is absorbed by the GLUT5 transporter that is not inhibited by JNJ-28431754-ZAE, it was postulated that the carbohydrate malabsorption and associated events would be prevented (e.g., effects on bone, urine calcium, and cell proliferation endpoints in the kidney and adrenal).

JNJ-28431754-ZAE was administered to rats maintained on either a standard diet or a 40% fructose diet at a dosage of 100 mg/kg, the same dosage associated with tumors in the kidney, adrenal medulla, and testes in the carcinogenicity study. An additional group was administered JNJ-28431754-ZAE at a dosage of 65 mg/kg on the fructose diet to match the exposure to that occurring in rats administered 100 mg/kg fed the standard diet. The groups used in the study are summarized below:

Group A:	Vehicle + Standard diet
Group B:	Vehicle + 40% Fructose diet
Group D:	65 mg eq./kg b.w./day + 40% Fructose diet
Group E:	100 mg eq./kg b.w./day + Standard diet
Group F:	100 mg eq./kg b.w./day + 40% Fructose diet

Observations and Results

Note that tox animals were housed 5/cage in each group.

Mortality:

- There was no test-article mortality. A total of 9 animals administered test-article were found dead or sacrificed during the course of the study.
- In the group administered 65 mg/kg, 1 animal was found dead on day 35. Postmortem examination revealed evidence of lung congestion and edema suggestive of gavage trauma. Additional animals were euthanized on days 38, 67, and 77 due to poor condition. Observations in these animals included decreased activity, circling movements, dyspnea, cold extremities, decubitus, ocular discharge, etc. in one or more affected animals.
- In the group administered 100 mg/kg and fed the standard diet, a single animal was sacrificed on day 161 due to poor condition (decreased activity, dehydration, piloerection, etc.).
- There were 4 animals sacrificed in the group administered 100 mg/kg and fed the fructose diet. Gavage trauma was the reason for the poor condition of an animal euthanized on day 36 based on the observation of esophageal perforation at necropsy. Additional animals euthanized demonstrated dyspnea, rough hair coat, narrowing of palpebral fissures, decreased activity, and/or cold extremities, etc.
- A single rat in the standard diet control TK group was euthanized, based on the appearance of crusty nose and piloerection, on day 49. Postmortem examination revealed brain malacia.

Reviewer Comment: Although there was no indication that treatment with test-article caused the death of animals, there appears to be an increase in clinical signs associated with drug treatment that led to the decision to euthanize animals. This is particularly true for the animals on the fructose diet. As there were minimal other clinical signs (see below), it is not clear why these animals were more affected by treatment.

Clinical Signs: Wet bedding was observed at a comparable incidence and duration from week 1 onward in all three groups administered JNJ-28431754-ZAE. This was associated with increased urine volumes related to the pharmacology of the compound. A yellow color to the feces was noted in all groups fed the fructose diet, including the controls, indicating it is related to the diet rather than to drug treatment. Soft feces were also noted in 1 or 2 cages of animals administered 100 mg/kg and fed the standard diet.

Body Weights: A slight loss of weight occurred at the start (day -8 to day -7) of feeding with the fructose diet, but the overall weight gains during the pre-dosing period were generally similar between the groups. Once treatment with JNJ-28431754-ZAE began, weight gain was lower in all drug treated groups leading to lower absolute weights. This was particularly true during the first 3 months of treatment. The group affected the most was the 100 mg/kg group fed the standard diet; effects on the two groups fed the fructose diet were similar even though the dosages were different. Coincident with the start of treatment, animals in the fructose control group gained more weight than the standard diet control group and continued to do so throughout the study. As a result, it is not possible to clearly establish how much of the weight gain in the drug treated fructose groups was due to test article or the fructose, even though absolute body weights remained lower than controls. Body weights and body weight gain data for representative periods are tabulated in the reviewer created table below:

day/interval	S0	F0	F65	S100	F100
		Body Weight/Body Weight Gain Diet/Dosage Group[1]			
-8	305	303	305	304	304
-7	310	301*	300**	305	300**
-1	346	339	341	341	342
0	355	345	348	351	351
7	382	381	357***	329***	360**
28	444	450	411***	400***	421*
91	542	557	482**	457***	487**
182	609	636	555	530*	559
-8 - -1	41	36	36	38	38
0 - 7	27	36**	9***	-22***	9***
0 - 28	89	105**	64***	49***	69***
0 - 91	189	217	140**	110***	139**
0 - 182	260	301	213	174**	216

1) S = standard diet; F = fructose diet; 0, 65, 100 = dose level
* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$ vs. S0 group

Feed Consumption: Food consumption was increased from the 1st week of dosing onwards and remained increased during the entire study at all dose levels tested. Control animals administered the fructose diet consumed slightly less than animals administered the standard diet (typically ~0.92x). Groups administered test-article consumed more than either control group (typically in the range of 20% to 35% more each week). The diet had minimal effect as

the consumption of the drug treated fructose fed rats ranged from 91% to 99% of the consumption of drug treated rats fed the standard chow.

Ophthalmoscopy: NA

Hematology: Slight decreases (~25% - 40% lower) in reticulocyte counts were noted in animals administered 100 mg/kg fed the standard diet relative to the standard diet control at the 1, 3, and 6 month time-points. Slight decreases in RBC, Hb, and Hct counts (~8% - 12% lower) were also evident at the 6-month time-point. In animals fed the fructose diet, the only effect noted was a decrease of ~12% in reticulocyte counts in the 100 mg/kg fructose group relative to the fructose control, seen only at the 6 month time-point. No effects were noted on coagulation parameters.

Clinical Chemistry:
- Control animals fed the fructose diet had higher levels of cholesterol, triglycerides, BUN, and ALP values at each time point as compared to control animals fed the standard diet. The increases in Chol, BUN, and ALP were all < 2x while the increases in Tri ranged from 3.7 to 4x.
- Treatment of standard diet animals with 100 mg/kg of JNJ-28431754-ZAE resulted in increased Ca, Phos, BUN, ALP, and ALT values, with a maximum increase of < 60% relative to standard diet controls. In animals on the fructose diet administered 65 or 100 mg/kg of JNJ-28431754-ZAE, increases of < 72% in BUN, ALP, and ALT levels relative to the fructose control group occurred at the various time-points but no effects on Ca or inorganic phosphorous levels. For both dietary treatments, Chol and Tri levels were lower in the drug treated groups than in their respective controls.
- When the groups administered test article were compared to each other, the fructose fed group showed higher BUN and ALP and lower levels of Ca, Phos, and ALT. These data suggested that the drug related increases in Ca and Phos levels can be prevented by the fructose diet.
- Data for each time point are shown in the Sponsor tables below:

Month 1 Sacrifice

Parameter	Unit	A	B	D:65	E:100	F:100
				Dosage Groups (mg eq/ kg) MALES		
Sodium	mmol/l	143 (0)	141 (0) **	142 (0)	142 (0) +	141 (0) *
Potassium	mmol/l	5.6 (0.1)	5.2 (0.1) *	5.0 (0.1) ***	5.8 (0.1) +++	5.2 (0.1) **
Chloride	mmol/l	104 (0)	103 (0) *	103 (0)	104 (0) ++	104 (0) ++
Calcium	mg/dl	9.7 (0.1)	10.0 (0.1) **	9.9 (0.1)	10.3 (0.1) *** +	9.8 (0.1)
Inorg. phosphorus	mg/dl	6.2 (0.1)	6.6 (0.2)	6.5 (0.1)	7.3 (0.2) *** ++	6.6 (0.1) *
Total protein	g/dl	5.1 (0.1)	5.6 (0.1) **	5.3 (0.1)	5.2 (0.0) +++	5.2 (0.1) +
Albumin	g/dl	3.7 (0.1)	3.8 (0.1)	3.6 (0.1)	3.7 (0.1)	3.5 (0.1)
Glucose	mg/dl	159 (7)	141 (5)	143 (4)	142 (3)	143 (3)
Cholesterol	mg/dl	65 (2)	80 (6) *	71 (3)	54 (3) * +++	71 (2)
Triglycerides	mg/dl	172 (5)	680 (72) ***	474 (63) *** +	105 (8) *** +++	474 (63) ***
Urea nitrogen	mg/dl	18.1 (0.4)	24.0 (0.9) ***	31.5 (1.3) *** +++	26.0 (0.9) ***	37.0 (1.8) *** +++
Creatinine	mg/dl	0.21 (0.01)	0.19 (0.01)	0.18 (0.01) *	0.18 (0.01)	0.19 (0.01)
Total bilirubin	mg/dl	0.06 (0.00)	0.05 (0.00)	0.06 (0.01)	0.04 (0.00) *	0.05 (0.01)
Alk. phosphatase	U/l	97 (6)	154 (6) ***	181 (7) *** +	150 (10) ***	180 (8) *** +
Aspartate aminotransferase	U/l	112 (3)	101 (4)	108 (4)	121 (4) ++	123 (13)
Alanine aminotransferase	U/l	43 (1)	36 (2) *	53 (2) *** +++	66 (4) *** +++	62 (6) * +++
Gamma glutamyl transferase	U/l	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

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

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Month 3 Sacrifice

Parameter	Unit	A	B	D:65	E:100	F:100
						Dosage Groups (mg eq/ kg)
					MALES	
Sodium		144	143	143	145	144
	mmol/l	(0)	(0)	(0)	(0)	(1)
				*	+	
Potassium		5.4	5.2	5.0	5.7	5.2
	mmol/l	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)
				**	* ++	
Chloride		106	104	105	106	106
	mmol/l	(0)	(1)	(0)	(0)	(0)
			*		+	+
Calcium		9.9	10.0	9.8	10.5	9.9
	mg/dl	(0.0)	(0.1)	(0.1)	(0.1)	(0.1)
					*** ++	
Inorg. phosphorus		5.7	6.1	5.9	7.2	6.1
	mg/dl	(0.2)	(0.1)	(0.1)	(0.1)	(0.2)
					*** +++	
Total protein		5.6	5.8	5.4	5.4	5.5
	g/dl	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)
				+	++	+
Albumin		3.6	3.5	3.2	3.5	3.3
	g/dl	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)
				*		*
Glucose		149	150	155	139	148
	mg/dl	(6)	(5)	(5)	(4)	(3)
Cholesterol		61	87	71	57	77
	mg/dl	(2)	(7)	(6)	(2)	(4)
			***	+	+++	**
Triglycerides		138	532	465	119	580
	mg/dl	(13)	(69)	(46)	(9)	(77)
			***	***	+++	***
Urea nitrogen		18.9	22.4	31.6	25.4	30.9
	mg/dl	(0.6)	(0.7)	(1.2)	(1.2)	(0.9)
			**	*** +++	*** +	*** +++
Creatinine		0.21	0.20	0.21	0.21	0.18
	mg/dl	(0.01)	(0.00)	(0.01)	(0.01)	(0.01)
						*
Total bilirubin		0.06	0.06	0.07	0.05	0.06
	mg/dl	(0.00)	(0.01)	(0.01)	(0.00)	(0.01)
					+	
Alk. phosphatase		87	117	168	111	188
	U/l	(7)	(8)	(5)	(8)	(12)
			*	*** +++	*	*** +++
Aspartate aminotransferase		99	91	87	111	93
	U/l	(4)	(6)	(4)	(4)	(3)
				*	+	
Alanine aminotransferase		47	36	47	72	56
	U/l	(2)	(2)	(3)	(4)	(3)
			**	++	*** +++	* +++
Gamma glutamyl transferase		0	0	0	0	0
	U/l	(0)	(0)	(0)	(0)	(0)

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

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Terminal Sacrifice

Parameter	Unit	A	B	D:65	E:100	F:100
Sodium	mmol/l	144 (0)	142 (0) **	143 (0)	144 (0) ++	143 (1)
Potassium	mmol/l	5.8 (0.1)	5.7 (0.1)	5.5 (0.1)	5.8 (0.1)	5.3 (0.1) * +
Chloride	mmol/l	103 (0)	102 (0)	103 (1) +	104 (1) ** ++	103 (0) ++
Calcium	mg/dl	9.9 (0.1)	9.8 (0.1)	9.8 (0.1)	10.4 (0.1) * ++	9.8 (0.1)
Inorg. phosphorus	mg/dl	5.8 (0.1)	5.4 (0.1)	5.9 (0.2)	6.9 (0.2) *** +++	5.8 (0.1)
Total protein	g/dl	5.9 (0.1)	5.9 (0.1)	5.8 (0.1)	5.6 (0.1) *** ++	5.7 (0.1)
Albumin	g/dl	3.6 (0.1)	3.4 (0.1)	3.4 (0.1)	3.4 (0.1)	3.2 (0.1) *
Glucose	mg/dl	150 (5)	160 (5)	146 (4)	152 (2)	151 (3)
Cholesterol	mg/dl	67 (4)	83 (5) *	77 (5)	59 (3) ++	71 (5)
Triglycerides	mg/dl	121 (14)	458 (52) ***	396 (65) ***	134 (20) +++	321 (78) **
Urea nitrogen	mg/dl	17.5 (0.6)	21.3 (0.5) ***	28.2 (1.4) *** +++	27.6 (0.8) *** +++	29.3 (1.3) *** +++
Creatinine	mg/dl	0.23 (0.01)	0.21 (0.00)	0.21 (0.01)	0.23 (0.01)	0.21 (0.01)
Total bilirubin	mg/dl	0.07 (0.01)	0.07 (0.01)	0.08 (0.01)	0.06 (0.00)	0.08 (0.01)
Alk. phosphatase	U/l	75 (5)	97 (9) *	157 (17) *** ++	100 (4) **	186 (22) *** ++
Aspartate aminotransferase	U/l	113 (9)	96 (6)	116 (7) +	139 (17) ++	99 (6)
Alanine aminotransferase	U/l	44 (2)	34 (1) **	53 (2) * +++	63 (8) * +++	58 (7) +++
Gamma glutamyl transferase	U/l	0 (0)	0 (0)	0 (0)	1 (0)	0 (0)

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

Urinalysis/Microalbumin: A consistent pattern of effects were seen at each measurement interval. Specific gravity and volume were increased while pH was reduced. In animals fed the fructose diet, proteins, phosphate (adjusted for creatinine), and NAG levels were increased while Na, K, Cl, and Ca levels (adjusted for creatinine) were all decreased compared to animals fed the standard diet. In all groups administered test article, glucose levels were increased as

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were Na, Cl, phosphate, NAG, and protein. In general, K and Mg levels were increased in the 100 mg/kg standard diet group but the increases were either smaller in magnitude (K) or levels decreased (mg) in the drug treated groups fed the fructose diet, though the levels were still higher than those recorded in the fructose controls. The level of Ca was increased only in the group administered 100 mg/kg and fed the standard diet; there was no increase in Ca levels in treated animals maintained on the fructose diet. Thus, feeding rats a fructose based diet prevented the excess calcium excretion induced by JNJ-28431754-ZAE when administered to rats maintained on a standard diet.

Urinary microalbumin and creatinine levels were also measured. At the timepoints evaluated, creatinine levels in the drug treated groups were lower than concurrent controls. Albumin levels tended to remain constant or increase in the drug treated groups relative to controls, although decreases were noted for a few time points. As a result of the alterations in creatinine and/or albumin levels, slight elevations in the urinary albumin/creatinine ratios were noted. The ratio data are summarized in the Sponsor table below:

Text Table 4.1 – 1. Urinary Albumin/Creatinine Ratio in Rats Receiving JNJ-28431754-ZAE[1]

Group	A	B	D	E	F
Dose (mg eq/kg/day)	0	0	65	100	100
Diet	Standard	Fructose	Fructose	Standard	Fructose
Month 1, 6 hr	0.02	0.02 (-)	0.04 (2X)	0.03 (+50%)	0.04 (2X)
Month 1, 18 hr	0.01	0.01 (-)	0.03* (3X)	0.03* (3X)	0.03*† (3X)
Month 3, 6 hr	0.02	0.03 (+50%)	0.09 (4.5X)	0.09 (4.5X)	0.07*† (3.5X)
Month 3, 18 hr	0.01	0.02 (2X)	0.04 (4X)	0.06 (6X)	0.07*† (7X)
Month 6, 6 hr	0.02	0.04 (2X)	0.07* (3.5X)	0.07 (3.5X)	0.12*† (6X)

[1]Mean and % difference or fold change from Group A (0 mg eq./kg/day standard diet)
*Statistically significant vs Group A (0 mg eq./kg/day standard diet)
†Statistically significant vs Group B (0 mg eq./kg/day fructose diet)
-No difference from Group A

Gross Pathology: There were effects on the intestinal track and liver associated with the fructose diet. In the intestinal track, animals maintained on the fructose diet (with or without JNJ-28431754-ZAE) had pale or yellow cecum contents that occasionally were of a firm consistency in the colon or rectum; a small cecum was also observed in some rats. Pale discoloration of the liver with swelling and a low incidence of prominent lobular pattern were also attributed to the fructose diet.

Treatment with JNJ-28431754-ZAE at 100 mg/kg body weight/day combined with the standard diet resulted in distention and soft contents of the cecum and/or colon and a low incidence of pale kidney discoloration. Similar effects were not observed in the drug treated fructose diet groups.
Animals administered JNJ-28431754-ZAE and fed either diet showed pale discoloration and/or swelling of the adrenal glands, kidney swelling (associated with increased weights) and a tendency towards increased pelvic dilatation (particularly at 6 months) and bladder dilatation, with a more frequent occurrence in the fructose diet groups than in the standard diet group. Key observations are tabulated in the Sponsor table below:

Tabulated overview of most relevant macroscopic changes (Table 4):

Terminally killed rats including deaths															
Dose group	A			B			D			E			F		
Diet	SD			FD			FD			SD			FD		
Dose (mg eq./kg/day)	0			0			65			100			100		
Time-point (months)	1	3	6	1	3	6	1	3	6	1	3	6	1	3	6
Number of animals	10	10	10	10	10	10	10	9	11	10	10	10	10	10	10
Adrenal glands															
- pale		1		1		1	4	2	4	2	3	5	5	5	6
- swollen				1			4	1	2		1	1	2	1	3
Kidneys															
- swollen							7	3	4		1	3	6	3	2
- pelvic dilatation		1						1	2		3	1	*1*	2	2
- pale									*1*	1	2	3			
Cecum															
- contents abnormal (pale or yellow)				6	5	1	8	6					7	7	2
- contents soft	3	1								9	3	7			
- distention	3	3	1				*1*			8	9	6			
- small				2		4			2			*1*			2
Colon and/or rectum															
- contents abnormal (pale, yellow and/or firm)				5	9	3	5	8	4				8	7	5
- contents soft										3		3			
- distention	1									3	1	3			
Liver															
- discoloration: pale			1	9	7	5	6	2	3		*1*		5	4	5
- swollen		1	2	2	6	5	3	5	2		1	1	4	6	5
- prominent lobular pattern					1	1		2					1	1	2
Urinary bladder															
- dilatation						1	1	2	4			3		1	4

Note: swelling = enlargement
italic: not considered toxicologically relevant

Organ Weights: At the three different necropsy time points, a similar pattern of effects were observed, although no always reaching statistical significance. Adrenal and kidney weights

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(absolute and body weight adjusted) were increased in the 3 drug-treated groups relative to the standard diet group. Absolute adrenal and kidney weights were also increased in the fructose diet control group, but the increases did not attain statistical significance following adjustment for body weight. The increases in organ weights were slightly greater in the drug treated fructose diet groups than in the drug treated standard diet group at months 1 and 3, but not at 6 months. In addition, coagulating gland, prostate, and seminal vesicle weights were reduced only in the drug treated standard diet group at each time point, indicating that the fructose diet may have protected against the induction of these effects. Testicular weights were increased in this same group, but only at the 3-month necropsy.

Histopathology
Battery Considered Adequate: yes
Peer Review Performed: yes

Microscopic effects were observed in the adrenal gland, bone, and kidney at each time point. In the adrenal gland, hypertrophy/vacuolization of the zona glomerulosa was observed in all drug treated groups and the fructose control group, but not the standard diet control group. The incidence was highest in the 2 drug treated and fructose fed groups. This observation is considered to be a response to the secretion of mineralocorticoids involved in electrolyte and water homeostasis that the zona glomerulosa is involved with. In addition, hypertrophy of the zona fasciculata was also seen in the 3 drug treated groups, most frequently at 6 months. Again this is likely a pharmacologic response probably related to increased secretion of glucocorticoids, secondary to the urinary loss of glucose.

Hyperostosis occurred in the sternum and stifle of virtually all rats administered 100 mg/kg and maintained on the standard diet, an effect considered related to increased intestinal calcium absorption. Similar observations were found in only single rats administered 65 or 100 mg/kg and fed the fructose diet, suggesting that the fructose diet prevented this effect.

In the kidney, tubular dilation was observed in the cortex and outer medulla of almost all rats administered the test article at each time point, regardless of dietary treatment. Dilation of the renal pelvis was also seen in some animals from all drug treated groups, but not to the same extent as the tubular dilation. Both effects were considered drug related, even though pelvic dilation was also observed in 1 or 2 animals administered either the standard or fructose diet alone. Swollen/vacuolated tubules and exfoliated renal tubular cells were also observed in drug treated rats fed the standard diet. Feeding a fructose diet decreased the occurrence of these effects, but did not completely eliminate them. Immunohistochemistry showed an increased incidence of KIM-1 positivity in the cortical tubules of all three drug treated groups at both 1 and 3 months. In the outer stripe of the outer medulla (OSOM), slightly more animals in the 100 mg/kg group fed the standard diet were affected than in the drug treated groups fed the fructose diet at 1 month, but the incidence was similar by 3 months. In the standard diet controls, one-half of the animals were positive at 1 month, but only a single animal was positive at 3 months. In the fructose controls, the majority of animals showed a positive response at each time point.

The incidences of relevant microscopic changes are summarized in the Sponsor table below:

Tabulated overview of most relevant microscopic changes (Table 5):

Terminally killed rats including deaths

Dose group	A			B			D			E			F		
Diet	SD			FD			FD			SD			FD		
Dose (mg eq./kg/day)	0			0			65			100			100		
Time-point (months)	1	3	6	1	3	6	1	3	6	1	3	6	1	3	6
Number of animals	10	10	10	10	10	10	10	9	11	10	10	10	10	10	10
Adrenal glands															
- Hypertrophy/vacuolization zona glomerulosa				1	1	2	7	8	10	2	2	5	10	10	10
- Hypertrophy zona fasciculata		1				1			4	1	2	3		1	3
Sternum: hyperostosis										8	8	8			
Stifle: hyperostosis								1		9	9	9		1	
Kidneys															
- CPN						3			3			2			3
- Dilated tubules							9	9	11	10	10	10	10	10	10
Grade 1							7	8	5	7	5	4	7	6	5
Grade 2							2	1	6	3	5	5	3	4	4
Grade 3												1			1
- Dilated pelvis	1		1		1	1	2	2	5	2	4	4	6	4	3
- Swollen/vacuolated tubules		1								2	8	5		4	1
- Exfoliated cells									1		4	7			
- *Hyperplasia pelvic epith.*			*1*			*1*				*1*	*1*	*3*			*2*
KIM-1 IHC*															
KIM-1 cortex multifocal	1				3		7	9		8	10		6	6	
Grade 1	1				3		7	9		8	8		6	6	
Grade 2											2				
KIM-1 OSOM multifocal	5	1		6	9		8	9		10	9		8	9	
Grade 1	4	1		6	7		7	9		5	4		6	4	
Grade 2	1				2		1			4	3		2	5	
Grade 3										1	2				
KIM-1 diffuse															
Grade 3 (unilateral)											2				

italic: not considered toxicologically relevant, but worth mentioning

*: for 6-month dosing period: see IHC-report

Additional immunohistochemistry analyses of selected tissues (kidney, adrenal, and bladder) measuring BrdU labeling and double-labeling to evaluate tissue proliferation, and KIM-1 at 6

months, was also performed separate from the main study. In the submission occasionally, the sponsor had referenced TIM-1 instead of KIM-1. They are the same but generally known as KIM-1. At the 1-month time point, there were no statistical differences in total kidney (cortex + medulla) uptake, although the level in the cortex was increased over control in the 100 mg/kg group fed the standard diet. In the adrenal, statistically significant increases were seen in the 100 mg/kg group fed the standard diet compared to the standard diet control and compared to the two drug treated fructose diet groups. The drug treated fructose groups were comparable to the fructose controls. The levels in the bladder were not interpretable as measurements were abnormally high in all groups, including controls, combined with high individual variability. Data are shown in the modified (to show only total counts for the kidney and to remove non-significant p-values for kidney and adrenal) Sponsor table and figures below:

Month 1

Table 4. a summary of mean value

Groups	BrdU-K-Total	BrdU-A
A	160±49	179±76
B	201±42	174±52
D	181±35	186±77
E	202±37	320±167
F	169±49	161±59
A vs E		p<0.01
A vs B		
A vs D		
A vs F		
B vs D		
B vs F		
E vs D		p<0.05
E vs F		p<0.01

Cx: cortex, OSOM: outer stripe outer medulla, K: kidney; A: adrenal

Figure 3. Kidneys: BrdU Labeling in the Total Area Tubular Epithelial Cells

Bonferroni's Multiple Comparison Test

G-A vs G-E, p<0.05; G-B vs G-D, p<0.05
G-A vs G-B, p<0.05; G-B vs G-F, p<0.05
G-A vs G-D, p<0.05; G-E vs G-D, p<0.05
G-A vs G-F, p<0.05; G-E vs G-F, p<0.05

Figure 4. Adrenal Medulla: BrdU Labeling in the Chromaffin cells

Bonferroni's Multiple Comparison Test

G-A vs G-E, p<0.05
G-A vs G-B, p<0.05
G-A vs G-D, p<0.05
G-A vs G-F, p<0.05
G-B vs G-D, p<0.05
G-B vs G-F, p<0.05
G-E vs G-D, p<0.05
G-E vs G-F, p<0.01

At month 3, similar increases in BrdU uptake were noted in the kidney and adrenal, with the 100 mg/kg group fed the standard diet showing increased counts relative to the standard diet control and or 1 or both drug treated fructose groups. The increase in counts in the kidney was related to increases in count in both the cortex and the medulla. In the adrenal, the 100 mg/kg standard diet group showed increased counts relative the standard diet controls and the drug treated fructose group; the drug treated fructose groups were similar to the fructose control. There was no indication of proliferation in the bladder. Data are shown in the modified Sponsor table below:

Month 3

Table 4. a summary of mean value

Groups	BrdU-K-Total	BrdU-A	BrdU-B
A	108±25	70±40	37±72
B	152±55	83±40	19±54
D	140±34	83±69	10±18
E	231±85	157±64	41±86
F	156±31	106±61	22±46
A vs E	p<0.001	p<0.01	
A vs B			
A vs D			
A vs F			
B vs D			
B vs F			
E vs D	p<0.01	p<0.05	
E vs F	p<0.05		

Cx: cortex, OSOM: outer stripe outer medulla, K: kidney; A: adrenal, B: bladder, and # means the mean value ± a standard deviation

Figure 3. Kidneys: BrdU Labeling in the Total Area Tubular Epithelial Cells — Bonferroni's Multiple Comparison Test

GA vs GE, p<0.001
GA vs GB, p>0.05
GA vs GD, p>0.05
GA vs GF, p>0.05
GB vs GE, p>0.05
GB vs GF, p>0.05
GE vs GD, p<0.01
GE vs GF, p<0.05

Figure 4. Adrenal Medulla: BrdU Labeling in the Chromaffin cells — Bonferroni's Multiple Comparison Test

GA vs GE, p<0.01
GA vs GB, p>0.05
GA vs GD, p>0.05
GA vs GF, p>0.05
GB vs GD, p>0.05
GB vs GF, p>0.05
GE vs GD, p>0.05
GE vs GF, p>0.05

At 6 months, increased counts in both the kidney cortex and medulla resulted in increases in total counts in the 100 mg/kg standard diet group compared to control and the fructose treated groups. The KIM-1 labeling showed an analogous pattern. In the adrenal, similar observations to those reported at the earlier time frames were noted. Bladder was not evaluated. Data are summarized in the modified Sponsor table below:

Month 6

Table 4. a summary of mean value

Groups	BrdU-K-Total	TIM-1-Total	BrdU-A
A	79±20	0.05909±0.02622	38±22
B	119±22	0.15783±0.10653	32±20
D	129±12	0.17957±0.07230	56±27
E	205±75	0.72311±0.59753	86±30
F	143±46	0.29978±0.20101	55±31
A vs B			
A vs D			
A vs E	p<0.001	p<0.001	p<0.001
A vs F	p<0.05		
B vs D			
B vs F			
E vs D	p<0.01	p<0.01	
E vs F	p<0.05	p<0.05	

Cx: cortex, Mdu: outer stripe outer medulla (OSOM), K: kidney; A: adrenal and # means the mean value ± a standard deviation

Figure 3. Kidneys: BrdU Labeling in the Total Area Tubular Epithelial Cells — Bonferroni's Multiple Comparison Test

GA vs GE, p<0.001
GA vs GB, p>0.05
GA vs GD, p>0.05
GA vs GF, p>0.05
GB vs GD, p>0.05
GB vs GF, p>0.05
GE vs GD, p<0.01
GE vs GF, p>0.05

Figure 4. Kidneys: TIM-1 Labeling in the Cortical Tubular Epithelial Cells — Bonferroni's Multiple Comparison Test

GA vs GE, p<0.001
GA vs GB, p>0.05
GA vs GD, p>0.05
GA vs GF, p>0.05
GB vs GD, p>0.05
GB vs GF, p>0.05
GE vs GD, p<0.01
GE vs GF, p>0.05

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Special Evaluations

Bone Biomarkers: As part of the study, 1,25dihydroxy vitamin D and parathyroid hormone levels (PTH) were measured.

Vitamin D and PTH levels were higher in the fructose control group than in the standard diet control group at each time point. Treatment with JNJ-28431754-ZAE led to lower levels of each parameter relative to the control group fed the same diet, although not all values were statistically different. Data are shown in the reviewer modified Sponsor tables below:

		After 1 month of Dosing					After 3 month of Dosing	
Group	Summary Information	Vit D pmol/L	PTH pg/mL		Group	Summary Information	Vit D pmol/L	PTH pg/mL
A	Mean	145.06	404.95		A	Mean	76.20	339.38
	SD	60.14	158.90			SD	53.49	129.97
	N	10	10			N	10	10
B	Mean	359.63 **	486.11		B	Mean	97.44	630.29
	SD	124.97	254.67			SD	39.11	398.73
	N	10	10			N	10	10
D	Mean	133.91 ^^	364.43		D	Mean	26.50* ^^	354.86
	SD	82.81	112.25			SD	0.00	225.31
	N	9	9			N	9	9
E	Mean	26.50 *** ^^^	75.57*** ^^^		E	Mean	26.50* ^^^	61.28*** ^^^
	SD	0.00	46.10			SD	0.00	34.08
	N	10	10			N	10	10
F	Mean	80.70 ^^^	293.63		F	Mean	26.50* ^^	324.00
	SD	46.93	126.08			SD	0.00	154.69
	N	10	10			N	9	9

		At the End of the 6 month Dosing	
Group	Summary Information	Vit D pmol/L	PTH pg/mL
A	Mean	30.18	314.81
	SD	11.64	80.01
	N	10	10
B	Mean	35.09	435.86*
	SD	18.90	125.93
	N	10	10
D	Mean	26.50	281.58
	SD	0.00	104.90
	N	8	8
E	Mean	26.50	40.42*** ^^^
	SD	0.00	12.37
	N	9	9
F	Mean	26.50	301.24
	SD	0.00	177.48
	N	7	7

Group A - Control Standard Diet
Group B - Control Fructose Diet
Group D - JNJ-28431754-ZAE 65 mg eq./kg b.w/day (Fructose Diet)
Group E - JNJ-28431754-ZAE 100 mg eq./kg b.w/day (Standard Diet)
Group F - JNJ-28431754-ZAE 100 mg eq./kg b.w/day (Fructose Diet)

Group B, D, E, F significantly different from Group A value:
* - $P' \leq 0.05$ ** - $P' \leq 0.01$ *** - $P' \leq 0.001$ (adjusted Wilcoxon)

Group D, E, F significantly different from Group B value:
$^{Y} \leq 0.05$ ^^ - $P' \leq 0.01$ ^^^ - $P' \leq 0.001$ (adjusted Wilcoxon)

Reviewer Comment: The Sponsor claims that feeding the fructose diet prevented the drug induced reduction in PTH levels seen in drug treated animals. Although this statement is true based on statistical analysis, the drug treated fructose groups still showed reductions of 25% to 49% as compared to an 82% reduction in the drug treated standard diet group from their respective controls.

Reproductive Hormones: The levels of LH and testosterone were measured at the 1 month time point. No effects were noted on the levels of either hormone.

Toxicokinetics: Blood samples were obtained at the completion of the study. In the drug treated fructose groups, exposure (both C_{max} and AUC) increased in a slightly less than proportional manner. Peak plasma concentrations were reached between 1.4 and 4.3 hours after dosing. These results also confirmed that exposure to test article at 100 mg/kg in the standard diet was comparable to 65 mg/kg in the fructose diet. Data are tabulated in the Sponsor table below:

Diet	Group	Dose (mg eq./kg b.w./day)	C_{max} (ng/mL)	t_{max} (h)	AUC_{0-24h} (h*ng/mL)
Fructose	D	65	31200 (2760)	1.33 (0.577)	421000 (32800)
Fructose	F	100	38700 (8600)	4.33 (2.52)	618000 (113000)
Standard	E	100	28900 (4720)	3.33 (1.15)	421000 (116000)

Standard diet ad libitum
Fructose diet ad libitum (glucose/galactose free diet)

Dosing Formulation Analysis: Dosing formulations were uniform and homogenous under conditions of the study.

Study title: 6-Month repeated dose oral and subcutaneous toxicity study of JNJ-28431754 in the male rat under standard diet and low calcium diet feeding conditions with a 1-month and 3-month interim kill

Study no.:	TOX10168 (EDMS-ERI-42074433)
Study report location:	JNJ, Raritan, NJ
Conducting laboratory and location:	Drug Safety Sciences, Beerse site Turnhoutseweg 30 B-2340 Beerse, Belgium
Date of study initiation:	Aug 16, 2011
GLP compliance:	yes
QA statement:	Yes
Drug, lot #, and % purity:	ZR600348PFA021, 97.6%

Key Study Findings

- Canagliflozin (all routes and diet type) significantly increased urine volume, urinary glucose and decreased body weight in male rats. The increased food intake was inadequate to prevent the urinary glucose-associated caloric loss.
- Canagliflozin by SC route was not well tolerated thus the SC groups were terminated prematurely at 3 month.

- SC route did not prevent glucose malabsorption and associated hypercalcium absorption, hypercalciuria and hyperostosis.
- Low Ca diet (0.1% Ca, 1/10 of normal calcium content) substantially reduced canagliflozin-induced increase in Ca absorption (much less at 3 and 6 months).
- Low Ca diet partially reversed Canagliflozin induced increase in hyperostosis.
- Low Ca diet appeared to have minimal effect on canagliflozin induced intestinal distention and soft content at 1 and 3 months.
- Low Ca diet did not improve canagliflozin induced renal changes marked by exfoliated cells and increase KIM-1 positivity.
- Low Ca diet did not prevent canagliflozin-induced proliferation of renal and adrenal cells. The study failed to show preventing Ca will block canagliflozin induced renal or adrenal cell proliferation. However, it did provide evidence supporting hypercalcium absorption as the potential cause of bone hyperostosis.

PK
- The Tmax after SC (2.3 to 2.6 hr) was shorter than after oral gavage (4 to 4.3 hrs) suggesting rapid absorption after SC than oral route. The diet itself had no notable impact on Tmax.
- The AUC and Cmax tend to be higher in rats 0.1% Ca diet whether the drug was administered by SC or oral route. Overall, the highest exposure was achieved by oral route in low Ca diet fed rats.

Reviewer Comments:

The low Ca diet reduced hyperostosis and calcium excretion but had minimal effect on drug-induced renal hyperplasia biomarkers suggesting that low Ca diet may not necessarily reverse canagliflozin induced renal changes noted in rats with chronic canagliflozin treatment. Whether lack of preventive effect of low Ca diet was due to other factors remains unresolved.

Methods   Doses: 0 and 100 mg/kg/day by oral and SC in standard and low Ca fed rats
                                    Treatment are abbreviated

Frequency of dosing: Daily

Route of   Oral gavage and Subcutaneous (SC)
administration:

Dose volume: 5 ml/kg/day for gavage and 10µl/ml for SC infusion

Formulation/Vehicle   0.5% w/v aqueous Methocel for oral route
:   16% HP-ß-CD (hydroxypropyl-ß-cyclodextrin) and 20% v/v ethanol in water

Species/Strain: Sprague Dawley rats (Crl:CD® (SD) IGS),   (b) (4)

Number/Sex/Group: 30/group, 7 groups

Age: 8 weeks old at the time of treatment

Weight: 274-348 g male rats

Satellite groups: 3 /group

Unique study design: This was a mechanistic study designed to assess whether a low Ca diet
                                    (0.1%) could prevent canagliflozin- induced hyperosteosis and other
                                    treatment related cell proliferation (kidney, adrenal and urinary bladder).
                                    There were 7 groups with 30 rats per group. Rats were treated with 100
                                    mg/kg by either oral or SC route. Male Sprague-Dawley rats were
                                    maintained on normal (ND) or low Ca diet (LC) for 6 month with interim
                                    sacrifice at 1 (Sept 21, 2011) and 3 months (Nov 23, 2011).   Due to SC
                                    injection site irritation/lesions, the SC rats were all sacrificed at the end of
                                    the 3 months rather. Only oral route rats reached the 6-month duration
                                    objective (Feb 23, 2012).   TK rats (3 rats/group, 7 groups) were treated in
                                    the same manner with data collected on Day 92 and 181. ALZET pumps
                                    were implanted about a week before the treatment. Pumps were loaded
                                    with 50 mg/ml of BrdU (5-bromo-2'-deoxyurindine, 10 µl/hr) to determine
                                    cell proliferation. The historical data was provided with the study.
                                    In addition to hematology, clinical chemistry and urinalysis, several renal
                                    and bone biomarkers were measured at 1, 3 and 6 months (urine N-acetyl
                                    glucosamine [NAG], gamma glutamyltransferase and serum PTH & VitD).
                                    Organ weight, gross necropsy, histopathology and immunohistochemistry
                                    analysis was performed at 1, 3 and 6 months.

Group A	ND-OR	Oral Vehicle + Standard diet (ND)
Group B	LC-OR	Oral Vehicle + 0.1% Low calcium diet (LC)
Group D	ND-ORHD	100 mg eq./kg b.w./day Oral (ORHD) + Standard diet
Group E	LC-ORHD	100 mg eq./kg b.w./day Oral + 0.1% Low calcium diet
Group F	ND-SC	Subcutaneous Vehicle + Standard diet
Group G	ND-SCHD	100 mg eq./kg b.w./day Subcutaneous + Standard diet
Group H	LC-SCHD	100 mg eq./kg b.w./day Subcutaneous + 0.1% Low Calcium diet

Observational endpoints/timing

Clinical Findings	Once a day
Body weights	Weekly
Food consumption	Weekly
Hematology	Under fasted conditions at 1-, 3- and 6-months
Clinical chemistry	Under fasted conditions at 1, 3- and 6-months
Urinalysis	Under fasted (food deprivation) at 1-, 3- and 6-months
Gross pathology	At the end of the 1-, 3- and 6-months (oral route)
Organ weights	At the end of the 1-, 3- and 6-months (oral route)

Histopathology

Adequate Battery: yes (x), no ( ) Peer review: yes

organ weights, tissues preserved and histopathology					
Tissue	Weighed	Fixed	HE	BrdU	Microscopy
animal identification		F			
administration site (F, G and H)		F	X		X
adrenal glands	W	F*	X	X	X
bone, sternum		F	X		X
bone, stifle, bilateral		both/F	X		unilateral
brain	W				
coagulating gland(s) + seminal vesicles + prostate#	W	F			
epididymides		F/Dav^x			
kidneys: both transverse sections	W	F*	X	X@	X
large intestine, cecum		F			
liver		F			
pancreas		F			
pituitary gland (Groups A and D: ½ frozen)		F*			
prostate (separately)	W	F			
small intestine, jejunum (on slide with kidneys)		F*	X	X pos. control for BrdU	
testes (both on 1 slide)	W	F*/Dav^x	X		X
urinary bladder		F*	X (1 month)	X (2 levels) (1 month)	X (1 month)
all tissues showing gross lesions		F	X tbd		X tbd

*		At terminal kill, these tissues were fixed in formalin for ≤ 48 hours and embedded
F		10% buffered formalin
Dav		Davidon's fixative
W		weighed in all terminally killed animals
X		processed and evaluated in all groups
@		additional staining for KIM-1 IHC
#		after weighing coagulating gland(s) + seminal vesicles + prostate, the prostate was dissected and weighed separately; the weight of coagulating gland(s) + seminal vesicles was calculated as the difference between the total weight minus prostate weight
x		after 1 and 3 months: fixed in formalin; after 6 months: fixed in Davidson's

Observations and Results

Mortality

- There were no drug-related deaths. Deaths seen in LC diet canagliflozin rat were likely related to diet / SC route.
- Rat #24 given 100 mg/kg SC canagliflozin fed LC diet was found dead on Day 10. Hemorrhagic GI content in this rat with no histopathology was likely related to LC diet. Rat #197 was sacrificed on Day 19 due to poor condition. Seven SC canagliflozin were terminated on Day 86 due to injection site reactions. SC canagliflozin rats #148 and 146 were sacrificed on Day 92 and 96, respectively. It appears that diet and route had worsened survival. Since canagliflozin had little impact on mortality in oral and standard diet, deaths were attributed to route and diet.

Clinical Signs

- As noted in other rat studies, canagliflozin resulted in bed wetting caused by urinary glucose induced osmotic diuresis.
- The low Ca diet rats had soft and yellow feces, irrespective of treatment.

6-month RD OR and SC tox study with a 1- and 3-month interim kill in the rat
JNJ-28431754-ZAE - OR/GAV - RAT   From Week 0 till 5, Day 0 till 29

Dosage Group (mg eq/ kg):	Group A X / N	Group B X / N	D: OR 100 X / N	E: OR 100 X / N
Feces, soft	0 / 30	0 / 30	0 / 30	30 / 30 ***
Wet urogenital region	0 / 30	0 / 30	0 / 30	6 / 30 *
Wet bedding	0 / 30	0 / 30	30 / 30 ***	30 / 30 ***
Feces, yellow	0 / 30	0 / 30	0 / 30	30 / 30 ***

Significance level computed with Fisher Exact probability test (two-tailed): * p < .05   ** p < .01   *** p < .001

6-month RD OR and SC tox study with a 1- and 3-month interim kill in the rat
JNJ-28431754-ZAE - OR/GAV - RAT   From Week 14 till 27, Day 93 till 183

Dosage Group (mg eq/ kg):	Group A X / N	Group B X / N	D: OR 100 X / N	E: OR 100 X / N
Feces, soft	0 / 10	0 / 10	0 / 10	10 / 10 ***
Wet bedding	10 / 10	10 / 10	10 / 10	10 / 10
Feces, yellow	0 / 10	0 / 10	0 / 10	10 / 10 ***
Feces, pale	0 / 10	10 / 10	0 / 10 ***	10 / 10

Significance level computed with Fisher Exact probability test (two-tailed): * p < .05   ** p < .01   *** p < .001

6-month RD OR and SC tox study with a 1- and 3-month interim kill in the rat
JNJ-28431754-ZAE - SC - RAT   From Week 0 till 5, Day 0 till 29

Dosage Group (mg eq/ kg):	Group F X / N	G: SC 100 X / N	H: SC 100 X / N
Feces, soft	0 / 30	0 / 30	30 / 30 ***
Skin lesion	30 / 30	24 / 30 *	21 / 30 **
Wet bedding	0 / 30	30 / 30 ***	30 / 30 ***
Feces, yellow	0 / 30	0 / 30	30 / 30 ***

Significance level computed with Fisher Exact probability test (two-tailed): * p < .05   ** p < .01   *** p < .001
(Significance computed versus the Group F dosage group)
X: Number of positive animals   N: Total number of animals

6-month RD OR and SC tox study with a 1- and 3-month interim kill in the rat
JNJ-28431754-ZAE - SC - RAT   From Week 5 till 14, Day 30 till 96

Dosage Group (mg eq/ kg):	Group F X / N	G: SC 100 X / N	H: SC 100 X / N
Feces, soft	0 / 20	0 / 20	18 / 18 ***
Skin lesion	20 / 20	19 / 20	18 / 18
Wet bedding	0 / 20	20 / 20 ***	18 / 18 ***
Feces, yellow	0 / 20	0 / 20	18 / 18 ***
Feces, pale	0 / 20	0 / 20	18 / 18 ***

Significance level computed with Fisher Exact probability test (two-tailed): * p < .05   ** p < .01   *** p < .001
(Significance computed versus the Group F dosage group)
X: Number of positive animals   N: Total number of animals

Body Weights

- Canagliflozin rats had lower BW and BW gain than corresponding controls. Excess urinary glucose and water losses were likely contributing factors. Substantial drug-induced glucosuria increased food intake. However, the increase in food intake did not compensate for the excess loss of calories in urine.
- There was minimal difference between LC diet and normal diet fed rats except for group H (LC diet SC treated) which had slightly higher BW and BW gain than group G (ND, normal standard diet SC treated).
- Overall, ND rats had slightly lower BW than SC rats, likely due to additional stress of injection site irritation.

Oral groups

1 month (Week 4)	B versus A	D versus A	E versus B	E versus D
BW	1.044x	0.911x	0.862x	0.987x
BWG	1.148x	0.680x	0.558x	0.943x
3 months (Week 13)	B versus A	D versus A	E versus B	E versus D
BW	1.073x	0.844x	0.796x	1.013x
BWG	1.165x	0.647x	0.572x	1.031x
6 months (Week 26)	B versus A	D versus A	E versus B	E versus D
BW	1.046x	0.799x	0.763x	0.998x
BWG	1.086x	0.646x	0.583x	0.979x

Feed Consumption

- Food intake in treated rats was significantly increased due significant glucosuria.
- Food intake was slightly lower in low Ca diet rats than standard diet fed rats.
- Food intake in SC rats was only slightly lower (0.995x) than oral vehicle or treated rats.

Oral groups

FC	B versus A	D versus A	E versus B	E versus D
1 month (Week 4)	0.762x	1.276x	1.276x	0.762x
3 months (Week 13)	0.806x	1.232x	1.165x	0.762x
6 months (Week 26)	0.742x	1.432x	1.482x	0.768x

Hematology and coagulation

- The hematology profile in oral and SC treated rats on LC diet were similar (increase in RBC, decrease in thrombocytes relative to respective controls).
- Reticulocytes were lower in orally treated rats vs. respective control.
- Reticulocytes were higher in the SC vehicle (1.4x) than gavage control at 3 months but not at 1 month likely due to SC injections/inflammation.
- There was a slight shortening of activated partial thromboplastin time (APTT) and prothrombin time (PT) in canagliflozin treated rats at 3 and 6 months independent of diet. Fibrinogen was increased in ND vehicle and SC treated rats. Whether the increase was related to SC injection is unknown.

Oral groups

1 month	B versus A	D versus A	E versus B	E versus D
Haemoglobin	-	-	1.082x	-
Haematocrit	-	-	1.061x	-
Reticulocytes	0.760x	0.651x	0.876x	1.023x
thrombocytes	-	-	0.627x	-
3 months				
Haemoglobin	-	-	1.090x	-
Haematocrit	-	-	1.062x	-
MCV	-	-	1.060x	-
MCH	-	-	1.079x	-
MCHC	-	-	1.021x	-
Reticulocytes	0.731x	0.666x	1.038x	1.140x
thrombocytes	-	-	0.605x	-
6 months				
Haemoglobin	-	-	1.070x	-
Haematocrit	-	-	1.028x	-
MCV	-	-	1.043x	-
MCH	-	-	1.085x	-
MCHC	-	-	1.039x	-
Reticulocytes	0.897x	0.663x	0.828x	1.120x
thrombocytes	-	-	0.681x	-

- No relevant findings

Clinical Chemistry

- Canagliflozin (OR or SC) in standard diet rats increased Ca, inorganic P, BUN, ALK and ALT in all treated groups compare respective control
- Canagliflozin LC diet rats increased Ca, inorganic P, BUN, ALP and ALT
- Canagliflozin LC diet rats had lower plasma Ca and BUN than Standard diet treated rats suggesting that the impact of canagliflozin was reduced when diet was low in Ca. Canagliflozin increased both plasma Ca and BUN in LC diet fed rats but to a lesser degree (less pronounced) than in ND fed rats.
- Transient increase in Trig and Chol was seen in standard diet controls vs. LC diet controls
- There was no apparent decrease in plasma glucose in any of the canagliflozin treated rats at 1, 3 or 6 months. It is not clear why since significant increase in urine glucose was noted in all treated rats. The sponsor attributed it to fed state prior to necropsy.

6-month RD OR and SC tox study with a 1- and 3-month interim kill in the rat
JNJ-28431754-ZAE - OR/GAV - SC - RAT

1M interim kill, recorded in week 4 till 5

Parameter	Unit	Dosage Groups (mg eq/ kg) MALES						
		A: OR 0	B: OR 0	D: OR 100	E: OR 100	F: SC 0	G: SC 100	H: SC 100
Calcium		9.7	9.8	10.3	9.8	9.6	10.5	10.3
	mg/dl	(0.0)	(0.1)	(0.1)	(0.1)	(0.0)	(0.1)	(0.1)
				*** ++		+	*** +++	*** +++
Inorg. phosphorus		6.7	7.2	7.6	7.2	5.9	7.0	7.0
	mg/dl	(0.2)	(0.1)	(0.2)	(0.2)	(0.1)	(0.2)	(0.1)
			*	*		** +++		
Urea nitrogen		18.8	19.4	27.3	24.0	22.7	31.0	24.0
	mg/dl	(0.5)	(0.8)	(1.3)	(0.8)	(0.5)	(1.4)	(1.2)
				*** +++	*** ++	*** ++	*** +++	*** +
Creatinine		0.20	0.22	0.19	0.19	0.23	0.20	0.22
	mg/dl	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.02)	(0.01)
				+	+	*		
Total bilirubin		0.05	0.07	0.04	0.06	0.03	0.04	0.06
	mg/dl	(0.01)	(0.01)	(0.00)	(0.00)	(0.00)	(0.00)	(0.01)
			*	+++		** +++	+++	
Glucose		152	146	144	177	173	155	171
	mg/dl	(6)	(4)	(2)	(10)	(5)	(4)	(4)
					+	* +++		* +++
Alk. phosphatase		108	131	163	224	113	127	174
	U/l	(4)	(6)	(12)	(19)	(5)	(8)	(10)
			**	*** +	*** +++			*** ++

Standard Error is shown between brackets if more than 2 animals
Significance versus A: OR 0 computed by Mann-Whitney U test (two-tailed): * P<.05 ** P<.01 *** P<.001
Significance versus B: OR 0 computed by Mann-Whitney U test (two-tailed): + P<.05 ++ P<.01 +++ P<.001

Experiment TOX10168   CLINICAL CHEMISTRY
6-month RD OR and SC tox study with a 1- and 3-month interim kill in the rat
JNJ-28431754-ZAE - OR/GAV - SC - RAT

3M interim kill, recorded in week 13 till 14

Parameter	Unit	Dosage Groups (mg eq/ kg) MALES						
		A: OR 0	B: OR 0	D: OR 100	E: OR 100	F: SC 0	G: SC 100	H: SC 100
Calcium		9.5	9.5	10.0	9.9	9.3	10.8	10.2
	mg/dl	(0.1)	(0.1)	(0.2)	(0.2)	(0.1)	(0.2)	(0.1)
				* ++			*** +++	*** ++
Inorg. phosphorus		6.1	5.8	6.9	6.7	6.0	6.9	7.2
	mg/dl	(0.1)	(0.1)	(0.2)	(0.2)	(0.1)	(0.1)	(0.1)
				** +++	* ++		** +++	*** +++
Glucose		165	135	136	142	157	137	151
	mg/dl	(10)	(5)	(11)	(5)	(10)	(6)	(7)
			*					
Urea nitrogen		17.2	16.1	26.8	22.7	22.8	28.7	26.2
	mg/dl	(0.6)	(0.8)	(1.5)	(0.9)	(0.8)	(1.5)	(0.9)
				*** +++	*** +++	*** +++	*** +++	*** +++
Creatinine		0.21	0.23	0.20	0.23	0.28	0.21	0.25
	mg/dl	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
			*		+	*	*** +++	**
Total bilirubin		0.05	0.06	0.05	0.07	0.04	0.04	0.06
	mg/dl	(0.00)	(0.01)	(0.01)	(0.01)	(0.00)	(0.00)	(0.01)
					**		* +	
Alk. phosphatase		79	83	152	122	85	143	150
	U/l	(4)	(3)	(16)	(9)	(8)	(12)	(18)
				*** +++	*** +++		*** ++	*** +++

Standard Error is shown between brackets if more than 2 animals
Significance versus A: OR 0 computed by Mann-Whitney U test (two-tailed): * P<.05 ** P<.01 *** P<.001
Significance versus B: OR 0 computed by Mann-Whitney U test (two-tailed): + P<.05 ++ P<.01 +++ P<.001

Experiment TOX10168   CLINICAL CHEMISTRY
6-month RD OR and SC tox study with a 1- and 3-month interim kill in the rat
JNJ-28431754-ZAE - OR/GAV - SC - RAT   6M terminal kill, recorded in week 27

| Parameter | Unit | Dosage Groups (mg eq/ kg) MALES | | | |
		A: OR 0	B: OR 0	D: OR 100	E: OR 100
Calcium	mg/dl	10.2 (0.1)	10.0 (0.1)	10.9 (0.1) *** +++	10.7 (0.1) ** +++
Inorg. phosphorus	mg/dl	5.8 (0.2)	5.4 (0.2)	6.8 (0.1) *** +++	7.0 (0.2) *** +++
Glucose	mg/dl	145 (3)	171 (8) *	189 (9) ***	164 (7) *
Urea nitrogen	mg/dl	16.2 (0.6)	14.7 (0.6)	28.8 (1.5) *** +++	23.8 (1.7) *** +++
Creatinine	mg/dl	0.23 (0.01)	0.26 (0.01)	0.25 (0.01)	0.24 (0.01)
Total bilirubin	mg/dl	0.05 (0.00)	0.08 (0.01) **	0.04 (0.00) +++	0.07 (0.01) *
Alk. phosphatase	U/l	68 (6)	69 (5)	113 (8) *** +++	120 (9) *** +++

Standard Error is shown between brackets if more than 2 animals
Significance versus A: OR 0 computed by Mann-Whitney U test (two-tailed): * P<.05 ** P<.01 *** P<.001
Significance versus B: OR 0 computed by Mann-Whitney U test (two-tailed): + P<.05 ++ P<.01 +++ P<.001

Experiment TOX10168   CLINICAL CHEMISTRY
6-month RD OR and SC tox study with a 1- and 3-month interim kill in the rat

1M interim kill, recorded in week 4 till 5   3M interim kill, recorded in week 13 till 14

| Parameter | Unit | Dosage Groups (mg eq/ kg) MALES | | |
		F: SC 0	G: SC 100	H: SC 100
Calcium	mg/dl	9.6 (0.0)	10.5 (0.1) ***	10.3 (0.1) ***
Inorg. phosphorus	mg/dl	5.9 (0.1)	7.0 (0.2) ***	7.0 (0.1) ***
Glucose	mg/dl	173 (5)	155 (4) *	171 (4)
Urea nitrogen	mg/dl	22.7 (0.5)	31.0 (1.4) ***	24.0 (1.2)
Creatinine	mg/dl	0.23 (0.01)	0.20 (0.02) *	0.22 (0.01)
Total bilirubin	mg/dl	0.03 (0.00)	0.04 (0.00) *	0.06 (0.01) ***
Alk. phosphatase	U/l	113 (5)	127 (8)	174 (10) ***

| Parameter | Unit | Dosage Groups (mg eq/ kg) MALES | | |
		F: SC 0	G: SC 100	H: SC 100
Calcium	mg/dl	9.3 (0.1)	10.8 (0.2) ***	10.2 (0.1) ***
Inorg. phosphorus	mg/dl	6.0 (0.1)	6.9 (0.1) ***	7.2 (0.1) ***
Glucose	mg/dl	157 (10)	137 (6)	151 (7)
Urea nitrogen	mg/dl	22.8 (0.8)	28.7 (1.5) **	26.2 (0.9) *
Creatinine	mg/dl	0.28 (0.01)	0.21 (0.01) ***	0.25 (0.01) *
Total bilirubin	mg/dl	0.04 (0.00)	0.04 (0.00)	0.06 (0.01) *
Alk. phosphatase	U/l	85 (8)	143 (12) **	150 (18) ***

Standard Error is shown between brackets if more than 2 animals
Significance versus F: SC 0 computed by Mann-Whitney U test (two-tailed): * P<.05 ** P<.01 *** P<.001

Bone Biomarkers

- As expected, LC diet control rats (group B) had elevated levels of 1,25 dihydroxyvitamin D (5 to 12x) and PTH (1.5x) compared to ND controls (group A). The impact was greater over time due to further deterioration in Ca homeostasis. The sharp rise was to normalize Ca levels. With only 1/10[th] of normal dietary Ca, the LC fed rats were likely Ca deficient.
- Oral canagliflozin decreased Vit D (0.19x to 0.89x) and PTH (0.33x to 0.096x) relative to standard diet fed control rats.
- Oral canagliflozin in LC diet rats reduced Vit D (0.14x to 0.77x) and PTH (1.07x to 0.126x) relative to LC diet control, suggesting that canagliflozin was still effectively suppressing Vit D and PTH especially at 3 and 6-months.
- When LC diet canagliflozin rats were compared to standard diet canagliflozin rats, canagliflozin suppressed Vit D and PTH less in LC diet rats than normal diet.

Bone biomarkers at 1 month

Daily Dose (mg eq./kg)	Group A OR	Group B OR		Group D OR 100		Group E OR 100		Group F SC		Group G SC 100		Group H SC 100	
No. of Animals	M:10	M:10		M:10		M:10		M:10		M:10		M:10	
Bone biomarkers[a,^]													
1.25(OH)2D	243.89	1261.21		26.50		177.62		53.78		26.50		95.22	
vs A		5.174	***	0.109	***	0.728		0.221	**	0.109	***	0.390	*
vs B						0.141	***			0.021	***	0.075	***
vs F										0.493		1.771	
PTH	267.38	401.45		88.20		432.57		282.45		104.63		411.99	
vs A		1.501	*	0.330	**	1.618	**	1.056		0.391	**	1.538	
vs B						1.078				0.261	***	1.026	
vs F										0.370	*	1.459	

^: Group means are shown. For treated groups and for group B and F, multiples of vehicles are shown. Statistical significance is based on actual data (not on the multiples of vehicles).
[a]: Significance computed by Mann-Whitney U test (two-tailed) :* p<0.05, ** p<0.01, *** p<0.001

[b]:Significance level computed by Fisher Exact probability test (two-tailed) :* p<0.05, ** p<0.01, *** p<0.001

[c]: */** Dunn's Test (Closed) at 5 % (*) or 1 % (**) level

[d]: One-Sided Exact Fisher Test :* p<=0.05, ** p<=0.01

[e]: BrdU: One way ANOVA, Bonferroni's Multiple Comparison Test * - p<0.05, ** - p<0.01, ***- p<0.001 (added if analysed; see IHC-report)
[#]: absolute value
- : No relevant findings   Note: Findings related to subcutaneous dosing were not reported in the CTD table

3-month interim kill in the rat

Daily Dose (mg eq./kg)	Group A OR	Group B OR		Group D OR 100		Group E OR 100		Group F SC		Group G SC 100		Group H SC 100	
No. of Animals	M:10	M:10		M:10		M:10		M:10		M:10		M:10	
Bone biomarkers[a,^]													
1.25(OH)2D	55.60	698.80		26.50		26.50		30.40		26.50		26.50	
vs A		12.568	***	0.477		0.477		0.547		0.477		0.477	
vs B						0.038	***					0.038	***
vs F										0.872		0.872	
PTH	257.38	459.71		67.92		164.49		399.23		46.68		368.14	
vs A		1.786	*	0.264	**	0.639		1.461		0.181	***	1.430	
vs B						0.358	**					0.801	
vs F										0.117	***	0.922	

6-Month RD oral tox

Daily Dose (mg eq./kg) No. of Animals	Group A OR M:10		Group B OR M:10		Group D OR 100 M:10		Group E OR 100 M:10	
Bone biomarkers[a,^]								
1,25(OH)2D	29.74		341.98		26.50		26.50	
vs A			11.499	***	0.891		0.891	
vs B							0.077	***
PTH	379.09		425.80		36.30		55.12	
vs A			1.123		0.096	***	0.145	***
vs B							0.126	***

- When ND oral control was compared to ND SC control, a significant decrease in Vit D levels were seen in SC control while PTH was slightly increase. It is not clear why the SC control rats had disturbed Vit D or PTH levels. One has to assume some sort of vehicle induced injection stress/inflammation.
- Since SC vehicle alone had resulted in unexpected changes in bone markers, any conclusion drawn from the treatment groups is likely to be in error even though the overall changes with canagliflozin appear to be similar in pattern to orally treated canagliflozin rats.

Subcutaneous groups

1 Month	F versus A	G versus F	H versus F	H versus G
1,25(OH)$_2$D	0.221x	0.493x	1.771x	3.593x
PTH	1.056x	0.370x	1.459x	3.924x
3 months				
1,25(OH)$_2$D	0.547x	0.872x	0.872x	1.000x
PTH	1.551x	0.117x	0.922x	7.886x

Urinalysis

- Urine data was collected over 6 and 24 hr time periods on WK 4, 13 and 26. Since the 6 hr and 24 hr urine parameters were generally similar, only the 6 hr data is discussed.
- Administration of canagliflozin by oral and SC route resulted in large increases in urine glucose and ketone bodies. The increase in urinary glucose was associated with significant increase in urine volume and decrease in pH.
- Canagliflozin in normal fed rats increased urine Ca, inorganic P, NAG (6-24 hr), protein, GGT and decreased electrolytes relative to respective control. The increase in protein and some of the renal biomarkers suggest that the increase in glomerular pressure/injury or pore size was permitting large protein molecules to escape which can eventually cause a decline in renal function.
- Low Ca diet rats treated with canagliflozin (SC & OR) had much smaller increase in urine Ca and Ca excretion, electrolytes and GGT than normal diet treated rats (OR & SC).

- Low Ca diet reduced calciuria but increased excretion of other electrolytes. However, LC diet did not prevent canagliflozin-induced increase in urine volume or glucose.
- SC administration of canagliflozin and vehicle resulted in notable increase in blood occult whether rats were maintained on normal diet or low Ca diet.   It was not clear if the hematuria was due to vehicle or the trauma from repeated SC injections.

6-month RD OR and SC tox study with a 1- and 3-month interim kill in the rat
JNJ-28431754-ZAE - OR/GAV - SC - RAT
Mean values per dosage group Periodical 1M, recorded in week 4 till 5, 6h

| Parameter | Unit | Dosage Groups (mg eq/ kg) MALES | | | | | | |
		A: OR 0	B: OR 0	D: OR 100	E: OR 100	F: SC 0	G: SC 100	H: SC 100
Volume		6.1	4.6	16.5	12.4	5.2	14.7	12.3
	ml	(0.4)	(0.4)	(0.7)	(0.6)	(0.4)	(0.6)	(0.4)
			**	*** +++	*** +++		*** +++	*** +++
Sodium		134.4	68.7	468.1	176.2	178.9	351.9	50.9
	mmol/g Crea	(14.8)	(8.9)	(41.2)	(24.7)	(22.7)	(24.9)	(8.4)
			***	*** +++	+++	+++	*** +++	***
Potassium		232.0	112.3	495.8	193.3	251.0	591.2	131.2
	mmol/g Crea	(14.1)	(6.9)	(24.2)	(22.2)	(13.0)	(21.8)	(15.3)
			***	*** +++	+	+++	*** +++	***
Chloride		159.9	65.2	545.1	160.1	209.0	436.1	63.9
	mmol/g Crea	(20.6)	(7.6)	(42.1)	(19.3)	(26.3)	(26.4)	(10.2)
			***	*** +++	+++	+++	*** +++	***
Calcium		0.162	0.037	2.443	0.201	0.364	2.117	0.373
	mg/mg Crea	(0.025)	(0.003)	(0.148)	(0.027)	(0.048)	(0.196)	(0.062)
			***	*** +++	+++	*** +++	*** +++	** +++
Phosphate		0.06	1.45	2.06	3.27	0.08	2.47	3.27
	mg/mg Crea	(0.02)	(0.14)	(0.16)	(0.21)	(0.03)	(0.17)	(0.17)
			***	*** ++	*** +++	+++	*** +++	*** +++
Glucose		0.17	0.20	501.48	513.86	16.56	621.26	495.47
	mg/mg Crea	(0.01)	(0.01)	(15.95)	(18.74)	(8.52)	(15.42)	(15.81)
			**	*** +++	*** +++	*** ++	*** +++	*** +++
Gamma glutamyl transferase		3602.4	3005.2	3446.4	3027.8	2839.0	3224.0	3649.2
	U/g Crea	(197.7)	(204.8)	(238.5)	(265.8)	(166.4)	(212.8)	(248.6)
			*			*	**	
N-Acetyl-ß-D-Glucosaminidase		20.8	26.2	57.7	61.7	36.4	85.5	73.0
	U/g Crea	(0.9)	(1.1)	(3.3)	(3.1)	(1.3)	(2.8)	(2.8)
			**	*** +++	*** +++	*** +++	*** +++	*** +++
Protein		1.1	0.9	1.9	2.2	1.7	2.6	3.5
	mg/mg Crea	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	(0.2)
				*** +++	*** +++	*** +++	*** +++	*** +++

Standard Error is shown between brackets if more than 2 animals
Significance versus A: OR 0 computed by Mann-Whitney U test (two-tailed): * P<.05 ** P<.01 *** P<.001
Significance versus B: OR 0 computed by Mann-Whitney U test (two-tailed): + P<.05 ++ P<.01 +++ P<.001

Mean values per dosage group Periodical 1M, recorded in week 4 till 5

Parameter	Unit	Dosage Groups (mg eq/ kg) MALES						
		A: OR 0	B: OR 0	D: OR 100	E: OR 100	F: SC 0	G: SC 100	H: SC 100
Protein Excretion	mg/24h	11.8 (1.3)	8.2 (0.5) *	18.5 (1.1) *** +++	18.3 (1.1) *** +++	13.5 (0.9) +++	17.3 (0.7) *** +++	22.0 (1.7) *** +++
Calcium Excretion	mg/24h	0.9 (0.2)	0.3 (0.0) ***	12.7 (0.9) *** +++	0.9 (0.1) +++	1.3 (0.2) +++	10.7 (1.2) *** +++	1.4 (0.4) +++

Standard Error is shown between brackets if more than 2 animals
Significance versus A: OR 0 computed by Mann-Whitney U test (two-tailed): * P<.05 ** P<.01 *** P<.001
Significance versus B: OR 0 computed by Mann-Whitney U test (two-tailed): + P<.05 ++ P<.01 +++ P<.001

6-month RD OR and SC tox study with a 1- and 3-month interim kill in the rat
JNJ-28431754-ZAE - OR/GAV - SC - RAT
Mean values per dosage group Periodical 3M, recorded in week 13, 6h

Parameter	Unit	Dosage Groups (mg eq/ kg) MALES						
		A: OR 0	B: OR 0	D: OR 100	E: OR 100	F: SC 0	G: SC 100	H: SC 100
Volume	ml	4.4 (0.3)	3.5 (0.3) *	16.9 (1.2) *** +++	14.5 (0.6) *** +++	4.4 (0.5)	13.2 (0.7) *** +++	11.7 (0.6) *** +++
Sodium	mmol/g Crea	76.0 (10.0)	33.0 (5.0) **	331.0 (48.5) *** +++	99.1 (26.0) ++	93.3 (12.2) +++	152.8 (20.7) ** +++	33.5 (5.3) **
Potassium	mmol/g Crea	145.0 (9.6)	59.4 (4.9) ***	428.4 (22.4) *** +++	160.0 (15.7) +++	179.8 (14.8) +++	436.1 (22.0) *** +++	114.6 (13.1) * +++
Chloride	mmol/g Crea	92.2 (12.6)	23.9 (2.8) ***	412.5 (46.7) *** +++	118.1 (17.3) +++	116.9 (15.2) +++	206.6 (22.2) *** +++	47.3 (6.3) ** ++
Calcium	mg/mg Crea	0.141 (0.028)	0.022 (0.001) ***	2.104 (0.133) *** +++	1.086 (0.069) *** +++	0.155 (0.031) +++	1.845 (0.105) *** +++	0.860 (0.067) *** +++
Phosphate	mg/mg Crea	0.02 (0.00)	0.74 (0.10) ***	2.17 (0.14) *** +++	3.34 (0.21) *** +++	0.03 (0.01) +++	2.30 (0.21) *** +++	3.30 (0.16) *** +++
Glucose	mg/mg Crea	0.15 (0.01)	0.17 (0.01)	457.37 (16.07) *** +++	471.11 (22.46) *** +++	0.19 (0.02) *	531.42 (19.57) *** +++	429.00 (18.68) *** +++
Gamma glutamyl transferase	U/g Crea	2966.0 (217.0)	2448.4 (277.6) *	2418.5 (247.4)	1971.8 (171.8) ***	3127.8 (227.9) +	3447.8 (340.6) +	3107.3 (249.9) +
N-Acetyl-ß-D-Glucosaminidase	U/g Crea	17.0 (1.1)	18.6 (0.9)	45.9 (2.5) *** +++	48.0 (3.5) *** +++	28.4 (2.5) ** ++	66.8 (2.8) *** +++	60.1 (3.4) *** +++
Protein	mg/mg Crea	0.9 (0.1)	0.6 (0.1) *	1.4 (0.1) *** +++	2.0 (0.2) *** +++	1.5 (0.1) *** +++	2.1 (0.1) *** +++	2.5 (0.2) *** +++

Standard Error is shown between brackets if more than 2 animals
Significance versus A: OR 0 computed by Mann-Whitney U test (two-tailed): * P<.05 ** P<.01 *** P<.001
Significance versus B: OR 0 computed by Mann-Whitney U test (two-tailed): + P<.05 ++ P<.01 +++ P<.001

6-month RD OR and SC tox study with a 1- and 3-month interim kill in the rat
JNJ-28431754-ZAE - OR/GAV - SC - RAT
Mean values per dosage group Periodical 3M, recorded in week 13

Parameter	Unit	Dosage Groups (mg eq/ kg) MALES A: OR 0	B: OR 0	D: OR 100	E: OR 100	F: SC 0	G: SC 100	H: SC 100
Protein Excretion	mg/24h	14.6 (2.8)	8.7 (0.7)	20.0 (1.5) * +++	21.3 (2.0) * +++	14.2 (1.4) ++	19.2 (1.1) +++	23.0 (1.1) * +++
Calcium Excretion	mg/24h	1.0 (0.3) ***	0.3 (0.0) *** +++	14.3 (0.7) *** +++	5.9 (0.4)	1.0 (0.3) +++	11.5 (0.8) *** +++	4.6 (0.7) *** +++

Standard Error is shown between brackets if more than 2 animals
Significance versus A: OR 0 computed by Mann-Whitney U test (two-tailed): * P<.05 ** P<.01 *** P<.001
Significance versus B: OR 0 computed by Mann-Whitney U test (two-tailed): + P<.05 ++ P<.01 +++ P<.001

6-month RD OR and SC tox study with a 1- and 3-month interim kill in the rat
JNJ-28431754-ZAE - OR/GAV - SC - RAT

Mean values per dosage group Terminal, recorded in week 26, 6h

Parameter	Unit	Dosage Groups (mg eq/ kg) MALES A: OR 0	B: OR 0	D: OR 100	E: OR 100
Volume	ml	5.4 (0.6)	5.2 (0.6)	18.0 (1.5) *** +++	15.3 (1.2) *** +++
Sodium	mmol/g Crea	62.9 (12.8)	53.7 (10.6)	271.6 (58.0) ** ++	125.8 (38.6)
Potassium	mmol/g Crea	118.2 (20.6)	53.0 (7.6) **	432.1 (59.9) *** +++	201.5 (25.7) * +++
Chloride	mmol/g Crea	77.6 (19.9)	38.3 (6.8)	353.8 (66.8) *** +++	143.8 (25.4) ++
Calcium	mg/mg Crea	0.159 (0.038)	0.029 (0.006) ***	2.109 (0.142) *** +++	1.509 (0.138) *** +++
Phosphate	mg/mg Crea	0.12 (0.04)	0.85 (0.17) ***	2.64 (0.47) *** ++	3.89 (0.32) *** +++
Glucose	mg/mg Crea	0.18 (0.04)	0.17 (0.02)	430.59 (26.12) *** +++	503.42 (32.02) *** +++
Gamma glutamyl transferase	U/g Crea	2449.1 (361.5)	1357.3 (184.2) **	2147.2 (454.1)	2042.3 (334.7)
N-Acetyl-ß-D-Glucosaminidase	U/g Crea	19.9 (2.4)	17.9 (1.5)	47.5 (4.3) *** +++	54.0 (5.5) *** +++
Protein	mg/mg Crea	0.7 (0.1)	0.4 (0.0) *	1.2 (0.1) * +++	2.6 (0.5) *** +++

Standard Error is shown between brackets if more than 2 animals
Significance versus A: OR 0 computed by Mann-Whitney U test (two-tailed):
* P<.05 ** P<.01 *** P<.001

Significance versus B: OR 0 computed by Mann-Whitney U test (two-tailed):
+ P<.05 ++ P<.01 +++ P<.001

Freshly Voided Urinalysis

The sposnor also collected freshly voided urine to detemine the crystalls sediments in the urine. Consistent with the earlier studies, canagliflozin reduced urine pH. Triple phosphate crytals were markedly decreased in both canagliflozin rats on normal diet (ND) and LC diet. The LC controls had only slighly lower crystalls than normal diet controls. Amorphous material was present in 8 of 10 rats of ND canagliflozin rats (absent in ND and LC controls) suggesting that canagliflozin was increasing sedimentation to some extent. There were no other notabel findings in the freshly voided urine samples.

Analsysis Ca Excretion in Low Calcium (LC) Diet and Normal Diet (ND)

Canagliflozin-induced Ca excretion was examined over time in rats on low Ca (LC). The impact of LC diet on Ca excretion decreased over time in canagliflozin treated rats.

UCA_CR	E versus A	H versus F
1 month		
0-6 hours	1.241x	1.025x
6-24 hours	1.500x	1.695x
3 months		
0-6 hours	7.702x	5.548x
6-24 hours	6.967x	5.047x
6 months		
0-6 hours	9.491x	

Since the dietary Ca was $1/10^{th}$ of normal diet, canagliflozin may have extracted Ca from other sources including enahnced Ca absorption caused by carbohydrate malabsorption. The changes in Ca excretion also correspond to changes in bone biomarkers.

Overall, canagliflozin increased urine volume, urine glucose, Ca and electrolytes, NAG, protein, GGT and ketone bodies relative to corresponding controls. However, when LC canagliflozin rats were comapred to ND canagliflozin rats, the LC rats had lower urianry Ca, electrolytes and GGT during the first month and to a small degree at 3 and 6 months suggesting that LC diet effect may have been blunted by hypercalcium absoption over time.

Gross Pathology

- LC diet alone was associated with white gastric content, pale discoloration and prominent lobular pattern/swelling of liver likely due to prominent glycogen/vacuolation.
- Injection sites appeared inflamed and ulcerated. SC vehicle (HP-ß-cyclodextrin) may have been responsible for the skin lesions and skin sores (abdominal area) and lung foci at 3 months.

Organ Weights

- Canagliflozin increased adrenal and kidney weight and decreased accessory sex gland, similar to the 6-month rat toxicology study. The severity was higher in the LC diet oral and LC diet SC.

- LC diet alone only slightly increased kidney weight in male rats at 3 and 6 months.
- SC administration of HP-ß-cyclodextrin (vehicle for SC) with or without canagliflozin caused slight increase in kidney weight at 1 and 3 months.

Immunohistochemistry of Kidneys at 1 month

Cortex
- There was no significant difference between orally treated canagliflozin and respective controls in BrdU labeling of the epithelial cell in the renal cortex.
- There was no significant difference between ND and LC rats suggesting that LC diet did not impact renal cell proliferation.
- There was no significant difference between SC canagliflozin on LC diet or SC normal diet.

Outer Stripe of the outer Medulla (OSOM)
- There was no difference in the tubular epithelium cell BrdU score of oral canagliflozin and controls (LC and ND vs. control).
- There was no difference between oral canagliflozin fed LC diet and normal diet control groups.
- There was a significant decrease in BrdU labeling of OSOM in canagliflozin SC ND rats relative to SC ND control.

Kidneys: BrdU Labeling in the Cortical Tubular Epithelial Cells
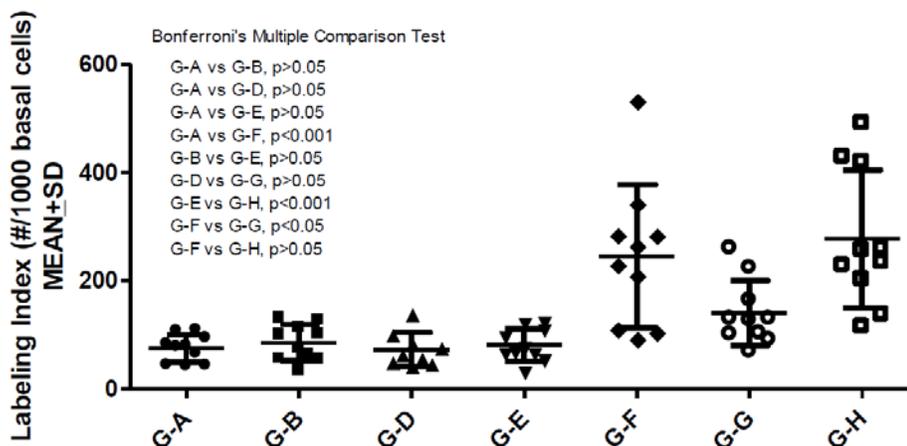
Bonferroni's Multiple Comparison Test
G-A vs G-B, p>0.05
G-A vs G-D, p>0.05
G-A vs G-E, p>0.05
G-A vs G-F, p<0.001
G-B vs G-E, p>0.05
G-D vs G-G, p>0.05
G-E vs G-H, p<0.001
G-F vs G-G, p<0.05
G-F vs G-H, p>0.05

Kidneys: BrdU Labeling in the OSOM Tubular Epithelial Cells

Bonferroni's Multiple Comparison Test

G-A vs G-B, p>0.05   G-E vs G-H, p>0.05
G-A vs G-D, p>0.05   G-F vs G-G, p<0.001
G-A vs G-E, p>0.05   G-F vs G-H, p<0.001
G-A vs G-F, p<0.001
G-B vs G-E, p>0.05
G-D vs G-G, p>0.05
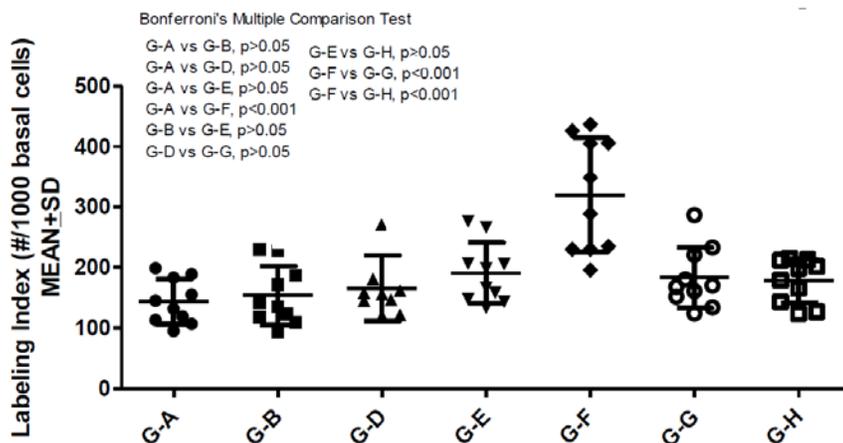
Kidneys: BrdU Labeling in the Total Area of Tubular Epithelial Cells

Bonferroni's Multiple Comparison Test

G-A vs G-B, p>0.05   G-B vs G-E, p>0.05
G-A vs G-D, p>0.05   G-D vs G-G, p>0.05
G-A vs G-E, p>0.05   G-E vs G-H, p<0.01
G-A vs G-F, p<0.001   G-F vs G-G, p<0.001
                      G-E vs G-H, p>0.05
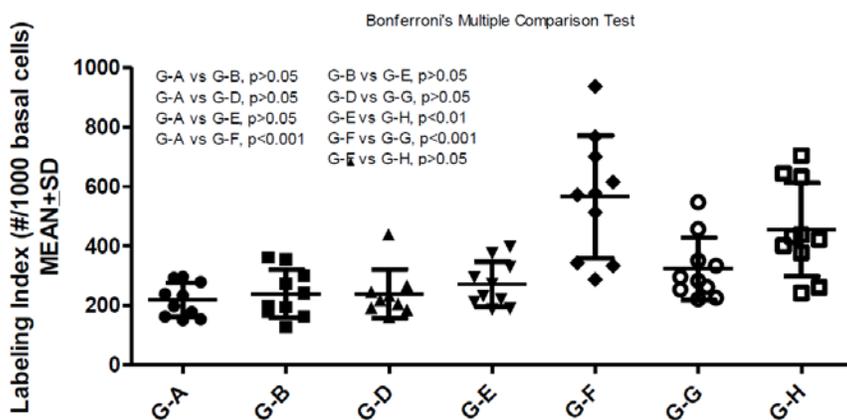
Immunohistochemistry of Adrenal
- There was no difference in BrdU medullary chromaffin cell labeling in the adrenal glands between canagliflozin groups (oral or SC) and their respective diet control groups.
- There was no significant difference between normal diet controls and canagliflozin given by oral route LC diet.

Adrenal Medulla: BrdU labeling in the Chromaffin Cells

Bonferroni's Multiple Comparison Test

G-A vs G-B, p>0.05   G-D vs G-G, p>0.05
G-A vs G-D, p>0.05   G-E vs G-H, p>0.05
G-A vs G-E, p>0.05   G-F vs G-G, p>0.05
G-A vs G-F, p>0.05   G-F vs G-H, p>0.05
G-B vs G-E, p>0.05

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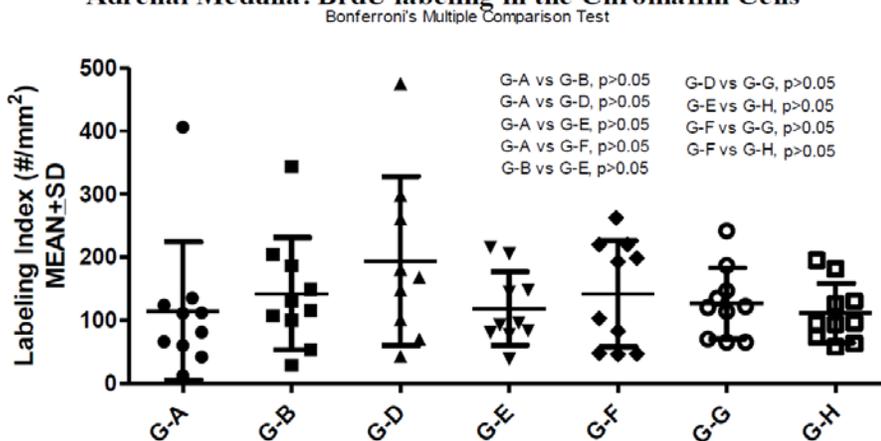
Immunohistochemistry of Bladder
- There was no or minimal BrdU urothelial cell labeling in the ventral part of the bladder.
- No significant difference in BrdU labeling was noted among groups.

Immunohistochemistry of Kidneys at 6 Month
- There was no significant difference between canagliflozin groups and respective control groups in BrdU labeling of cortical tubular cells.
- There was a significant increase in BrdU labeling in the cortical tubular cells in the LC diet control and normal diet control suggesting that the LC diet alone had an impact on cortical tubular cells differentiation making drug effect rather difficult to sort out.
- There was no significant increase in BrdU labeling of the cortical tubular cells in canagliflozin groups on LC diet and normal diet control group.
- Although there was a slight trend toward an increase in BrdU labeling of OSOM tubular cells in the canagliflozin groups compared to standard diet, there was no significant difference in BrdU labeling of OSOM tubular cells of canagliflozin groups and respective diet control groups.

Kidneys: BrdU Labeling in the Cortical Tubular Epithelial Cells

6-month

Bonferroni's Multiple Comparison Test

G-A vs G-B, $p<0.01$
G-A vs G-D, $p>0.05$
G-A vs G-E, $p>0.05$
G-B vs G-E, $p>0.05$
G-D vs G-E, $p>0.05$

Labeling Index (#/1000 basal cells) MEAN±SD

Kidneys: BrdU Labeling in the OSOM Tubular Epithelial Cells

6-month

Bonferroni's Multiple Comparison Test

G-A vs G-B, $p<0.05$
G-A vs G-D, $p>0.05$
G-A vs G-E, $p<0.05$
G-B vs G-E, $p>0.05$
G-D vs G-E, $p>0.05$

Labeling Index (#/1000 basal cells) MEAN±SD

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Kidneys: BrdU Labeling in the Total Area Tubular Epithelial Cells

6-month

Bonferroni's Multiple Comparison Test

G-A vs G-B, p<0.01
G-A vs G-D, p>0.05
G-A vs G-E, p<0.05
G-B vs G-E, p>0.05
G-D vs G-E, p>0.05

Kidneys: TIM-1 Labeling in the Cortical Tubular Epithelial Cells

6-month

Bonferroni's Multiple Comparison Test

G-A vs G-B, p>0.05
G-A vs G-D, p>0.05
G-A vs G-E, p>0.05
G-B vs G-E, p>0.05
G-D vs G-E, p>0.05

Histopathology

Adequate Battery: Yes,
Peer Review: Yes

Histological Findings
- LC diet control rats had high incidence of bone atrophy and low incidence of adrenal hypertrophy and or/vacuolation of the zona glomerulosa.
- At 3 months, LC diet control rats had high incidence of multifocal KIM-1 positivity in the outer renal medulla (OSOM). Similar observation was seen in other canagliflozin treated rats at 3 months but with higher severity.
- Canagliflozin administration increased adrenal zona fasciculata hypertrophy, renal tubule dilatation and renal pelvis dilatation at 1 month after oral and SC route maintained on ND and at 3 month in all canagliflozin treated groups. Additional changes in all canagliflozin groups at 3 months included increased swollen/vacuolated tubular cytoplasm in the OSOM/inner cortex with exfoliated cells.
- There was an increase in KIM-1 positivity in cortex and OSOM in Canagliflozin oral normal diet rats and Canagliflozin oral LC diet rats.   LC diet did not prevent KIM-1 signal.

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- At 6 months, the LC diet control rats had slight non-significant increase in KIM-1 in the OSOM with significant increase in cell proliferation in both cortex and OSOM. Similar observation was seen in the LC diet canagliflozin treated rats.
- Both SC injected ND control rats and SC LC rats has swollen /vacuolated cortical tubules with increased KIM-1 positivity in the kidneys. It is difficult to make sense of this except that SC injections independent of diet or treatment had increased vacuolation and KIM-1 positivity in rats.
- Canagliflozin dosed ND rats (oral and SC) had high incidence of bone hyperostosis.
- Consistent with previous studies, renal pelvis mineralization was noted in canagliflozin ND rats at 6-month.
- Canagliflozin ND rats (SC and oral) had the highest incidence and severity of multifocal KIM-1 positivity in the OSOM at 1 month but at 3 months only few of them had increased KIM-1 positivity (cell proliferation).
- LC diet appeared to inhibit KIM-1 positivity in the OSOM at 1 and 3 months in canagliflozin rats but the benefit of the LC diet at 6 months was diminished. The data appears to show a link between Ca availability and excretion and kidney histopathology initially but the limitations of preventive role of LC is puzzling since one would have expected greater preventive role for LC diet over time.
- Hyperostosis was evident in rats on ND rats treated with oral or SC canagliflozin suggesting that canagliflozin route was less consequential while hyperostosis was significantly lower in the low Ca diet rats.
- LC diet rats had low incidence of adrenal hypertrophy and vacuolization of the adrenal zona glomerulosa.
- SC and oral canagliflozin rats on LC and ND had slight to moderate increase in adrenal gland weight, kidney weight, decrease in sex organ accessory (atrophy in some instances) and pale or swollen adrenal gland. The highest incidence of pale or swollen adrenal glands were seen in LC diet canagliflozin rats.
- Minimal mineralization of renal pelvis was seen in ND canagliflozin rats at 6 months.
- Skin lesions were seen in rats with all SC administration (control as well as canagliflozin rats) suggesting skin irritation by the vehicle. This may have played a role in increased WBC, fibrinogen and AST. The lesions were the reason for early termination of the SC rats at 3-months rather than the originally designed necropsy at 6 months.

Immunohistochemistry Analysis
- At 3-month, higher incidence of multifocal KIM-1 (also known as TIM-1) positivity in the outer stripe of outer medulla (OSOM) was seen in LC diet and all canagliflozin treated groups. The treated rats had even slightly higher KIM-1 positivity.
- At 6-months, the LC diet rats has slightly higher KIM-1 positivity (nonsignificant) in the OSOM with significant increase in cell proliferation in both cortex and OSOM, comparable to LC canagliflozin rats.
- The pale kidneys had swollen vacuolated cortical tubules and higher cortical KIM-1 positivity and higher BrdU-count. The sponsor attributed this to the vehicle, HP-ß-cyclodextrin.

- Overall, low calcium diet prevented bone hyperostosis but there was minimal impact on adrenal (cell proliferation) or renal biomarkers (exfoliated cells and increased KIM-1) to suggest a preventive role for low dietary calcium. The study failed to show Ca to play a role in renal and adrenal proliferation.

Toxicokinetics:
- The Tmax after SC (2.3 to 2.6 hr) was shorter than after oral gavage (4 to 4.3 hrs) suggesting rapid absorption after SC than oral route. The diet itself had no notable impact on Tmax.
- The AUC and Cmax tend to be higher in rats on 0.1% Ca diet whether the drug was administered by SC or oral route. Oral administration resulted in higher exposure than SC route. Therefore, highest exposure was achieved by oral route in low Ca diet fed rats.

Day	Route	Diet	Group/Dose	C_{max} (ng/mL)	t_{max} (h)	AUC_{0-24h} (h*ng/mL)
92	SC	Standard	G/100	24300 (721)	2.67 (1.15)	372000 (16800)
92	SC	0.1% Low Calcium	H/100	26500 (4990)	2.33 (1.53)	480000 (112000)
181	oral	Standard	D/100	27800 (6380)	4.00 (0.00)	461000 (95700)
181	oral	0.1% Low Calcium	E/100	30800 (7040)	4.33 (2.52)	541000 (100000)

SC: subcutaneous

Dosing Formulation Analysis

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

Study Title: 7-Month Mechanistic Repeated Dose Oral Toxicity Study of JNJ-28431754-ZAE in the male rat (hormonal evaluation).

Study #	TOX10108
Study report location	Janssen, Belgium
CRO/Laboratory name and location	Janssen Research and Development, Beerse, Belgium
Date of study initiation	June 7, 2011
GLP compliance statement	Yes
GLP issues identified	None
QA statement	yes
Drug, lot #, and % purity	JNJ-28431754-ZAE; batch ZR600348PFA271; purity 99.8%

Key Study Findings

- Consistent with earlier studies in male rats, canagliflozin (100 mg/kg) increased bed wetting and decreased BW and BW gain and increased food consumption.

- Canagliflozin significant increased serum LH levels (up to 2 fold) during the first 5 of the 7-month study. The increase in LH was associated with a decrease in accessory sex gland weight.

- There was no change in serum testosterone. Terminal serum LH and testosterone was unchanged at the end of the 6-month high fructose diet study. The terminal LH levels were below the detection limits in the rat carcinogenicity study while testosterone levels were significantly decreased.

- Changes in the adrenal, renal and bone (hyperostosis) were also consistent with the earlier canagliflozin studies in rats.

- The kidney findings were also consistent with the earlier studies (tubular dilatation in all rats, minimally increased pelvic dilatation, swelling/vacuolization of the renal tubular cytoplasm in the outer stripe of the outer medulla and inner cortex (14/30) with minimal presence of exfoliated cells (10/30), mineralization in the renal pelvis (8/30) with a minimal increase in hyperplasia of the pelvic epithelium and a tendency towards increased mineral deposits in the cortex and medulla).

- PK values were similar to previous studies in rats and supports adequate drug exposure (AUC_{0-24} = 35900 ng.h/ml, Cmax=24100 ng/ml, Tmax= 3 hrs).

Reviewer Comments: This study was designed to test the hypothesis that canagliflozin induced increase in Leydig cell hyperplasia and tumors were caused by elevated LH in male SD rats. The role of LH overstimulation of rat Leydig cells with nearly 10 fold more LH receptor density than humans is well documented in the literature. This study found a significant treatment-related increase in LH during the first 5 of the 7-month in male rats. There was no change in testosterone levels at any time point. The significant perturbation in LH in early in life would have been sufficient to cause Leydig cell hyperplasia and eventual tumors in male rats. The reviewer concludes that canagliflozin induced increase in LH (2 fold) as valid mode of action for

rat Leydig cell tumors. Since clinical studies found no change in LH or testosterone and human Leydig cells have significantly fewer LH receptors than rats, the testicular Leydig cell tumors are clinically irrelevant.

Methods

Doses	0 and 100 mg/kg/day with standard diet
Frequency of dosing	daily
Route of administration	oral gavage
Dose volume	10 ml/kg (Day 1-69) and 5 mL/kg (Day 70 onward)
Formulation/Vehicle	Aqueous suspension containing 0.5 % (w/v) Methocel (F4M Premium EP). New bottle of the formulation was used each day of dosing/week.
Species/Strain	SD(Crl: CD) rat, _(b) (4)_
Number/Sex/Group	30 males/group
Age	8 weeks at dosing initiation
Weight	274 to 320g on Day 0 of dosing
Satellite groups	15 (3/group for TK analysis)
Unique study design	Yes – standard parameters + serum LH and testosterone at one time point during the study and at 0, 4, 6 and 24 hrs after dosing at the end of the study. Endocrine hormone analysis (carotid arterial blood) was carried out at _(b) (4)_
Deviation from study protocol	Minimal

Study Objective: This study was designed to test the hypothesis that the increase in LH was responsible for overstimulation of testicular Leydig cells, resulting in Leydig cell hyperplasia and ultimately testicular tumors in the 2-year rat carcinogenicity study.

Male rats maintained on standard chow were administered with 100 mg/kg canagliflozin or vehicle for up to 7 months. Serum LH and testosterone levels were measured every month. The original study duration was 6 months but due to dehydration at the time of sample collection at month6, canagliflozin treatment was extended to 7 months for an additional blood collection opportunity. The study design is shown in table:

Dosage group assignment			
		V	**H**
		Vehicle	High
Dose level (mg eq./kg/day)		0	100
Concentration (mg eq./ml)		0	10/20
Dose volume (ml/kg/day)		10/5	10/5
Male Numbers	Main	1 - 30	61 - 90
	TK	31-33	91 - 93

Observations and Results

Mortality: There was no test-article related mortality. During WK 3 to 6 of dosing, 4 control animals died without prior clinical signs, likely due to gavage error. One TK rat died on Day 155 due to gavage error (congested lungs).

Clinical Signs: Wet bedding was observed with all canagliflozin rats, suggestive of pharmacological response (glucosuria-induced diuresis).

Body Weights: Canagliflozin significantly reduced BW (0.8x the control) and BW gain (0.6x the control).

Week/Day	Dosage Group (mg eq/ kg) Males		Week/Day	Dosage Group (mg eq/ kg) Males	
	Vehicle	High:100		Vehicle	High:100
1 / 7	340 (3.1)	310 *** (1.6)	16 / 112	537 (8.8)	446 *** (5.5)
2 / 14	370 (3.6)	341 *** (2.4)	17 / 119	535 (8.7)	440 *** (5.6)
3 / 21	394 (4.3)	353 *** (2.8)	18 / 126	543 (8.9)	448 *** (6.3)
4 / 28	408 (5.3)	366 *** (3.3)	19 / 133	545 (9.1)	456 *** (6.1)
5 / 35	418 (5.5)	369 *** (3.8)	20 / 140	555 (9.3)	467 *** (6.3)
6 / 42	431 (6.6)	377 *** (4.2)	21 / 147	551 (9.0)	457 *** (6.5)
7 / 49	447 (7.1)	392 *** (4.5)	22 / 154	557 (9.4)	464 *** (6.6)
8 / 56	460 (7.4)	406 *** (5.0)	23 / 161	566 (9.6)	466 *** (6.1)
9 / 63	474 (7.8)	418 *** (5.3)	24 / 168	574 (9.6)	477 *** (6.5)
10 / 70	488 (8.2)	426 *** (5.2)	25 / 175	579 (9.6)	487 *** (6.9)
11 / 77	500 (8.4)	435 *** (5.1)	26 / 182	580 (9.5)	420 *** (5.2)
12 / 84	513 (8.5)	439 *** (5.9)	27 / 183	569 (9.3)	453 *** (5.7)
13 / 91	514 (8.6)	441 *** (5.1)	27 / 189	578 (9.4)	476 *** (6.1)
14 / 98	521 (8.8)	443 *** (5.4)	28 / 196	589 (9.9)	490 *** (6.4)
15 / 105	530 (8.8)	453 *** (5.9)	29 / 202	579 (10.2)	477 *** (9.2)
16 / 112	537 (8.8)	446 *** (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

Feed Consumption: Food consumption was increased (1.3x the control) throughout the canagliflozin treatment.

Ophthalmoscopy: Not performed
Hematology: Not performed

Endocrine Analysis
- Canagliflozin significantly increased LH during the first 5 months of the study.
- There was no change in testosterone in either control or canagliflozin group.
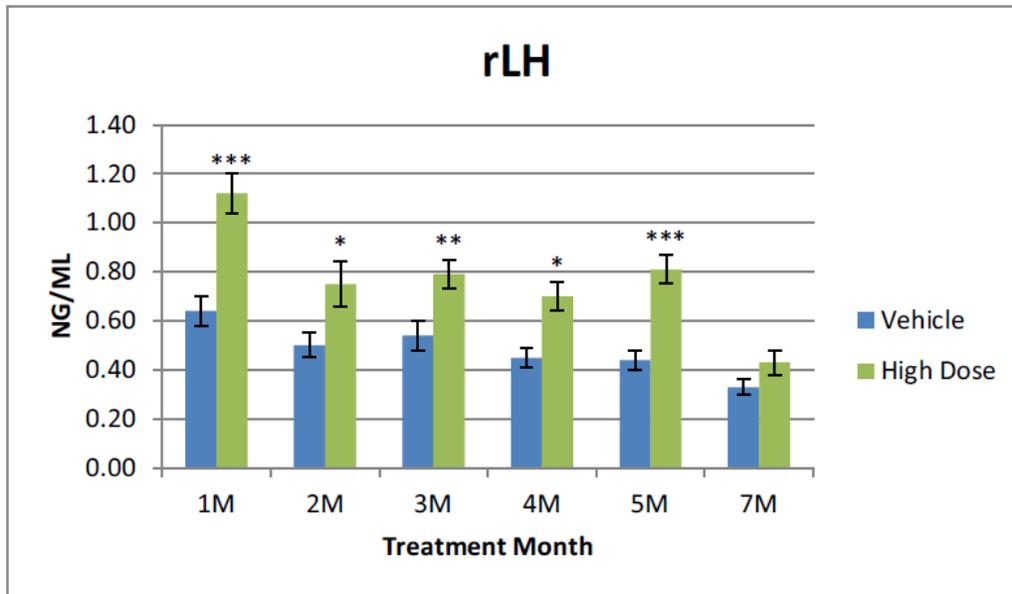
Serum concentrations of LH in male rats treated with vehicle or 100 mg eq./kg canagliflozin for 203 days.   * = P<0.05, ** = P<0.01, *** = P<0.001.
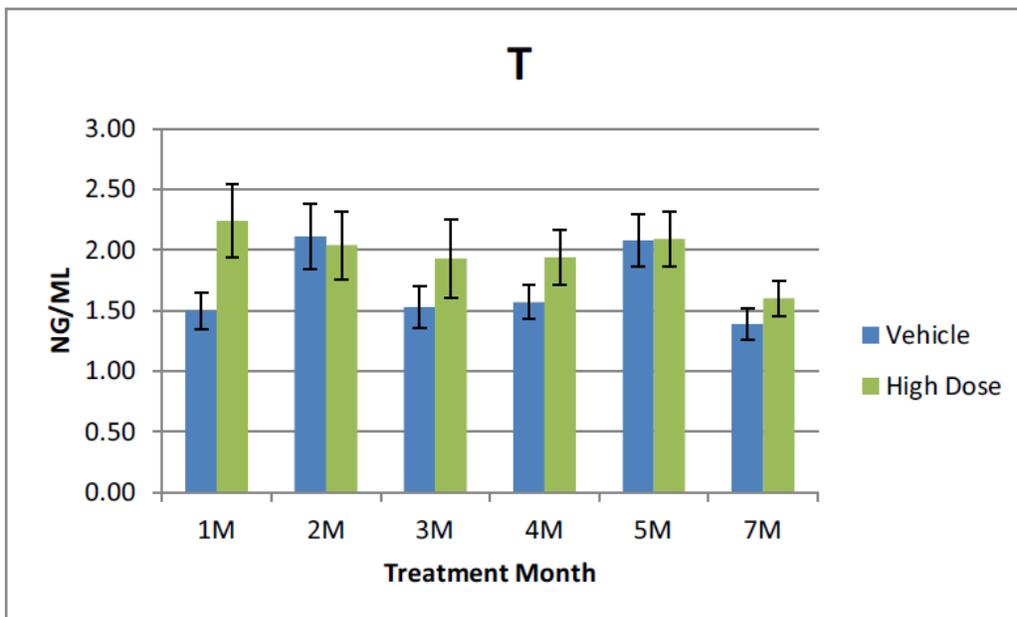
Serum concentrations of testosterone in male rats treated with vehicle or 100 mg eq /kg canaglifozin for 203 days.

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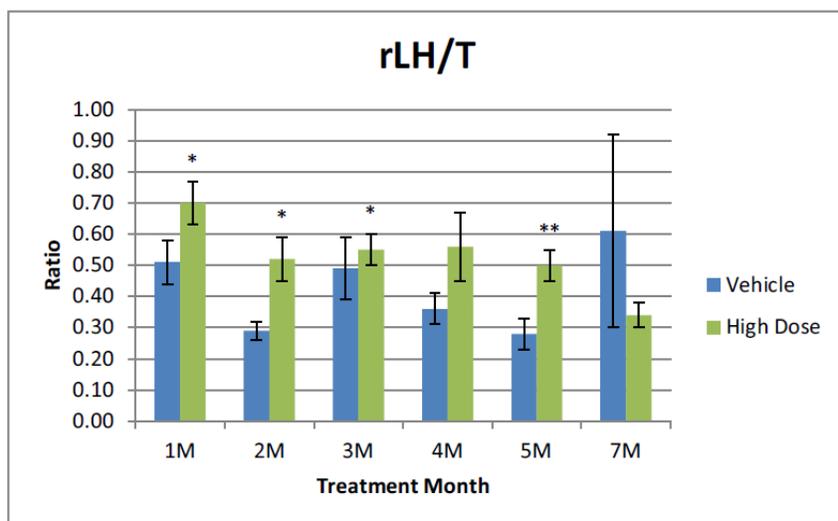
Ratio of rat luteinizing hormone to testosterone in male rates treated with vehicle or
100 mg eq. /kg canaglifozin for 203 days. * = P<0.05, ** = P<0.01.

Reviewer's note: Canagliflozin increased LH levels during the first 5 months. Testosterone
levels were unaffected. The increase in LH was associated with a decrease in the secondary
accessory sex organ weight and increase in testicular interstitial cell hyperplasia, suggesting
that a marked hormonal perturbation had occurred early with canagliflozin. The increase in LH
therefore supported the "hormonal perturbation hypothesis" put forward by the sponsor,
namely, excess LH was resulting in overstimulation of the sensitive rat Leydig cells leading to
hyperplasia and ultimately tumors. Since the disruption of the negative feedback loop by low
testosterone can result in elevated LH, the driving force behind elevated LH remains
unresolved. Whether the increase in LH was due to episodic decrease in testosterone or new
higher LH set point caused by canagliflozin effect on glucose metabolism is not clear.

Clinical Chemistry: Not analyzed.
Urinalysis/Microalbumin: Not analyzed
Gross Pathology: Treatment related discolorations of the adrenal gland and kidneys were
noted.

	Vehicle	Canagliflozin
Daily Dose (mg/kg)	0	100
No. of Animals	M:30	M:30
Gross Pathology		
Animals Examined	30	30
ADRENAL GLANDS		
- Discoloration: pale	2	23
- Swollen	1	3
KIDNEYS		
- Discoloration: pale	1	8
- Swollen	0	1
LARGE INT. COLON		
- Contents: soft	0	2
- Distention	0	2
LARGE INT.CECUM		
- Contents: soft	3	16
- Distention	3	19

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Organ Weights: Consistent with earlier rat studies, canagliflozin increased kidney weight and reduced prostate, seminal vesicles and coagulating glands.

The changes in absolute weight and relative weights.

	Vehicle	Canagliflozin	
Daily Dose (mg/kg)	0	100	
No. of Animals	M:30	M:30	
Organ Weights[e,f]			
MEAN WEIGHT			
FINAL BODY WEIGHT	562.8	0.801	**
ADRENAL GLANDS	0.06429	1.058	
KIDNEYS	3.08	1.136	**
PROSTATE+ SEMINAL VESICLES+ COAGULATING GLANDS	4.42	0.830	**
PROSTATE	1.82	0.791	**
SEMINAL VESICLES+ COAGULATING GLANDS	2.6	0.858	**
MEAN % BODY WEIGHT			
ADRENAL GLANDS (%body)	0.01158	1.302	**
KIDNEYS (%body)	0.54909	1.419	**
MEAN % BRAIN WEIGHT			
ADRENAL GLANDS (%brain)	2.9	1.110	**
KIDNEYS (%brain)	139.4	1.191	**
PROSTATE+ SEMINAL VESICLES+ COAGULATING GLANDS (%brain)	199.6	0.871	**
PROSTATE (%brain)	82.25	0.831	**
SEMINAL VESICLES+ COAGULATING GLANDS (%brain)	117.4	0.899	

Histopathology

Battery Considered Adequate: Adequate for the objectives of the study. Microscopic examinations of the tissues were limited to target organs identified in the earlier studies (adrenal, bone kidney, testes and tissues showing gross lesions).

Peer Review Performed: Not mentioned.

- Microscopic changes in the target organs (renal, adrenal, bone, testes) were consistent with earlier canagliflozin studies in rats.
- Adrenal gland hypertrophy/vacuolization (zona glomerulosa) was observed in canagliflozin rats.
- Hyperostosis occurred in the sternum and stifle of virtually all rats treated with 100 mg/kg canagliflozin.
- Renal tubular dilation was observed in the cortex and outer medulla of almost all rats administered with canagliflozin. Mineralization of pelvis (8/30) was seen in some of the treated rats.
- Testicular interstitial hyperplasia was noted in 7/30 rats treated with canagliflozin.

Relevant Microscopic Findings:

	Vehicle	Canagliflozin	
Daily Dose (mg/kg)	0	100	
No. of Animals	M:30	M:30	
Histopathology[d,g]			
Animals Examined	30	30	
ADRENAL GLANDS, No. Examined	29	30	
- Hypertrophy/vacuolization zona glomerulosa	0	13	**
Grade 1		13	
BONE, STERNUM, No. Examined	30	30	
- Hyperostosis	0	25	**
Grade 1		13	
Grade 2		11	
Grade 3		1	
BONE, STIFLE, No. Examined	30	30	
- Hyperostosis	1	30	**
Grade 1	1	16	
Grade 2	0	13	
Grade 3	0	1	
KIDNEYS, No. Examined	30	30	
- Dilatation pelvic	1	4	
Grade 1	1	3	
Grade 2		1	
- Dilatation tubule(s)	0	29	**
Grade 1		19	
Grade 2		9	
Grade 3		1	
- Exfoliated cells	0	10	**
Grade 1		10	
- Hyperplasia pelvic epithelium	2	7	
Grade 1	2	6	
Grade 2		1	
- Mineralization cortex	2	6	
Grade 1	2	6	
- Mineralization medulla	0	4	
Grade 1		4	
- Mineralization pelvis	0	8	**
Grade 1		5	
Grade 2		1	
Grade 3		1	
Grade 4		1	
- Swollen/vacuolated tubules	2	14	**
Grade 1	1	14	
Grade 2	1		
TESTES, No. Examined	30	30	
- Hyperplasia interstitial cell	1	7	*
Grade 1	1	5	
Grade 2		2	

d   Statistical analysis method used: one-sided Fisher Exact test.
g   Absolute values   ; * - p<0.05, ** - p<0.01, *** - p<0.001

Toxicokinetics: Blood canagliflozin analysis confirmed drug exposure in the study.   The AUC, Cmax and Tmax were comparable to other rat studies.

Day	Dose (mg eq./kg b.w./day)	C_{max} (ng/mL)	t_{max} (h)	AUC_{0-24h} (h*ng/mL)
182	100	24100	3.00	359000
202	100	24500	3.00	356000

Dosing Formulation Analysis: The concentrations, homogeneity and stability of canagliflozin in the solutions were within the predefined acceptance criteria (±10%). The test article in the different formulations was stable for up to 21 days when stored refrigerated (2-8°C).

> **Study Title:** Four-Week Oral Bone Biomarker Investigative Toxicity Study of JNJ-28431754 in Female rats (TOX 8707

The effect of canagliflozin on 8-week old young fast growing female rats was compared to 6-month old female SD rats in a 4-week non-GLP study. Bone formation markers (serum osteocalcin, serum propeptide amino-terminal of type 1 procollagen) and bone resorption markers (serum crosslinked C-telopeptide of type 1 collage, urine deoxypyridinoline/creatinine ratio) were measured in both age groups.

Although the study was relatively short, canagliflozin had significantly greater impact on the growing bones of the young female rats than older rats. Canagliflozin bone hyperostosis was seen only in young rats.

- Canagliflozin reduced plasma glucose by approximately 50% in both young and old to similarly relative to respective control (114 mg/dl)
- Urine glucose increased nearly 3000 fold in both young and old.
- Urine volume increased in both by 2 to 3 fold, slightly more in the older rats
- Urine Ca was increase by 4 fold in old vs. 9 fold in young. The nearly 2 fold higher urinary Ca in the young may explain why hyperostosis was limited to young rats
- Plasma Ca biomarkers (VitD , Calcitonin, PTH) decreased in both young and old rats
- Plasma bone biomarkers were decreased in young rats and old except for bone resorption which remained unchanged in old rats.
- Urine Ca biomarkers were decreased in both young and old
- **Hyperostosis was seen only in the young rats**
- Trabecular bone volume, surface and separations were decreased and trabecular thickness, number and density were increased in young rats. Osteoid volume was increased in old rats and decreased in young rats.
- Canagliflozin AUC on Day 27 were similar

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Species/Strain: Rat/ Crl:CD(SD)		Duration of Dosing: 4-Weeks		Study No.: TOX8707
Age at First Dose: 6 months; 8 weeks		Route: Orally by gavage		
Date of First Dose: 07 February 2008				
Vehicle/Formulation: 0.5% hypromellose solution				GLP Compliance: non-GLP

Daily Dose (mg/kg)	0 (Control, 6-month-old))	0 (Control, 8-week-old)	150/100 (6-month-old) [a]	150/100 (8-week-old) [a]
Toxicokinetics:				
No. of Animals	F:4	F:4	F:4	F:4
Died or Sacrificed Moribund	-	-	2[b]	-
$AUC_{0-\infty}$ (ng·h/mL), Day 0	BLLOQ	BLLOQ	4280000	1060000
AUC_{0-24h} (ng·h/mL), Day 27	BLLOQ	BLLOQ	431000[b]	401000
C_{max} (ng/mL), Day 0	BLLOQ	BLLOQ	65200	53000
C_{max} (ng/mL), Day 27	BLLOQ	BLLOQ	23100[b]	25800
No. of Animals	F:10	F:10	F:10	F:10
Noteworthy Findings				
Died or Sacrificed Moribund	-	-	4[c]	-
Body Weight (g)[d]	406.18	241.84	0.95×	0.97×
Body Weight Gain (g)	-0.81	14.03	2.27	10.14
Food Consumption (g/day)[d]	22.03	21.69	1.38× **	1.33× **
Clinical Observations				
Absent feces	-	-	3	-
Decreased feces	-	-	8	-
Soft feces	-	-	6	-

[a] Due to toxicity and mortality in the 6-month old rats dosed at 150 mg/kg/day, the dose of both 150 mg/kg groups was lowered to 100 mg/kg on Day 6.
[b] N = 2 due to early deaths on Day 5
[c] Two deaths occurred, one on Day 5 (3509) and one on Day 7 (3503); two accidental deaths occurred on Day 28 following blood collection for clinical pathology evaluation
BLLOQ = Below lower limit of quantification
- = No noteworthy findings
** - p<0.01

Daily Dose (mg/kg)	0 (Control, 6 month old))	0 (Control, 8-week-old)	150/100 (6-month-old)	150/100 (8-week-old)
No. of Animals	F:10	F:10	F:8	F:9/10[a]
Hematology/Coagulation (Week 4)	-	-	-	-
Serum Chemistry[b] (Week 4)				
ALT (IU/L)	-	28.7	-	1.88×***
AST (IU/L)	-	94.3	-	1.50×
UREA N (mg/dL)	19.2	16.8	1.63×	1.99×
CA (mg/dL)	11.17	10.71	0.93×	0.95×
PHOS (mg/dL)	-	9.98	-	0.95×
TP (g/dL)	-	6.40	-	0.91×
ALB (g/dL)	4.61	4.07	0.92×	0.90×
GLU (g/dL)	113.6	114.2	0.52×	0.51×***
TRIG (mg/dL)	84.9	32.5	1.69×*	2.14 ×
Urinalysis[a] (Week 4)				
URVOL (mL)	8.70	7.90	2.92×***	2.32×**
SP GR	1.0360	1.0344	1.49×	1.63×***
GLU/CREAT (mg/mg)	0.094	0.109	2389.65×***	2678.17×***
GLU-EXCRETED (mg/hr)[b]	0.753	0.543	2746.52×***	2819.64×***
CA/CREAT (mg/mg)	0.211	0.085	4.11×	8.80×***
CA-EXCRETED (mg/hr)[b]	1.778	0.428	4.52×	9.38×***
MG/CREAT (mg/mg)	0.206	0.200	4.65×	6.19×
MG-EXCRETED (mg/hr)[b]	1.810	1.046	4.87×	6.13×
PHOS/CREAT (mg/mg/)	1.589	2.389	2.53×	2.58×***
PHOS-EXCRETED (mg/hr)[b]	13.102	12.258	2.80×	2.62×
NAG/CREAT (U/mg)	0.013	0.016	1.92×**	2.13×***
NAG-EXCRETED (U/hr)[b]	0.108	0.085	2.08×***	2.11×
NA/CREAT (mmol/mg)	0.052	-	2.09×***	-
NA-EXCRETED (mmol/hr)[b]	0.430	-	2.29×	-

[a] At end of dosing period. For controls, group means are shown. For treated groups, multiples of control/baseline are shown. Statistical significance is based on actual data (not on the multiples of control/baseline).

[b] Excreted values are based on 16 hour urine sample collection.
- = No noteworthy findings
* - p<0.05, ** - p<0.01, *** - p<0.001

Four-Week Oral Bone Biomarker Investigative Toxicity Study of JNJ-28431754-ZAE in Female Rats

Daily Dose (mg/kg) No. of Animals [a]	0 (Control, 6 month old)) F:10	0 (Control, 8-week-old) F:10	150/100 (6-month-old) F:6/5 [a]	150/100 (8-week-old) :9[a]
Serum Biomarkers for Calcium Homeosasis[b]				
1,25-Dihydroxyvitamin D (pmol/mL)	34.58	326.00	0.29×[c]	0.06×*
25-Hydroxyvitamin D (ng/mL)	13.12	20.58	0.76×*	0.57×***
Calcitonin (pg/mL)	159.1	129.0	0.28×*	0.58×
Intact Parathyroid hormone (pg/mL)	5468.9	2775.0	0.38×*	0.38×***
Insulin (ng/mL)	0.78	0.71	0.32×**	0.27×**
Serum Biomarkers for Bone Turnover)[b]				
Osteocalcin (ng/mL) – bone formation	8.51	26.35	0.67×*	0.52×***
Crosslinked C-telopeptide of type 1 collagen (ng/mL) – bone resorption	22.9	108.2	1.11×	0.77×
Propeptide amino-terminal of type I procollagen (ng/mL) – bone formation	1.32	5.29	0.77×*	0.42×***
Urinary Biomarkers for Calcium Homeosasis[c]				
Urinary deoxypyridinoline/creatinine ratio (nM/mM) – bone resorption	16.49	167.66	0.68×	0.57×***

[a] Number of animals for serum/urinary parameters: 1,25 Dihydroxyvitamin D n=5; all other calcium homeostasis biomarker parameters n=6
[b] At end of dosing period. For controls, group means are shown. For treated groups, multiples of control/baseline are shown. Statistical significance is based on actual data (not on the multiples of control/baseline).
[c] Excreted values are based on 16 hour urine sample collection.
     -    = No noteworthy findings
* - p<0.05, ** - p<0.01, *** - p<0.001
Note: Bone turnover marker is a generic term that covers both bone formation and resorption markers.

Four-Week Oral Bone Biomarker Investigative Toxicity Study of JNJ-28431754-ZAE in Female Rats

Daily Dose (mg/kg) No. of Animals	0 (Control, 6 month old)) F:10	0 (Control, 8-week-old) F:10	150/100 (6-month-old) F:8	150/100 (8-week-old) F:10
Gross Pathology	-	-	-	-
No. of Animals	F:10	F:10	F:6	F:10
Organ weights [a]				
Kidney (g)	2.44	1.73	1.13x **	1.14x*
Kidney mean % body (g)	0.64061	0.77751	1.27x	1.23x
No. of Animals	F:10	F:10	F:10	F:10
Histopathology				
Bone Sternum hyperostosis minim	-	-	-	9
Minimal	-	-	-	9
Bone stifle hyperostosis	-	-	-	10
Mild	-	-	-	8
Moderate	-	-	-	2
Histomorphometry[a]				
Trabecular bone volume (%)	15.54	13.84	0.73×	2.58×***
Bone surface/bone volume (mm/mm²)	36.7	62.6	1.34×*	0.75×***
Trabecular thickness (μm)	56.7	32.1	0.78×*	1.36×***
Trabecular number (mm⁻¹)	2.695	4.260	0.89×	1.92×***
Trabecular separation (μm)	370.9	212.4	2.49×	0.39×***
Mineralized trabecular bone volume (%)	15.54	13.82	0.73×	2.59×***
Osteoid surface (%)	0.463	0.822	2.45×	0.50×
Osteoid thickness (μm)	2.05	2.84	1.11×	1.16×
Osteoid volume, bone referent (%)	0.042	0.142	10.70×	0.43×
Osteoid volume, tissue referent (%)	0.007	0.019	0.88×	1.17×

[a]At end of dosing period. For controls, group means are shown. For treated groups, multiples of control/baseline are shown. Statistical significance is based on actual data (not on the multiples of control/baseline).

 - = No noteworthy findings
* - p<0.05, ** - p<0.01

Study Title: 1-Month repeated dose oral toxicity study of J⬛⬛⬛⬛⬛⬛ (b) (4) (dapagliflozin) in the male SD rat (TOX 10359

(b) (4)

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11   Integrated Summary and Safety Evaluation

Canagliflozin (JNJ-28431754-ZAE) is a selective sodium-glucose co-transporter 2 (SGLT2) inhibitor designed to lower plasma glucose by reducing the renal glucose threshold independent of insulin. Under normal conditions, filtered glucose is reabsorbed back to blood by the renal tubular SGLT2 (90%) and SGLT1 (10%) transporters.   The reabsoption of glucose is effective up to 180 mg/dl glucose concentrations (glucose threshold). In diabetics, the glucose threshold is increased to 240 mg/dl. As a selective SGLT2 inhibitor, canagliflozin has been shown to reduce renal glucose threshold to as low as 70 mg/dl in diabetic patients, resulting in a significant spillage/excretion of glucose into urine.

Canagliflozin was nearly 160 fold more selective to renal SGLT2 (IC_{50} 4.1 nM) than SGLT1 (IC_{50} 664 nM), making the renal tubules the primary target organ for the drug pharmacology. The selectivity may diminish after high oral doses, where local intestinal levels can reach high enough to inhibit SGLT1.   The pharmacology effect of canagliflozin on intestinal SGLT1 may have indeed been responsible for most of adverse findings in rats. In the in vitro IC_{50} studies, canagliflozin was nearly as effective in inhibiting SGLT2 in mice as it was in rats, dogs and humans (similar IC_{50}). However, in the in vivo studies, SD rat was far more sensitive to canagliflozin than other species, making rat an ideal model to test the toxicity of canagliflozin. The order of in vivo activity was rats > > dogs > rabbits > mice.   The chronic toxicity of canagliflozin was tested in rats and dogs while reproductive toxicity was assessed in rats and rabbits and found no teratogenic signal (reviewed by Dr. Mink separately). The 2-year carcinogenicity of canagliflozin was determined in mice and rats.

Impurities and degrdanats

Canagliflozin batches used in the nonclicnial and clincial studies had a purity greater than 97%. The Impurities identified during the bacth analsysis were within the specification, requireing no further analysis. However, in the 4-month safety update during the NDA review cycle, the sponsor notified the agency of presence of a genotoxic degradant (b) (4) in the drug product in the 18-month stability study.   The degradant was present in drug substance and drug product, reaching concentrations up to (b) (4) , signficaintly greater than the 1.5 µg/day dose limit set forth in the guidanc for genotoxic impurities for chronically used pharmacuticals. Upon further analysis, the (b) (4) moeity was found to be responsible entity for the weak genotoxicity of degrdant. Since humans are exposed up to 1000 fold higher levels of (b) (4) on daily basis in the diet (edible oils, tooth past), the specifications for the degrdant was raised to (b) (4) for 100 and 300 mg tablets, respectively.

Safety Pharmacology

Canagliflozin pharmacological activity is limited to renal SGLT2 and to a small extent intestinal SGLT1 at very high concentrations, thus as expected, it had minimal or no direct effect on CNS, cardiovascular, and respiratory system. The small decrease in blood pressure noted in the clinical studies was therefore likely to the diuretic effect of glucose. There was no data to show a direct canagliflozin effect on blood pressure or heart rate.

ADME

Oral dose of canagliflozin is well absorbed in all 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. 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 female 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 binding was 97.3 and 39.8%, respectively. Kidney as both the site of the drug pharmacology and clearance, play a pivotal role on canagliflozin disposition and activity. Therefore, the greater the severity of renal impairment, the greater is the drug exposure in humans. However, since drug efficacy depends on effective GFR, the greater exposure does not translate to higher efficacy.   Indeed, decrease in GFR can significantly diminish drug efficacy. Since GFR is known to decrease by 1 ml/min per year after the age 40 in humans, the clinical efficacy is predicted to be lower in old diabetics with no renal reserve capacity.

Canagliflozin is extensively metabolized by uridine diphosphate glucuronosyltransferase (UGT) to *O*-glucuronide metabolites (M7 by UGT1A9 & M5 by UGT2B4) in humans but no to the same extent in animals.   CYP enzymes had minimal role in metabolism of canagliflozin in humans. The two *O*-glucruonide metabolites are inactive and highly water soluble. Although plasma exposure to M7 and M5 was low in rats, the analysis bile found significantly greater M7 (12%) and M5 (4%) exposure than previously reported in rat sugesting that rats do make the metabolites but they are rapidly hydrolizeded back to the parnt drug.   Overall, all the canagliflozin metabolites in humans were present in animals qualitatively, but quantitatively animals had lower exposure to M7 and M5. Since the two metabolites are inactive, highly water soluble and readily filtered by the kidney, they were considered safe.

Chronic Toxicology Studies

The chronic toxicity studies identified two key target organs, kidney and bone. Per canagliflozin pharmacology, the decrease in renal glucose threshold results in significant increase in urinary glucose excretion and associated diuresis and weight loss, especially in rats where significant dose-related increase in renal tubule and pelvic dilatation occurred. The dilations also extended to the urethra and bladder in rats. With no evidence of renal tubule hyperplasia, changes in the kidney and urinary tract were considered an adaptive response to polyuria. Although there was a significant dose-dependent increase in the incidence of renal tubule hyperplasia in the 15-day preliminary 2-year rat carcinogenicity safety report, re-examination of the renal slides by the pathology-working group found no meaningful increase in the incidence of renal tubule hyperplasia. Whether canagliflozin had any proliferative effect on renal tubules in rats remains to be seen. There were minimal renal findings in mice and dogs, likely due to limited diuresis.

Based on potential underlying causes and morphological characteristics, canagliflozin effect on bone could be considered somewhat two distinct adverse findings, an acute effect noted

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in the trabecular bone and chronic effect noted in the compact bone. The acute effect on trabecular bone was seen only in rats (not seen in mice, dogs or humans) caused by hypercalcium absorption while the decrease in compact bone density and strength seen in the older rats and dogs appeared to be related to weight loss and Ca metabolism. Canagliflozin consistently caused trabecular bone hyperostosis (stifle, sternum and femur) in young rats in as few as 4 days of high doses of canagliflozin and over time at as low as ≥ 4 mg/kg.   Hyperostosis was generally seen at one site at low doses in females (higher drug exposure than male rats), then expanding to more sites in both sexes at higher doses (≥20 mg/kg, ≥4x the MRHD) and persisted until the end of the studies (6 to 24 months). Canagliflozin effect on bone was greater when the treatment was initiated at young age of 8 weeks in rats undergoing rapid growth. In fact, old rats (6 months) did not develop hyperostosis.   Since hyperostosis was present at the end of the 6-month chronic toxicology and at 24 months in rat carcinogenicity study suggests that once it is formed at young age, it persist until the end of the treatment. In these animals, hyperostosis was characterized by increase in trabecular bone volume and number and decrease in trabecular separation and osteoid surface. There was little change in trabecular thickness. Associated with the early signs of hyperostosis were the significant decreases in Ca biomarkers (i.e. 1,25 dihydroxyvitamin D and serum PTH at ≥ 20 mg/kg; ≥4x the MRHD) suggesting that a substantial increase in Ca absorption had taken place. The decrease in Ca regulating hormones (i.e. PTH, VitD) and increase in Ca deposition (i.e. tissue mineralization, hyperostosis) and urinary excretion was an attempt to normalize excess incoming Ca. Therefore, calciuria was not caused by the diuretic effect, but rather by the hypercalcium absorption in rats.   Indeed, the mechanistic studies supported the hypercalcium absorption as the primary mode of action for hyperostosis and calciuria. When dietary Ca was limited in the low Ca diet (1/10th or normal diet), or when the carbohydrate malabsorption was prevented, there was a substantial decrease in hyperostosis and calciuria suggesting that hyperostosis was caused by hypercalcium absorption in young rapidly growing rats. Although there was no hyperostosis in old rats or humans, there is still a small risk that young children undergoing rapid bone growth or exposed in utero may be vulnerable to canagliflozin effect on bone growth.

The compact bone effect was observed in chronic rat and dog toxicology studies, generally at top doses (≥12x the MRHD).   The compact bone effect was generally marked by decreases in bone turnover enzymes and lower bone density and strength. Canagliflozin decreased rat femur bone area at ≥ 20 mg/kg(≥ 4x the MRHD) and femoral mineral density and shaft strength at 100 mg/kg (≥12x MRHD). Femoral shaft maximal load and energy decreased by as much as 15 and 25% in HD male rats, respectively. Mechanical strength of L5 lumbar vertebral bone was decreased at all doses in male (< 1x the MRHD) and at 100 mg/kg in female rats. The decrease in bone mineral density correlated with the decrease in osteocalcin in both male and female rats at ≥ 20 mg/kg (≥4x the MRHD). The changes observed in the compact bone appeared to correlate to the decrease in BW in male (- 26%) and female rats (-20%). Similar pattern was also seen in dogs. Male dogs that lost weight also had lower femur mineral density. The decrease in bone density in humans was suspected to be due to weight loss. In female dogs, neither the BW nor the mineral density was decreased supporting the sponsor's assertion that the decrease in bone density in clinical studies was related to decrease in body weight.   What remains to be seen is the

long-term effect of canagliflozin on Ca absorption, excretion and deposition. Since Ca is essential to bone density and strength, any shift however slight may have long-term impact on bone health.

Carcinogenicity Studies

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. In mice, canagliflozin doses as high as 100 mg/kg (7 to 14x the clinical dose of 300 mg QD AUC basis) did not increase the incidence of any type of tumor. Canagliflozin had no notable effect on mortality, survival rate, body weight or organ weight. There were sufficient numbers of animals in each group surviving to the end of the study and with adequate drug exposure to make the study valid and acceptable. The mouse study provided adequate coverage for canagliflozin and its' prominent human inactive O-glucuronide metabolites (M5 and M7). The safety margins for NOAEL dose of 100 mg/kg in male and female mice were 7x and 14x the MRHD, based on AUC, respectively.

In rats, canagliflozin significantly increased the incidence of renal tubule and adrenal pheochromocytoma tumors at top dose of 100 mg/kg in male and female rats and testicular Leydig cell tumors at all doses in male rats. The two renal tumors in the MD male rats (P>0.05) were considered spontaneous by the pathology working group (PWG) based on amphophilic vacuolated appearance. Only the basophilic appearing renal tubule tumors were considered treatment related. The few incidences of liver and bladder tumors observed in the treated rats were not significantly different from control in the pair wise comparison and were generally within the historical range from different labs.

Canagliflozin increased the incidence and severity of chronic progressive nephropathy (CPN), renal tubule hypertrophy, and pelvic mineralization. The severity of CPN ranged from minimal to moderate and was not considered by the reviewer or by the PWG to be sufficiently severe enough to increase the incidence of renal tumor.

The emergent tumors seen in the SD rat here are frequently observed with compounds that inhibit intestinal carbohydrate absorption. For example, acarbose a complex oligosaccharide known to delay digestion of ingested carbohydrates, increased the incidence of the same tumors in rats. When the diet was modified to prevent carbohydrate malabsorption, both the renal and adrenal tumors were no longer present at the end of the 2-year acarbose study, suggesting that carbohydrate malabsorption was responsible for the adrenal and renal tumors in SD rats.   This mode of action was also proposed by the sponsor for canagliflozin and supported by 6-month mechanistic studies in male rats. Exactly how carbohydrate malabsorption results in renal tumors is not clear. The sponsor had hypothesized calcium hyperabsorption in an acidic intestinal environment caused by bacterial fermentation of unabsorbed carbohydrates as the potential mode of action. The role of hypercalcium absorption was supported by data that canagliflozin can result in significant calciuria, bone hyperostosis and tissue mineralization marked by significant decrease in Vit D and PTH in rats.

Carbohydrate Malabsorption Hypothesis

Although canagliflozin is more than 160 fold more selective to renal tubule SGLT2, bolus oral dosing of 100 mg/kg, can result in significant local exposure, high enough to inhibit intestinal SGLT1. As a transporter of intestinal glucose and galactose, inhibition of SGLT1 by canagliflozin can technically lead to carbohydrate malabsorption similar to acarbose. The division considered the carbohydrate malabsorption hypothesis as a reasonable mode of action for the tumors but requested specific mechanistic studies supporting it.

The carbohydrate malabsorption/ hypercalcium absorption hypothesis was tested in two 6-month mechanistic studies where the dietary carbohydrate or Ca was modified to prevent renal tubule and adrenal cell proliferation. Since there was no notable renal tubule hyperplasia in the 6-month rat study, the sponsor chose Brdu and Kim1 (immunohistochemistry stains) as renal and adrenal cell proliferation markers.

Proposed carbohydrate malabsorption and calcium hyperabsorption hypothesis

In the first 6-month study, the carbohydrate portion (i.e. fructose and sucrose) was replaced with 40% high fructose (HF). Since fructose is transported via glucose transporter type 5 (GLUT5), its absorption is unaffected by SGLT1 inhibition, therefore HF fed rats should have minimal renal and adrenal cell proliferations. Renal and adrenal proliferation (Brdu and KIM-1) were substantially lower in the HF canagliflozin rats than standard diet canagliflozin rats, suggesting that preventing carbohydrate malabsorption can substantially reduce renal tubule and adrenal cell proliferation. Furthermore, HF diet resulted in significant decline in calciuria and hyperostosis suggesting that carbohydrate malabsorption indeed was responsible for hypercalcium absorption, bone hyperostosis, common observations in standard chow fed canagliflozin treated rats.

In the second 6-month study, male rats were fed a 0.1% low calcium diet (LC) to prevent hypercalcium absorption and sequelae, renal and adrenal cell proliferation. The LC diet contained $1/10^{th}$ of calcium in the standard rat chow. LC diet failed to reduce renal or adrenal cell proliferation biomarkers. In fact, both were increased. However, LC diet significantly reduced calciuria and bone hyperostosis suggesting that enhanced Ca absorption was pivotal for bone hyperostosis and calciuria. Ca was likely coming from the diet and not from the bone. The study did not support the role of calcium as the final step in carbohydrate malabsorption hypothesis. However, it did provide evidence for hypercalcium in hyperostosis. Hypercalcium absorption was pivotal to bone hyperostosis but not necessarily as the proximal step in the carbohydrate malabsorption hypothesis as proposed by the sponsor.
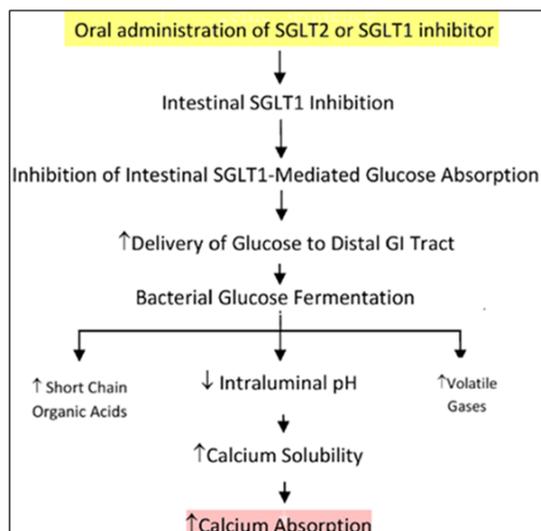
Testicular Leydig Cell Tumors
Canagliflozin induced a significant dose-dependent increase in the incidence of testicular Leydig cell tumors (1/65, 8/65, 20/65 and 24/65 in C, LD, MD and HD, respectively) in male rats with no safety margin (<1x the MRHD). Based on published literature, the sponsor hypothesized that elevation in LH may have played a pivotal role since rat Leydig cells are known to have nearly 10 fold more LH receptors than humans [1,2], making rats extremely sensitive to increased LH. Increased LH levels have been consistently associated with an increase in Leydig cell tumors in rats. To explore the role of LH, the sponsor performed a 7-month mechanistic study to monitor the monthly changes in LH and testosterone in male rats maintained on standard rodent chow. Canagliflozin significantly increased LH levels during the first 5 months. Testosterone was unchanged. The increase in LH levels supported LH role as the potential cause of Leydig cell tumors in rats. Since clinical studies found no change in LH, the Leydig cell tumors are considered clinically irrelevant. It should be noted that Leydig cell tumors were also observed in acarbose carcinogenicity studies in rats. However, unlike the renal and adrenal tumors, preventing carbohydrate malabsorption did not prevent testicular tumors suggesting a different mode of action (i.e. increase in LH).

In summary, toxicological assessment of canagliflozin identified kidney (rat only) and bone as the primary target organs. The dilatation of renal tubule, pelvis and urinary tract appeared to be an adaptation to persistent diuresis caused by the osmotic effect of excreted glucose in rats. Absence of renal tubule dilatation and nadir diuresis in mice and dogs appeared to support the adaptive response. Trabecular bone hypostasis was caused by hypercalcium absorption with fast growing young rats being the most vulnerable. Older rats with stable bone growth were not as susceptible to hyperostosis as young rats. Compact bone effect developed over time with chronic use of high dose of canagliflozin in both rats and dogs with strong correlation to weight loss. Since high dose canagliflozin may affect body weight and Ca metabolism, long term clinical monitoring of bone strength and density in diabetics may be advisable.

Canagliflozin was not carcinogenic in mice. However, in rats, it increased the incidence of renal tubule and adrenal tumors in both sexes at 100 mg/kg (12-21x the MRHD) and testicular tumors at all doses in males (no safety margin). Canagliflozin induced carbohydrate malabsorption appeared to be the mode of action for renal and adrenal tumors.

Testicular Leydig cell tumors in rats were caused by their sensitivity to increase in LH (high density of LH receptors in rat Leydig cell than in humans). Since there was no change in LH in humans, testicular tumors were not clinically relevant. With no significant increase in renal and adrenal tumors at 30 mg/kg, the safety margin for renal and adrenal tumors was 5x to 7x the clinical dose of 300 mg QD based on AUC.

Safety margin for canagliflozin

Species	Daily Dose, (mg/kg)	Canagliflozin AUC$_{0-24}$ (μg.h/ml)	NOAEL, (mg/kg) M/F	AUC Safety margins, (Animal /Human)	
				male	Female
13-Week mouse Study	30	M:65.9 F:102		2	3
	100	M:235 F:354	100	9	14
	300	M:651 F:736		25	28
13-Week rat study	4	M:12.6 F:21.3	M: 4	<0.5	<0.8
	20	M:74.4 F:94.6	F:20	3	4
	100	M:365 F:470		14	18
6-Month rat study	4	M:14.1 F:21.6	< 4	<1	<1
	20	M:68.5 F:88.3		3	3
	100	M:321 F:379		12	14
12-Month Dog Study	4	M: 52.2 F: 67		2	2.6
	30	M: 262 F: 264	M:30	10	10
	100	M: 529 F: 503	F:100	20	19
104-Week Mouse Carci Study	10	M:11.3 F:36.4		<1	1
	30	M:47.7 F: 102		2	4
	100	M:194 F:353	100	**7**	**14**
104-Week Rat Carci Study	10	M:38.3 F:62		2	3
	30	M:118 F:188	30	5	7
	100	M:316 F:560		**12**	**21**
Maximum Clinical Dose: Canagliflozin , 300 mg		**26.1**			

1. Prentice DE., Meikle AW. (1995). A review of drug-induced Leydig cell hyperplasia and neoplasia in the rat and some comparisons with man. Human and Experimental Toxicology 14, 562-572. 2.

2. Clegg E. et al. (1997). Leydig cell hyperplasia and adenoma formation: mechanisms and relevance to humans. Reprod Toxicol 11, 107-121

12   Appendix/Attachments

Expert Report on Histological Changes in Rat Kidney Associated with oral exposure to canagliflozin by Dr. [b) (4)

Prior to the PWG, the sponsor enlisted the assistance of expert renal pathologist, [b) (4) The objective of the analysis was to determine the key histopathological events that would support a mode of action underlying the development of renal tumors. [b) (4) reviewed the renal slides at the JNJ facility in NJ between March 28 and April 4, 2011. His report was submitted to the agency on behalf of the sponsor (Sept 13, 2011). It should be noted that [b) (4) also participated in the PWG evaluation held on June 24, 2011 (final report, Aug 12, 2011).

Studies, groups, and animal numbers for which kidneys were examined.

Two-week oral toxicity study (TOX7633)
Control males, 0 mg/kg – animal nos. 1001 through 1010.
High-dose males, 150 mg/kg – animal nos. 4001 through 4010.

One-month repeated dose oral toxicity study (TOX9582)
Group VM, control males 0 mg/kg – animal nos. 1 through 10
Group H2, high-dose males 100 mg/kg – animal nos. 81 through 90.

Three-month repeated dose oral toxicity study (TOX9667)
Group VM, control males 0 mg/kg – animal nos. 1 through 10.
Group H2M, high-dose males 100 mg/kg – animal nos. 81 through 90.

Thirteen-week oral toxicity study (TOX8150)
Group 1, control males 0 mg/kg – animal nos. 1001 through 1010.
Group 4, high-dose males 100 mg/kg – animal nos. 4001 through 4010.

Six-month repeated dose oral toxicity study (TOX8574)
Group VM, control males 0 mg/kg – animal nos. 1 through 19.
Group HM, high-dose males 100 mg/kg – animal nos. 61 through 80.
Group HF, high-dose females 100 mg/kg – animal nos. 161 through 179.

Two-year oral gavage carcinogenicity study (TOX8986)
Group VM, control males 0 mg/kg – animal nos. 1 through 65.
Group LM, low-dose males 10 mg/kg – animal nos 101 through 160.
Group MM, mid-dose males 30 mg/kg – animal nos. 201 through 265.
Group HM, high-dose males 100 mg/kg – animal nos. 301 through 365.
Group HF, high-dose females 100 mg/kg – animal nos. 721, 730, 740, 741, 747, 752, 756.

Diagnostic criteria for renal tumors was based mainly on [b) (4) (Hard et al, 1995, Hard el al, 1999) recommended by the Society of Toxicology Pathology.
Per [b) (4) analysis, the most persistent and underlying observation was increased patency and dilation of the tubules and collecting ducts. The severity and incidence increased with duration of canagliflozin treatment and generally enveloped the renal system. For example, tubule dilation in the 1-month study was expanded to include cortical ducts in the 3-month study in treated male rats with occasional evidence of exfoliation of cells in the lumen of

proximal tubules of the deep cortex. In the 6-month study, the patency and dilation of distal tubules in the cortex and collection tubules in the OSOM was clearly notable in both male and female rats. [(b) (4)] found no apparent change in the severity of CPN between control and HD males in the 6-month study.

Mild patency /dilation of distal tubules in the cortex and collecting tubule in the outer medulla was also noted in nearly all HD males and was therefore considered the most prominent non-neoplastic finding in the 2-year rat study. [(b) (4)] considered approximately 20% of these rats to have small, ill-defined areas of tubule basophilia with minimal to mild retrograde nephropathy. Similar to the JNJ study pathologists, [(b) (4)] noted mineralization of renal pelvis and associated transitional cell hyperplasia of the urothelium. He added that approximately 40% of these animals had one to several dilated cortical tubules displaying tubule hypertrophy.

An example of renal tubule hypertrophy slide was provided in the report (HD male #306 in the 2-year rat study) depicting mildly dilated eosinophilic tubules with lining cells protruding into the lumen. This male rat had treatment-related (basophilic) renal tubule adenoma (not depicted in the slide).
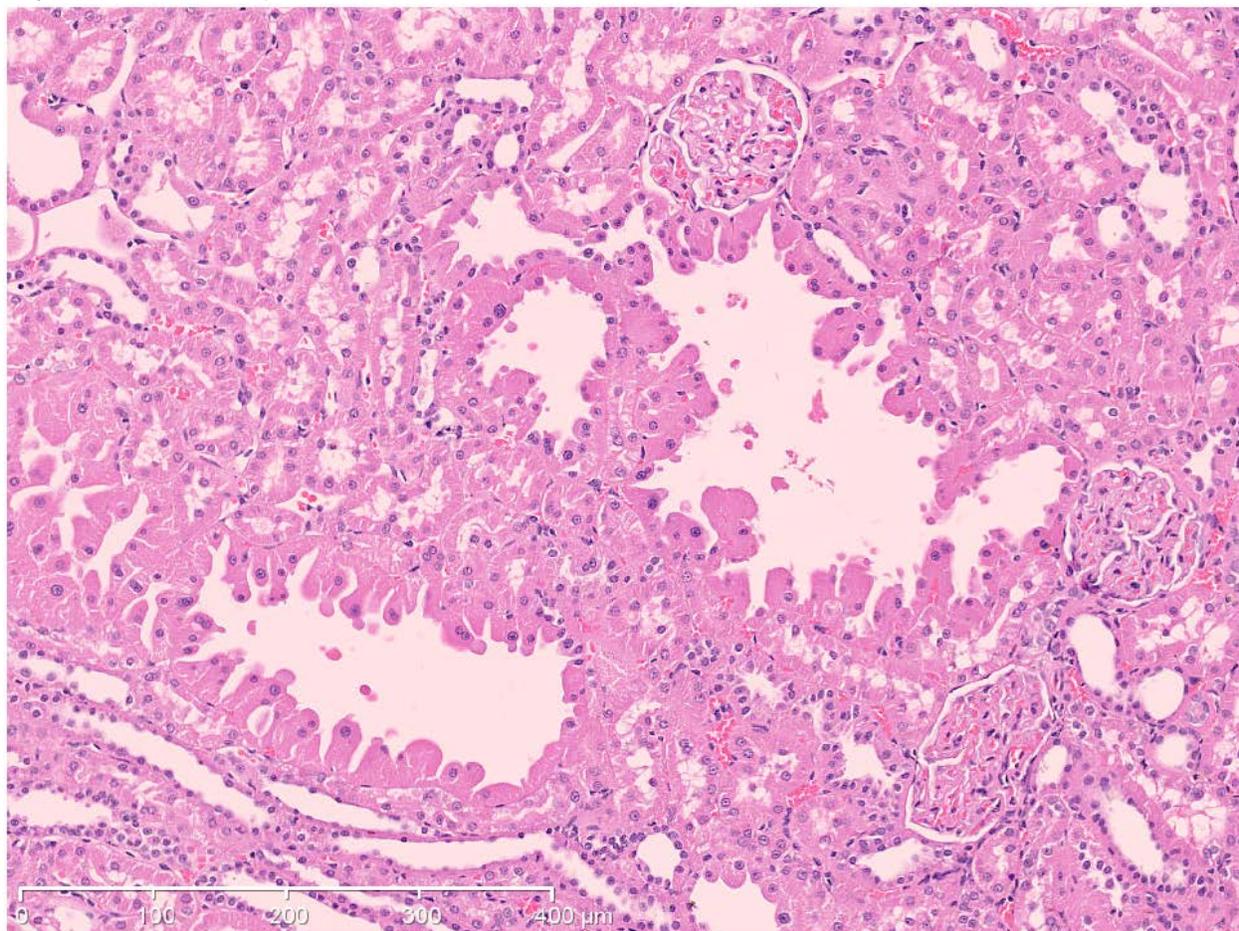
Examples of tubule hypertrophy in the cortex, showing hobnail-shaped, enlarged cells, often with apical nuclei.

[(b) (4)] stated that the deep cortex of some HD rats had trace nuclear variability in size involving proximal tubules and occasional karyomegaly which were occasionally observed in the hypertrophic tubules. [(b) (4)] concluded that there was an absence of tubule hyperplasia as a general response to canagliflozin, which contradicts the initial rat safety report submitted by the sponsor and what the agency has seen with other SGLT2 inhibitors. [(b) (4)]

24-Month Rat Study

[(b) (4)] agreed with the incidence of renal tubule tumors in rats. Collectively, there were 12 renal tubule tumors in the HD males, 8 in HD females and 2 in MD males (Adenoma: 222 and 259). The tumors in all HD rats except for one (HD male rat #309) appeared basophilic with well-formed tubular differentiation. The carcinoma in the HD male (#309) had a mixture of solid, clear cell and tubular areas. The tumors appeared to have originated from both cortex and OSOM based on distribution of the small lesions.   There was no association with hyperplasia except for rat # 306 which has atypical tubule hyperplasia.
The two MD male rats with renal tubule tumor had different characteristics. The adenoma in MD rat #222 consisted of solid or lobular areas of mixed clear and basophilic cells, without differentiation of the tumors seen in the HD rats. The MD rat #259 with carcinoma had the amphophilic-vacuolar tumor type and an atypical hyperplastic lesion of the same type described in Hard et al, 2008.

[(b) (4)] concluded that the most common non-neoplastic treatment related change was the increased dilation of the cortical distal tubules and outer medulla of collecting tubules, likely due to increased urine flow. Canagliflozin appeared to have caused increased tubule hypertrophy in the cortex over time to adapt to increased filtration. He added that pelvic mineralization along with transitional cell hyperplasia frequently occurs in older rats but could have been also due to a canagliflozin-related increase in Ca absorption and excretion. He stated that renal tubular tumors occur by several processes (listed below).

1. direct DNA reactivity (eg several nitrosocompounds),
2. indirect DNA reactivity mediated by oxidative stress (eg potassium bromate),
3. sustained cytotoxicity/regeneration mediated directly by the chemical but unassociated with DNA reactivity (eg chloroform),
4. sustained cytotoxicity/regeneration associated with an indirect physiological perturbation as occurs with alpha-2$_u$-globulin nephropathy (eg d-limonene),
5. interaction with spontaneous CPN (eg ethyl benzene).

[(b) (4)] noted that none of the lesions seen in the canagliflozin rat study (tubule dilation, hypertrophy and pelvic mineralization) would have caused renal tubule tumors. If canagliflozin had caused renal tumors by sustained cytotoxicity and regeneration, there would have been histological evidence of injury such as single cell deaths in the tubules. Further, it was unlikely that CPN had a role since the severity was low. Only end-stage CPN has been associated with renal tumors (Hard et al, 2011). The absence of hyaline droplet accumulation and granular

casts in the 3-month studies and the absence of linear papillary mineralization in the carci study suggest that alpha-2U globulin nephropathy was also not involved.   Since the morphology of most of the tumors was remarkably uniform, a genetic cause of the tumors cannot be excluded thus he recommended DNA analysis for potential genetic link and whether rats with amphophilic renal tumor appearance were full or half sibling. The liver DNA analysis performed later by the sponsor found no genetic link among the rats with amphophilic-vacuolated (A-V) spontaneous renal tubule tumors (2 MD and 1 HD male rats).

In summary, nearly all the HD renal tumors had morphology different from the amphophilic-vacuolar (A-V) phenotype which occurs spontaneously (Hard et al, 2008). He added that one of the two MD male rat renal tumors was spontaneous due to A-V appearance and thus should not be grouped together with others. However, the second MD male rat renal tumor (#222) appeared to have a transition from A-V type to solid conventional basophilic tumor epithelium. His exact wording regarding the MD male rat tumors are shown below.

> Because of the remarkable uniformity of the tumors and in some cases, multiplicity within individual rats, an inherent genetic cause in this particular batch of animals cannot be excluded, although the dose-related distribution conflicts with this possibility. The morphology of these tumors is very different from the amphophilic-vacuolar (A-V) phenotype that has been recently identified as exclusively of spontaneous origin (Hard et al, 2008). However, one of the 2 neoplasms recorded in the mid-dose males was of this A-V type and should not be grouped together with the tumors linked to canagliflozin. The second tumor in the mid-dose males showed a transition from the typical A-V morphology to solid more conventional basophilic tumor epithelium (Hard et al, 2008).

Reviewers note:

(b) (4) characterized one of the two MD male rat (#259) renal tubule tumors as spontaneously occurring amphophilic-vacuolar (A-V) phenotype which is significantly different than the rest of the rats with treatment related basophilic tubule cell renal tumors.   His description of the renal tumor in the second MD male rat (#222) suggested a transition from amphophilic to basophilic tumor type. He did not characterize the renal tumor in this rat (#222) and 1 HD male (#309) as spontaneous. Based on his assessment, the renal tumors in one MD and all HD rats were treatment related suggesting that there was a dose-response relationship between renal tumors and canagliflozin dose. Further, he added that canagliflozin did not produce any of the signals/lesions known to lead to treatment-related renal tubule tumors in rats, thus the mode of action remains unknown.   In the absence of renal tubule hyperplasia and tubule cell degeneration and regeneration in rats, the author chose the hypercalcium absorption hypothesis proposed by the sponsor. Carbohydrate malabsorption/Ca hyperabsorption is reasonable but with no data to exclude a direct treatment effect on renal SGLT2, the contribution of renal SGLT2 to renal tumors remains unresolved.

Renal Tumor Diagnosis by the Pathology Working Group (PWG)

The sponsor established a pathology working group to examine the renal slides from the 2-year rat carcinogenicity study. The primary purpose of the PWG was to characterize the spontaneous (unrelated to treatment) and treatment-related renal changes in the 2-year carcinogenicity and rat toxicology studies. Since there were no renal tumors in the 2-year mouse study, only the rat slides were examined by the PWG.   It should be noted that (b) (4) had already examined the renal glass slides unblinded and provided an expert opinion to the sponsor (reviewed earlier). The criteria for spontaneous renal tumor diagnosis were based on descriptions provided by Hard el al (2008, 2011).

The PWG was chaired by non-voting member,   (b) (4) from   (b) (4)
                He organized and presented the material to the six independent consulting pathologists with expertise in rodent renal pathology. The 2-day meeting on June 29-30, 2011 was held at   (b) (4) where coded slides were reviewed by the members. The final report was signed off on Aug 12, 2011. The FDA had was not informed or involved in the establimnet or diagnostic criteria used by the PWG. The agency became aware of the PWG when the report was submitted for review. The names of the panel members and observers from the sponsor's team are listed below.

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PWG Panel Members:
                                          (b) (4)      Chairperson
                                                       PWG Participant

                                                       PWG Participant
                                                       PWG Participant
                                                       PWG Participant
                                                       PWG Participant
                                                       PWG Participant

Observers:
    Dr. Rao Mamidi, Johnson & Johnson PDRUS, Raritan, NJ
    Dr. Sandra De Jonghe, Johnson & Johnson PDRBE, Beerse, Belgium
    Dr. Calvert Louden, Johnson & Johnson PDRUS, Raritan, NJ
    Dr. Mark Johnson, Johnson & Johnson PDRUS, Raritan, NJ
    Dr. Kirk Ways, Johnson & Johnson PDRUS, Raritan, NJ
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According to the sponsor, after an introductory background information by the sponsor (canagliflozin team leader, Dr. Ways and toxicology team leader, Dr. Johnson from JNJ), the chair of the PWG   (b) (4) provided an overview of the four chronic rat toxicology studies to be reviewed by the PWG along with photomicrographs of the representative renal changes and questions to be discussed and addressed by the PWG. The meeting was overseen by the canagliflozin team from JNJ. Following the initial introduction, the JNJ observers were relocated to an adjacent room while PWG conducted the review but they remained available to answer member's questions.

The objective of the PWG was to determine if any of the tumors were spontaneous and to determine if any of the non-proliferative renal changes observed in the rat carcinogenicity and toxicology studies were related to or predictive of the renal tumors seen in the 2-year rat carcinogenicity study. The PWG members were asked to answer and keep in mind the following questions when examining the rat renal tissue slides.

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1. What is the NOEL for renal tubule tumors?
   a) Do you consider any of the tumors to be spontaneous?
   b) Are renal tubule tumors at the mid dose treatment-related?

2. What non-neoplastic changes were observed in the kidney?
   a) Other than renal tubule tumors, are there other treatment-related histologic findings of possible significance (hyperplasia, etc.)?
   b) Are there morphologic changes suggesting cell damage or tubular cell injury that would be relevant to the pathogenesis of cell proliferation?
   c) What is the dose response for non-neoplastic changes that could be of relevance to increased tubular cell proliferation?

3. Is there a correlation between the incidence/severity of CPN and the renal tubule tumors?
   a) Is the CPN sufficient to explain the formation of renal tubule tumors?

4. Was there any correlation between the increase of urinary volume and the incidence/severity of CPN/renal tubule tumors?

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As noted, the PWG also examined the renal slides from the rat toxicology studies listed in table below to characterize spontaneous and canagliflozin-related renal changes. The 2-WK and 1-month studies were not reviewed due to the absence of drug-related renal lesions. The 3-month rat study #TOX9667 was canagliflozin (14, 20 and 100 mg/kg) in combination with 300 mg/kg metformin.

Study No.	Study Description	Kidney Slides Selected for PWG Examination
TOX8150	13-week rat	All kidney sections from control (0 mg/kg/day) and high dose (100 mg/kg/day) male rats from the main and recovery groups
TOX9667	3-month rat	All kidney sections from control (0 mg/kg/day) and high dose (100 mg/kg/day) male rats
TOX8574	6-month rat	All kidney sections from control (0 mg/kg/day) and high dose (100 mg/kg/day) male rats
TOX8986	24-month rat	All kidney sections with a diagnosis reported by the study pathologist of renal tubule hyperplasia (simple or atypical), renal tubule adenoma, and renal tubule carcinoma from the control (0 mg/kg/day), mid-dose (30 mg/kg/day) and high dose (100 mg/kg/day) male and high dose (100 mg/kg/day) female rats
TOX8986	24-month rat	All kidney sections for 15 male rats from each dose group that did not have a previous diagnosis of a proliferative lesion (hyperplasia, adenoma or carcinoma) to evaluate the presence and severity of non-proliferative lesions (i.e., Chronic Progressive Nephropathy, Tubular Hypertrophy, etc.). Animals selected were the last 15 animals in each group without proliferative lesions previously diagnosed.

The slides with proliferative renal lesions from the 2-year rat study were coded to permit independent diagnosis of hyperplasia and neoplasia. As noted earlier, the purpose of examination of the toxicology renal slides (unblinded) was to determine if any of the non-proliferative changes were related to neoplastic changes seen in the rat carcinogenicity study. Each member recoded his diagnosis and comments on worksheets prepared by _____ (b) (4) After examination of each slide, the consensus was reached when majority agreed on a diagnosis. Prior to discussion of consensus findings by the PWG, the group identity for each slide was provided by the sponsor. The consensus was recorded by the chair, _____ (b) (4).

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Criteria for Diagnosis
The sponsor stated that the criteria for diagnosis and nomenclatures for renal tumors were based on the Society of Toxicologic Pathology publications (Thurman et all, 1995, Hard et al, 1995, 1999). The criteria for spontaneous renal tumor diagnosis were based on publication by Hard el al (2008, 2011). Criteria for atypical tubule hyperplasia as an obligate precursor for renal tubule tumors were based on 2005 publication by Hard and Seely.

The PWG findings compared to the study pathologist:
13-WK rat study, TOX8150 (Canagliflozin dose:0, 4, 20 and 100 mg/kg):
The PWG agreed with the study pathologist's conclusion that there were no drug-related renal histopath changes in the 3-month rat study.

3-Month canagliflozin-metformin combination study, TOX9667 (Canagliflozin/metformin dose: 0, 4/300, 20/300, 100/300, 100/0 and 0/300 mg/kg):
The study pathologist had identified a drug-related increase in minimal renal tubular dilatation at 100 mg/kg in male rats. The PWG confirmed the original finding of increased tubular dilatation of the distal cortical tubules in the HD males.

6-Month rat study, TOX8574 (Canagliflozin dose: 0, 4, 20 and 100 mg/kg):
The study pathologist had identified renal tubular dilatation in the cortex and medulla with cellular debris in the tubular lumen in several animals at 4, 20 and 100 mg/kg, attributed to increased urine volume. There was no renal tubule hyperplasia in the 6-month study. However, transitional cell hyperplasia was frequently observed in the HD rats. The study pathologist had concluded that the incidence of mineralization of either cortex, medulla, papilla or pelvis and pelvis dilatation were similar to background lesions of the same age of rats and thus unlikely to be of toxicological significance.

The PWG confirmed the presence of tubular dilation of the distal cortical tubules in the cortex and collecting tubules in the HD males and the presence of occasional exfoliated cells in the lumen of proximal tubules of the deep cortex. According to the PWG there were no differences in the severity of CPN between control and HD males. There were no differences in mineralization, cysts or pelvic dilatation or basophilic tubules. The transitional cell hyperplasia was recognized as hyperplasia of the epithelial lining of the papilla and fornix by the PWG. The PWG assessment of the renal findings in the 6-month rat study was generally in agreement with the study pathologist.

2-Year rat study
The PWG confirmed the presence of renal tubule tumors in the HD male and female rats in the 2-year carcinogenicity study; however, they considered the renal tumors in the MD males as spontaneous and therefore unrelated to canagliflozin. This conclusion was based on the amphophilic-vacuolar appearance of the MD tumors, which is consistent with spontaneous but not drug-induced tumor types (Hard publication). The amphophilic appearance refers to tissues that can take both acidic (eosinophilic) and basic stains (basophilic). Based on the PWG's conclusions, drug-related tumors occurred only in the high dose males and females. According to the PWG, there was no atypical tubular hyperplasia in MD and HD males with renal tumors.

In female rats, the PWG also identified 8 renal tumors (**5** adenoma: #709. 721, 730, 747, 755, **2** carcinoma: 752, 756 and **1** with adenoma and carcinoma: #740). All renal tubule neoplasms in HD female rats were considered well-differentiated basophilic renal tumors related to canagliflozin.

The incidence of renal tubule tumor and tubule hyperplasia (atypia) identified by the PWG in male rats are shown in table below.

Renal Tubular Tumors and Hyperplasia in the 24-Month Rat Oral Carcinogenicity Study

Dose (mg/kg/d):	0		10		30		100	
No. of Animals:	M: 65	F: 65	M: 65	F: 64	M: 64	F: 65	M: 65	F: 65
Renal tubular tumors basophilic incidence	-	-	-	-	-	-	**11**	**8**
Adenoma renal tubule, basophilic	-	-	-	-	-	-	**8**	**7**
Carcinoma renal tubule, basophilic	-	-	-	-	-	-	**4**	**2**
Adenoma renal tubule, AV (spontaneous)	-	-	-	-	1	-	-	-
Carcinoma renal tubule, AV (spontaneous)	-	-	-	-	1	-	1	-
Hyperplasia simple	3	-	-	-	-	-	6	-
Grade 1	3	-	-	-	-	-	5	-
Grade 2	-	-	-	-	-	-	1	-

Incidences in bold indicate a treatment related effect.
AV = amphophilic-vacuolar (a spontaneous tumor type); F = female; M = male

(b) (4) analysis (reviewed ealier) had only considered one MD male rat (#259) with renal tumor as spontaenous. He classified the second MD male (#222) to have a transition stage from amphophiphilic-vacuolar to basophilic tubule cell tumor and sugegsted examining DNA liver for possible genetic linkage them. Spontaneous amphophilic renal tumors have been known to occur in rats that are full or half sibling.   The analysis of liver DNA found no geneitic relationship among the three rats thus making the spontaneous occurance of three amphophilic renal tumors in a single study a very rare occurance.

The most prominent non-neoplastic finding according to the PWG was an increased incidence of minimal to mild dilation of distal tubules in the cortex and collecting tubules in the outer medulla in HD males. Mineral deposits were present in the lumen of the renal pelvis and associated hyperplasia of the epithelial lining of the papilla and urothelium of the kidney pelvis present in some, often associated with chronic inflammation.   Urothelial hyperplasia was only present and associated with mineralization in the lumen of the renal pelvis. A few males had focal/multifocal dilated cortical tubules that displayed tubular hypertrophy.

The incidence of CPN determined by the PWG was nearly unchanged; however, the severity was ranked 1 to 2 grades lower (0 to 4 scale) relative to the study pathologist. The PWG also noted a significant background tubular hypertrophy of cortex. The high incidences of renal tubule hyperplasia submitted initially in the safety report were no longer diagnosed as such. It

appears that most of the hyperplasias were diagnosed as hypertrophy.   In absence of the proliferative lesion such as renal tubule hyperplasias, there was no clear explanation of how canagliflozin may have caused renal tubule tumors. The PWG did not consider CPN or mineralization as potential contributing factors to canagliflozin induced renal tubule tumors in rats. The only conclusion the PWG could reach was the likely exaggerated pharmacological activity of canagliflozin on tubule dilatation.

<u>Histographic Example of Non-Spontaneous (treatment-related) Renal Tubule Tumors</u>
The PWG primarily used the basophilic appearance of the renal tubule cell tumors to distinguish treatment related tumors from spontaneous renal tumors. Based on basophilic appearance of renal tubular adenomas and carcinomas, there were 11/12 HD male and 8/8 female rats diagnosed as treatment related. Examples of treatment related basophilic renal tubule adenoma (male rat #322, image A and C) and carcinoma (female rat# 740, image B and D) are shown below.
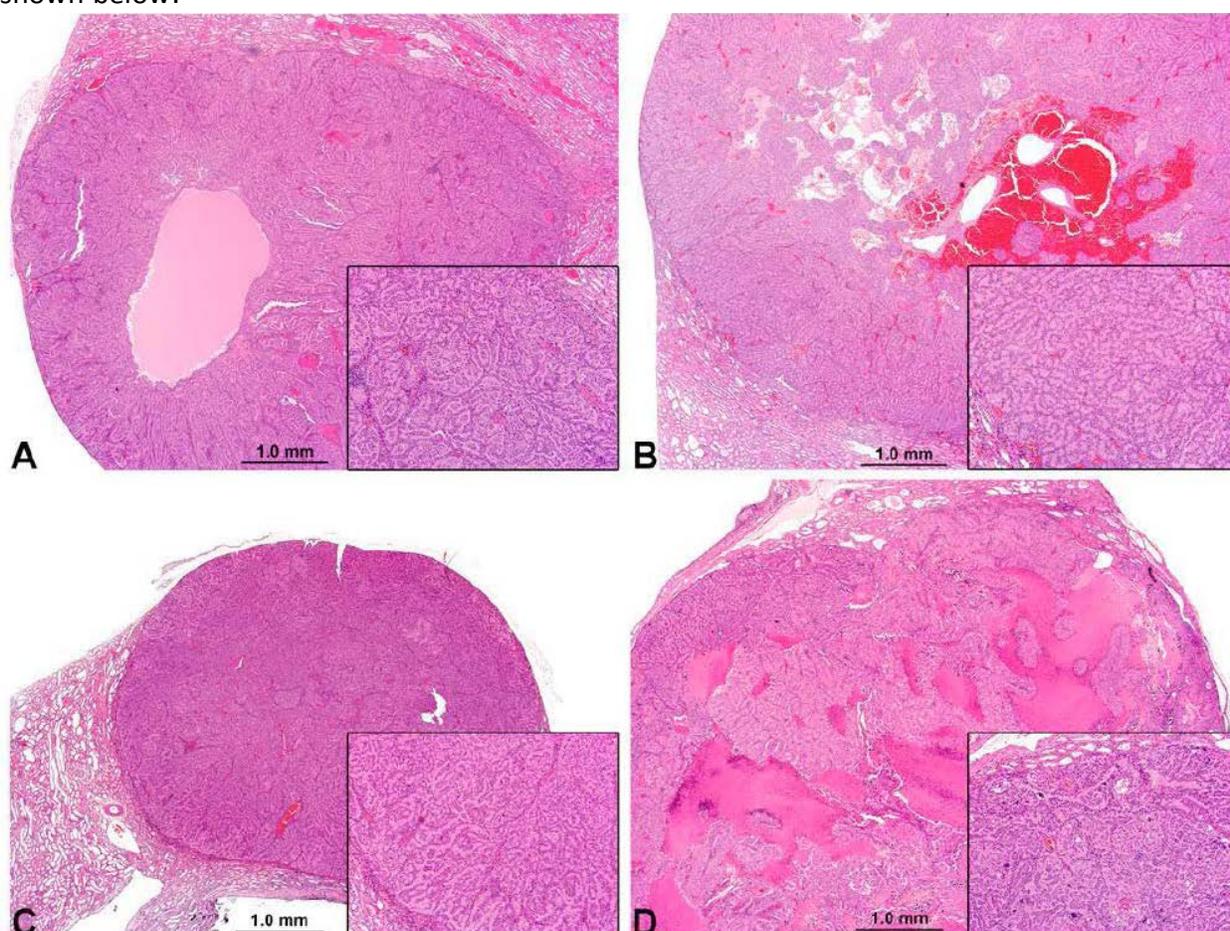
Basophilic renal tubule tumors with well-formed tubular differentiation (A) Tubule adenoma, 100 mg/kg/day, male rat, animal number 322, (B) Tubule carcinoma, 100 mg/kg/day, male rat, animal number 333, (C) Tubule adenoma, 100 mg/kg/day, female rat, animal number 740, (D) Tubule carcinoma, 100 mg/kg/day, female rat, animal number 756. Inserts at high magnification demonstrate the similar basophilic tubular morphology of the renal cell tumors in the 100 mg/kg/day dose group. H&E.

Histographic Example of Spontaneous (unrelated to treatment) Renal Tubule Tumors

The PWG considered amphophilic (stained with eosinophilic or basophilic stains) vacuolar renal tubular tumors as spontaneous tumors and not related to treatment. This classification was applied to 2 MD (#222, #259) and 1 HD male rats (#309). Since spontaneous renal tumors are rare and occur more frequently in siblings, the DNA of the three rats with spontaneous tumors was tested. The three rats were not genetically related (not siblings). Examples of spontaneous amphophilic -vacuolated renal tubule adenoma (male rat #222, image A) and carcinoma (male rat# 259, image B, male #309, image C) are shown below.
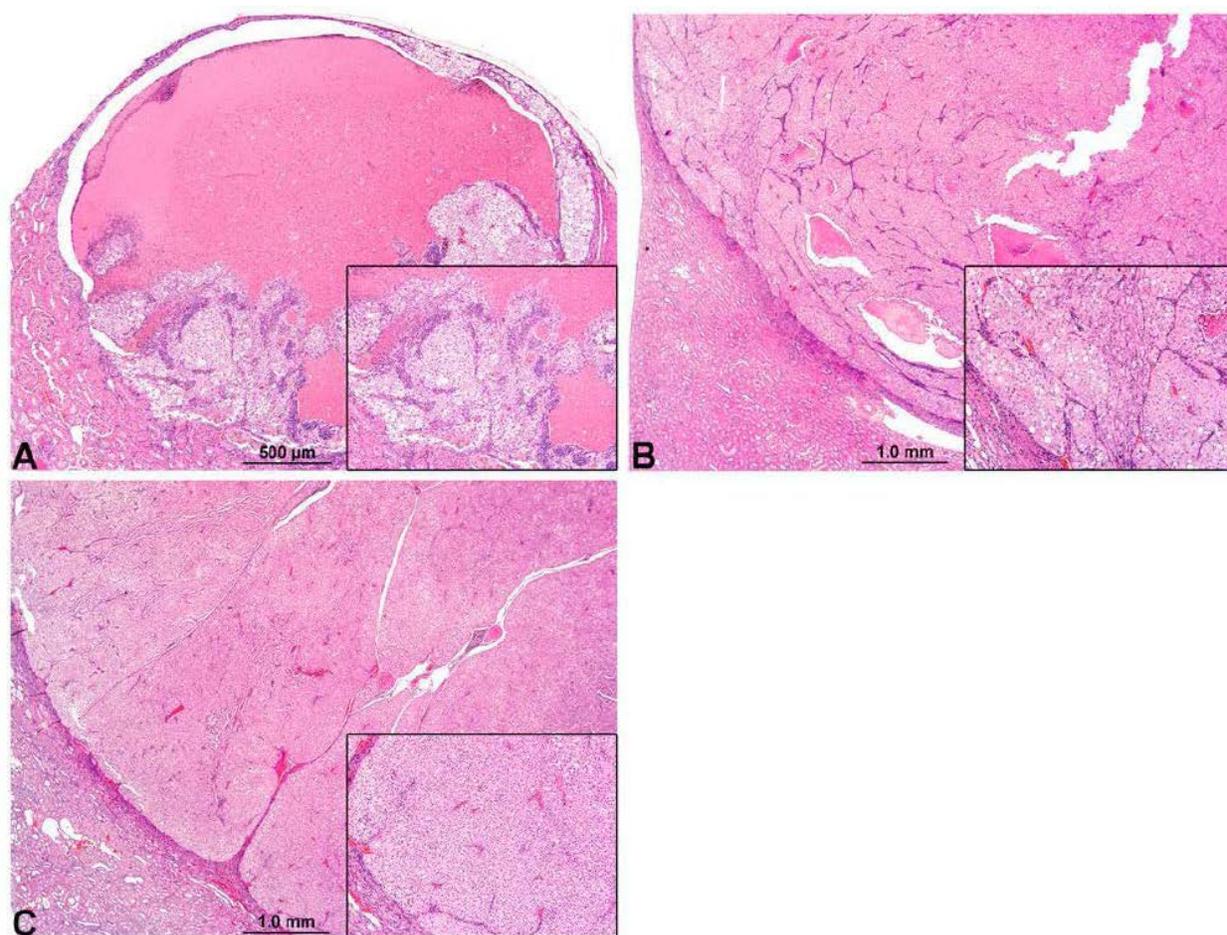
Amphophilic-vacuolar (AV) variant of renal tubule tumors. (A) Tubule adenoma, 30 mg/kg/day, male rat, animal number 222, (B) Tubule carcinoma, 30 mg/kg/day, male rat, animal number 259; (C) Tubule carcinoma, 100 mg/kg/day, male rat, animal number 309. Inserts at higher magnification demonstrate solid lobules of eosinophilic/amphophilic staining cells with vacuolated cytoplasm. H&E.

The renal tissue diagnosis by the study pathologist and PWG are provided in the table below. A detailed description of renal pathology by the PWG was not provided. Based on the table below, the difference between study pathologist and PWG was minor except for significantly fewer renal tubule hyperplasia and CPN which were graded lower by the PWG.

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Proliferative lesions in the 2-Year SD Rat Carcinogenicity Study Diagnosed by the Study Pathologist and PWG

Group	Dose (mg/kg/day)	Sex	Animal Number	Study Pathologist Diagnosis*	Pathology Working Group Diagnosis
Vehicle Control	0	Male	19	Hyperplasia Simple, grade 1; CPN, grade 1	CPN, grade 1
Vehicle Control	0	Male	22	Hyperplasia Simple, grade 1; CPN, grade 3	CPN, grade 2
Vehicle Control	0	Male	49	Hyperplasia Simple, grade 1	CPN, grade 1
Mid	30	Male	209	Hyperplasia Simple, grade 1; CPN, grade 3	CPN, grade 2
Mid	30	Male	220	Hyperplasia Atypical, grade 1; CPN, grade 4	CPN, grade 3
Mid	30	Male	222	Adenoma Renal Tubule; CPN, grade 3	Tubule Adenoma; CPN, grade 2
Mid	30	Male	246	Hyperplasia Simple, grade 1	CPN, grade 1
Mid	30	Male	258	Hyperplasia Simple; CPN 4	CPN, grade 3
Mid	30	Male	259	Carcinoma; CPN, grade 2; Hyperplasia Renal Tubule, grade 1	Tubule Carcinoma; Tubule Hyperplasia, Atypical; CPN, grade 1
Mid	30	Male	265	Hyperplasia Simple; CPN 2	CPN, grade 1
High	100	Male	301	Adenoma; CPN, grade 3; Hyperplasia Simple Tubule, grade 1	Tubule Adenoma; CPN, grade 1
High	100	Male	306	Adenoma; CPN, grade 3; Hyperplasia Renal Tubule, grade 1	Tubule Adenoma; CPN, grade 2
High	100	Male	309	Carcinoma; CPN, grade 3; Hyperplasia Simple, grade 2	Tubule Carcinoma; CPN, grade 1
High	100	Male	310	Adenoma; CPN, grade 2; Hyperplasia Simple, grade 1	Tubule Adenoma; CPN, grade 1
High	100	Male	314	Adenoma; CPN, grade 2	Tubule Carcinoma; CPN, grade 1
High	100	Male	321	Adenoma; CPN, grade 2	Tubule Adenoma; CPN, grade 1
High	100	Male	322	Adenoma; CPN, grade 4; Hyperplasia Simple, grade 1	Tubule Adenoma; CPN, grade 3
High	100	Male	332	Adenoma; CPN, grade 2	Tubule Adenoma; CPN, grade 1
High	100	Male	333	Adenoma; Carcinoma; CPN, grade 3; Hyperplasia Simple, grade 1	Tubule Carcinoma; Tubule Adenoma; CPN, grade 1
High	100	Male	341	Hyperplasia Renal Tubule, grade 2; CPN grade 3	CPN, grade 2
High	100	Male	350	Hyperplasia Simple, grade 1; CPN grade 2	CPN, grade 1
High	100	Male	351	Adenoma; CPN, grade 3	Tubule Adenoma; CPN, grade 2
High	100	Male	355	Carcinoma; CPN, grade 2	Tubule Carcinoma; CPN, grade 1
High	100	Male	360	Hyperplasia Simple, grade 1; CPN grade 3	CPN, grade 1
High	100	Male	364	Adenoma; CPN, grade 2; Hyperplasia Simple, grade 1	Tubule Carcinoma; Tubule Adenoma; CPN, grade 1
High	100	Female	702	Hyperplasia Simple, grade 1; CPN grade 3	CPN, grade 3
High	100	Female	709	Adenoma	Tubule Adenoma
High	100	Female	721	Adenoma; CPN, grade 3	Tubule Adenoma; CPN, grade 1
High	100	Female	722	Hyperplasia Renal Tubule, grade 3	CPN, grade 2
High	100	Female	730	Adenoma; CPN, grade 2	Tubule Adenoma; CPN, grade 1
High	100	Female	734	Hyperplasia Simple, grade 1; CPN grade 3	CPN, grade 2
High	100	Female	740	Adenoma; Carcinoma; CPN, grade 3	Tubule Carcinoma; Tubule Adenoma; CPN, grade 2
High	100	Female	741	Adenoma; CPN, grade 3; Hyperplasia Renal Tubule, grade 1	Tubule Hyperplasia Atypical; CPN, grade 2
High	100	Female	743	Hyperplasia Renal Tubule, grade 1; CPN, grade 3	CPN, grade 2
High	100	Female	747	Adenoma; CPN, grade 3	Tubule Adenoma; CPN, grade 1
High	100	Female	752	Adenoma; CPN, grade 2	Tubule Carcinoma; CPN, grade 1
High	100	Female	755	Hyperplasia Renal Tubule, grade 4; CPN, grade 3	Tubule Adenoma; CPN, grade 2
High	100	Female	756	Carcinoma; CPN, grade 4	Tubule Carcinoma; CPN, grade 3
High	100	Female	761	Hyperplasia Simple, grade 1; CPN, grade 2	CPN, grade 1

*Draft Data, Individual Animal Data Sheets, 24-May-11

24-Month Repeated Dose Oral Carcinogenicity Study in the `MALE Rat` `Non-proliferative Lesions`

Group	Dose (mg/kg/day)	Sex	Animal Number	Study Pathologist Diagnosis*	Pathology Working Group Diagnosis
Vehicle Control	0	Male	51	CPN, grade 1; Mineralization Pelvis, unilateral, grade 1	CPN, grade 1; Mineralization Pelvis, unilateral, grade 1
Vehicle Control	0	Male	52	Tubule Dilatation, grade 3; CPN, grade 2; Dilatation Pelvic, unilateral, grade 3; Hyaline Droplets, grade 2	Tubule Dilatation, grade 3; CPN, grade 1; Dilatation Pelvic, unilateral, grade 3
Vehicle Control	0	Male	53	Tubule Dilatation grade 2; CPN, grade 4; Dilatation pelvic, bilateral, grade 4; Inflammation Pelvis, unilateral, grade 2	Tubule Dilatation grade 2; CPN, grade 1; Dilatation pelvic, bilateral, grade 4; Inflammation Pelvic, unilateral, grade 2
Vehicle Control	0	Male	54	Tubule Hypertrophy grade 1; Cyst(s) focal, bilateral, grade 2	Tubule Hypertrophy grade 1; Cyst(s) focal, bilateral, grade 2
Vehicle Control	0	Male	55	Tubule Hypertrophy, grade 1; CPN, grade 1; Cyst(s), focal, unilateral, grade 1; Mineralization Pelvis, unilateral, grade 1	Tubule Hypertrophy, grade 1; CPN, grade 1; Cyst(s), focal, unilateral, grade 1; Mineralization Pelvis, unilateral, grade 1
Vehicle Control	0	Male	56	Basophilia Tubule, bilateral, grade 3; Inflammation chronic focal, unilateral, grade 3	Basophilia Tubule, bilateral, grade 3; Inflammation chronic focal, unilateral, grade 3
Vehicle Control	0	Male	57	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 1; CPN, grade 2; Cyst, grade 3; Hyperplasia Transitional Cell, grade 1; Mineralization Pelvis, grade 1	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 1; CPN, grade 1
Vehicle Control	0	Male	58	Mineralization cortex, unilateral, grade 1	Autolysis
Vehicle Control	0	Male	59	CPN, grade 1; Inflammation Pelvis, acute, unilateral, grade 2	CPN, grade 1; Inflammation Pelvis, acute, unilateral, grade 2
Vehicle Control	0	Male	60	Tubule Dilatation, grade 1; CPN, grade 3; Mineralization Pelvis, grade 1	Tubule Dilatation, grade 1; CPN, grade 2
Vehicle Control	0	Male	61	"without abnormalities"	Autolysis
Vehicle Control	0	Male	62	Tubule Dilatation, grade 1; CPN, grade 3; Inflammation Pelvis, focal, bilateral, grade 1; Atrophy Fat, grade 2	Tubule Dilatation, grade 1; CPN, grade 3; Inflammation Pelvis, focal, bilateral, grade 1
Vehicle Control	0	Male	63	"without abnormalities"	"without abnormalities"
Vehicle Control	0	Male	64	"without abnormalities"	"without abnormalities"
Vehicle Control	0	Male	65	CPN, grade 1; Inflammation Pelvis, bilateral, grade 1	CPN, grade 1; Inflammation Pelvis, bilateral, grade 1
Low	10	Male	151	Tubule Hypertrophy, grade 1; CPN, grade 3	Tubule Hypertrophy, grade 1; CPN, grade 2
Low	10	Male	152	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 2; CPN, grade 2; Atrophy Fat, bilateral, grade 2; Mineralization Cortex, bilateral, grade 1	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 2; CPN, grade 1; Atrophy Fat, bilateral, grade 2; Mineralization Cortex, bilateral, grade 1
Low	10	Male	153	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 2; CPN, grade 1; Mineralization Papilla, unilateral, grade 1	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 2; CPN, grade 1; Mineralization Papilla, unilateral, grade 1
Low	10	Male	154	CPN, grade 1	CPN, grade 1
Low	10	Male	155	CPN, grade 1; Hyaline Droplets, Cortex, bilateral, grade 2	CPN, grade 1; Hyaline Droplets, Cortex, bilateral, grade 2
Low	10	Male	156	Tubule Dilatation, grade 1; CPN, grade 1; Hyperplasia Transitional Cell, focal, unilateral, grade 1	Tubule Dilatation, grade 1; CPN, grade 1; Hyperplasia transitional cell, focal, unilateral, grade 1
Low	10	Male	157	Tubule Dilatation, grade 1; CPN, grade 2	Tubule Dilatation, grade 1; CPN, grade 1
Low	10	Male	158	Tubule Dilatation, grade 1; CPN, grade 1	Tubule Dilatation, grade 1; CPN, grade 1
Low	10	Male	159	Tubule Dilatation, grade 1; CPN, grade 1; Hyaline Droplets, Cortex, bilateral, grade 2	Tubule Dilatation, grade 1; CPN, grade 1; Hyaline Droplets, Cortex, bilateral, grade 2
Low	10	Male	160	Tubule Dilatation, grade 1; CPN, grade 1; Hyperplasia Transitional Cell, focal, unilateral, grade 1	Tubule Dilatation, grade 1; CPN, grade 1; Hyperplasia Transitional Cell, focal, unilateral, grade 1
Low	10	Male	161	Tubule Dilatation, grade 1; CPN, grade 3; Mineralization Cortex, unilateral, grade 1	Tubule Dilatation, grade 1; CPN, grade 2; Mineralization cortex, unilateral, grade 1
Low	10	Male	162	Tubule Dilatation, grade 1; CPN, grade 1; Mineralization Pelvis, bilateral, grade 2	Tubule Dilatation, grade 1; CPN, grade 1; Mineralization Pelvis, bilateral, grade 2
Low	10	Male	163	Tubule Dilatation, grade 1; CPN, grade 2; Hyperplasia Transitional Cell, focal, unilateral, grade 1; Mineralization Pelvis, unilateral, grade 3	Tubule Dilatation, grade 1; CPN, grade 1; Hyperplasia Transitional Cell, focal, unilateral, grade 1; Mineralization Pelvis, unilateral, grade 3

24-Month Repeated Dose Oral Carcinogenicity Study in the MALE Rat   Non-proliferative Lesions

Group	Dose (mg/kg/day)	Sex	Animal Number	Study Pathologist Diagnosis*	Pathology Working Group Diagnosis
Low	10	Male	164	Tubule Dilatation, grade 1; CPN, grade 1; Hyperplasia Transitional Cell, focal, reactive unilateral, grade 1; Inflammation Pelvis, focal, unilateral, grade 1; Mineralization pelvis, multifocal, unilateral, grade 2	Tubule Dilatation, grade 1; CPN, grade 1; Hyperplasia Transitional Cell, focal, reactive unilateral, grade 1; Inflammation pelvis, focal, unilateral, grade 1; Mineralization pelvis, multifocal, unilateral, grade 2
Low	10	Male	165	CPN, grade 4; Dilatation pelvic, bilateral, grade 4; Mineralization vascular, unilateral, grade 3	CPN, grade 2; Dilatation pelvic, bilateral, grade 4; Mineralization vascular, unilateral, grade 3
Mid	30	Male	248	Tubule Dilatation, grade 1; CPN, grade 2	Tubule Dilatation, grade 1; CPN, grade 2
Mid	30	Male	249	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 1; CPN, grade 1	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 1; CPN, grade 1
Mid	30	Male	250	CPN, grade 2; Hyperplasia Transitional Cell, focal, unilateral, grade 1	CPN, grade 1; Hyperplasia Transitional Cell, focal, unilateral, grade 1
Mid	30	Male	251	CPN, grade 2; Dilatation Tubule, grade 1	CPN, grade 1
Mid	30	Male	252	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 1; CPN, grade 2; Mineralization Papilla, unilateral, grade 2	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 1; CPN, grade 1; Mineralization Papilla, unilateral, grade 2
Mid	30	Male	253	CPN, grade 1; Hyperplasia Transitional Cell, focal, unilateral, grade 1; Mineralization Pelvis, unilateral, grade 4	CPN, grade 1; Hyperplasia Transitional Cell, focal, unilateral, grade 1; Mineralization Pelvis, unilateral, grade 4
Mid	30	Male	254	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 1; CPN, grade 1	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 1; CPN, grade 1
Mid	30	Male	255	Tubule Dilatation, grade 1; CPN, grade 1	Tubule Dilatation, grade 1; CPN, grade 1
Mid	30	Male	256	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 1; CPN, grade 3; Dilatation Pelvic, bilateral, grade 3; Inflammation Acute, Multifocal, unilateral, grade 3; Mineralization Vascular, unilateral, grade 2; (Peri) Vasculitis, grade 2	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 1; CPN, grade 2; Dilatation Pelvic, bilateral, grade 3; Inflammation Acute, Multifocal, unilateral, grade 3; Mineralization Vascular, unilateral, grade 2
Mid	30	Male	257	CPN, grade 2; Cyst(s), focal, unilateral, grade 1; Dilatation Tubule, grade 1	CPN, grade 1; Cyst(s), focal, unilateral, grade 1
Mid	30	Male	260	CPN, grade 3	CPN, grade 2
Mid	30	Male	261	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 1; CPN, grade 2; Hyperplasia Transitional Cell, multifocal, unilateral, grade 1; Inflammation Pelvis, acute, unilateral, grade 2; Mineralization Pelvis, grade 2	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 1; CPN, grade 1; Hyperplasia Transitional Cell, multifocal, unilateral, grade 1; Inflammation Pelvis, acute, unilateral, grade 2
Mid	30	Male	262	Tubule Dilatation, grade 2; CPN, grade 3; Hyperplasia Transitional Cell, focal, unilateral, grade 1; Hyperplasia Mesenchymal, focal, unilateral, grade 3; Mineralization Pelvis, unilateral, grade 4; Mineralization Vascular, bilateral, grade 1	Tubule Dilatation, grade 2; CPN, grade 2; Hyperplasia Transitional Cell, focal, unilateral, grade 1; Hyperplasia Mesenchymal, focal, unilateral, grade 3; Mineralization Pelvis, unilateral, grade 4; Mineralization Vascular, bilateral, grade 1
Mid	30	Male	263	Tubule Dilatation, grade 1; CPN, grade 2; Cyst(s), focal, unilateral, grade 1	Tubule Dilatation, grade 1; CPN, grade 1; Cyst(s), focal, unilateral, grade 1
Mid	30	Male	264	CPN, grade 1; Mineralization vascular, bilateral, grade 1	CPN, grade 1; Mineralization vascular, bilateral, grade 1
High	100	Male	346	Dilatation Pelvic, grade 1; Tubule Dilatation, grade 2; CPN, grade 2; Hyperplasia Transitional Cell, multifocal, bilateral, grade 1; Mineralization Medulla Multifocal, bilateral, grade 1; Mineralization Vascular, unilateral, grade 1	Tubule Dilatation, grade 2; CPN, grade 1; Hyperplasia Transitional Cell, multifocal, bilateral, grade 1; Mineralization Medulla Multifocal, bilateral, grade 1; Mineralization Vascular, unilateral, grade 1
High	100	Male	347	Tubule Dilatation, grade 3; CPN, grade 4; Hyperplasia Transitional Cell, Multifocal, bilateral, grade 2; Mineralization Cortex, bilateral, grade 2; Mineralization Medulla, Multifocal bilateral, grade 1; Mineralization Papilla, bilateral, grade 3; Mineralization Pelvis, Multifocal, bilateral grade 2	Tubule Dilatation, grade 3; CPN, grade 3; Hyperplasia Transitional Cell, Multifocal, bilateral; Mineralization Cortex, bilateral, grade 2; Mineralization Medulla, Multifocal bilateral, grade 1; Mineralization Vascular, unilateral, grade 1; Mineralization Pelvis, Multifocal, bilateral grade 2

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24-Month Repeated Dose Oral Carcinogenicity Study in the MALE Rat   `Non-proliferative Lesions`

Group	Dose (mg/kg/day)	Sex	Animal Number	Study Pathologist Diagnosis*	Pathology Working Group Diagnosis
High	100	Male	348	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 2; CPN, grade 2; Hyperplasia transitional cell, multifocal, bilateral, grade 1; Mesenchymal Cell Tumor Benign, Cortex/Outer Medulla, unilateral (benign neoplasm); Mineralization Medulla, Multifocal, bilateral, grade 1; Mineralization Pelvis, bilateral, grade 1; Mineralization Papilla, bilateral, grade 1	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 2; CPN, grade 1; Mesenchymal Cell Tumor Benign, Cortex/Outer Medulla, unilateral (benign neoplasm); Mineralization Medulla, Multifocal, bilateral, grade 2; Mineralization Pelvis, bilateral, grade 1; Mineralization papilla, bilateral, grade 1
High	100	Male	349	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 2; Inflammation Pelvis, grade 3; Mineralization, medulla, grade 2; Vacuolization, grade 1; CPN, grade 1; Hyperplasia Transitional Cell, grade 2	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 2; CPN, grade 1
High	100	Male	352	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 2; CPN, grade 2; Dilatation Pelvic, grade 2; Hyperplasia Transitional Cell, grade 2; Mineralization Medulla, grade 1; Mineralization Pelvis, grade 1	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 2; CPN, grade 1
High	100	Male	353	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 1; CPN, grade 3; Hyperplasia Transitional Cell, focal, unilateral, grade 1; Mineralization Pelvis, unilateral, grade 3	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 1; CPN, grade 1; Hyperplasia Transitional Cell, focal, unilateral, grade 1; Mineralization Pelvis, unilateral, grade 3
High	100	Male	354	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 2; CPN, grade 2; Mineralization Medulla, Multifocal, bilateral, grade 2; Mineralization Vascular, unilateral, grade 1; Mineralization Papilla, bilateral, grade 1	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 2; CPN, grade 1; Mineralization Medulla, Multifocal, bilateral, grade 2; Mineralization Vascular, unilateral, grade 1; Mineralization Papilla, bilateral, grade 1
High	100	Male	356	Hyaline droplets, grade 3; Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 2; CPN, grade 2; Hyperplasia Transitional Cell, Multifocal, mainly left bilateral, grade 3; Mineralization Medulla, multifocal, bilateral, grade 1; Mineralization Cortex, bilateral, grade 1; Mineralization Vascular, bilateral, grade 3	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 2; CPN, grade 1; Hyperplasia Transitional Cell, Multifocal, mainly left bilateral, grade 3; Mineralization medulla, multifocal, bilateral, grade 1; Mineralization Cortex, bilateral, grade 1; Mineralization Vascular, bilateral, grade 3
High	100	Male	357	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 2; CPN, grade 3; Hyperplasia Transitional Cell, focal, unilateral, grade 2; Mineralization Medulla, focal, bilateral, grade 1; Mineralization Vascular, bilateral, grade 2	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 2; CPN, grade 2; Hyperplasia Transitional Cell, focal, unilateral, grade 2; Mineralization Medulla, focal, bilateral, grade 1; Mineralization Vascular, bilateral, grade 2
High	100	Male	358	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 3; CPN, grade 3; Hyperplasia Transitional Cell, multifocal, bilateral, grade 2; Mineralization Medulla, Multifocal, unilateral, grade 1; Mineralization Papilla, bilateral, grade 2; Mineralization Pelvis, mainly right, bilateral, grade 4; Mineralization Vascular, unilateral, grade 2; Dilatation Pelvic, grade 1	Tubule Hypertrophy, grade 1; Tubule Dilatation, grade 3; CPN, grade 2; Hyperplasia Transitional Cell, multifocal, bilateral, grade 2; Mineralization Medulla, Multifocal, unilateral, grade 1; Mineralization Papilla, bilateral, grade 2; Mineralization Pelvis, mainly right, bilateral, grade 4; Mineralization Vascular, unilateral, grade 2

24-Month Repeated Dose Oral Carcinogenicity Study in the MALE Rat <mark>Non-proliferative Lesions</mark>

Group	Dose (mg/kg/day)	Sex	Animal Number	Study Pathologist Diagnosis*	Pathology Working Group Diagnosis

High	100	Male	359	Tubule Dilatation, grade 3;
				CPN, grade 4;
				Dilatation Pelvic, bilateral, grade 1;
				Mineralization Medulla, multifocal,
				   bilateral, grade 2;
				Mineralization Vascular, bilateral, grade 2
					Tubule Dilatation, grade 3;
					CPN, grade 3;
					Dilatation Pelvic, bilateral, grade 1;
					Mineralization medulla, multifocal,
					   bilateral, grade 2;
					Mineralization Vascular, bilateral, grade 2

High	100	Male	361	CPN, grade 2;
				Mineralization Cortex, unilateral, grade 1;
				Mineralization Papilla, bilateral, grade 1;
				Mineralization Medulla, Multifocal,
				   bilateral, grade 1;
				Mineralization Vascular, unilateral, grade 2
					CPN, grade 1;
					Mineralization Cortex, unilateral, grade 1;
					Mineralization Papilla, bilateral, grade 1;
					Mineralization Medulla, Multifocal,
					   bilateral, grade 1;
					Mineralization Vascular, unilateral, grade 2

High	100	Male	362	Tubule Dilatation, grade 2;
				CPN, grade 1;
				Cyst(s), focal, unilateral, grade 3;
				Mineralization Medulla, Multifocal, unilateral,
				   grade 1;
				Mineralization Pelvis, bilateral, grade 2;
				Atrophy Fat, grade 2
					Tubule Dilatation, grade 2;
					CPN, grade 1;
					Cyst(s), Focal, unilateral, grade 3;
					Mineralization Medulla, Multifocal, unilateral,
					   grade 1;
					Mineralization Pelvis, bilateral, grade 2

High	100	Male	363	Tubule Hypertrophy, grade 1;
				Tubule Dilatation, grade 3;
				CPN, grade 3;
				Hyperplasia Transitional Cell, focal,
				   unilateral, grade 1;
				Mineralization Medulla, focal,
				   bilateral, grade 1
					Tubule Hypertrophy, grade 1;
					Tubule Dilatation, grade 3;
					CPN, grade 2;
					Hyperplasia Transitional Cell, focal,
					   unilateral, grade 1;
					Mineralization Medulla, focal,
					   bilateral, grade 1

High	100	Male	365	Tubule Dilatation, grade 3;
				CPN, grade 3;
				Atrophy Fat, bilateral, grade 1;
				Inflammation Acute, rather diffuse, with Tubular
				   Necrosis, unilateral, grade 4;
				Inflammation Pelvis, bilateral, grade 3;
				Mineralization Cortex, bilateral, grade 1;
				Mineralization Papilla, bilateral, grade 1;
				Mineralization Medulla, Multifocal,
				   bilateral, grade 1;
				Mineralization Pelvis, bilateral, grade 1;
				Hyperplasia Transitional Cell, grade 2;
				Mineralization Vascular, grade 2
					Tubule Dilatation, grade 3;
					CPN, grade 2;
					Atrophy Fat, bilateral, grade 1;
					Inflammation Acute, rather diffuse, with
					   Tubular Necrosis, unilateral, grade 4;
					Inflammation Pelvis, bilateral, grade 3;
					Mineralization Cortex, bilateral, grade 1;
					Mineralization Papilla, bilateral, grade 1;
					Mineralization Medulla, Multifocal,
					   bilateral, grade 1;
					Mineralization Pelvis, bilateral, grade 1

*Draft Data, Individual Animal Data Sheets, 24-May-11

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The responses of PWG to the sponsor's questions are provided in verbatim in italic blue:

Do you consider any of the tumors to be spontaneous?

"Male rat (animal no. 259) in the mid dose group (30 mg/kg/day) had a carcinoma of the amphophilic-vacuolar tumor type (Thurman et al, 1995; Hard et al, 2008). Renal tumors of this cell type have a familial predisposition and are considered to be spontaneous incidental tumors unrelated to treatment. Additionally, one carcinoma in a high dose male (animal no. 309) and the adenoma in mid-dose male (animal number 222) were characterized by a mixture of vacuolated cells, basophilic tubule cells and included areas having features of the amphophilic-vacuolated cell type of renal tubule tumor. These two neoplasms were distinctly different from the well differentiated basophilic tubule cell tumors that were present in the high dose male and female groups; these two tumors were considered to be incidental, spontaneous tumors unrelated to treatment."

Reviewer note:
Based on the description provided by the PWG, the amphophilic staining renal tumor in male #259 may have been spontaneous even though this animal had an atypical hyperplastic tubule lesion. By classifying this tumor spontaneous, the PWG considered this atypical hyperplastic tubule had no role in renal tumorigenesis.   The basophilic renal tubule cells in rat #222 and #309 were more likely treatment related as they were not classified by ⬚ (b) (4) as spontaneous. The only distinguishing criteria the PWG provided was the mixed basophilic staining in the slide they examined. The evidence for spontaneous nature of the renal tumors in rat 222 and 309 appears very weak considering that these rats were not genetically related and there were no such tumors, spontaneous or otherwise, at lower doses or in control male and female rats. Even though the PWG examined the slides in a blinded manner, they appeared to have been well informed about the objectives set by the sponsor (Question below).

Are renal tubule tumors at the MD treatment related?

"In addition to the amphophilic-vacuolar renal tubule carcinoma in animal no. 259, a male rat (animal no. 222) in the mid-dose group had a renal tubule adenoma. This tumor consisted of solid proliferation of a mixture of clear and basophilic cells, without the tubule differentiation of the high-dose tumors.
Except for one of the carcinomas, the neoplasms in the high-dose (100 mg/kg/day) were basophilic and were similar in morphology with well-formed tubule differentiation. One carcinoma in the 100 mg/kg/day (animal no. 309) was characterized by a mixture of solid, clear cell, and tubular areas including some areas having features of the amphophilic-vacuolar cell type of renal tubule cell neoplasm.
There are clear morphologic differences which distinguish the two renal tubule tumors present in the mid-dose group from the tumors that were present in the high-dose group. One of these two tumors in the mid-dose group is clearly not related to treatment (animal no. 259) but represents a cell type that has a familial predisposition and is considered to be spontaneous. The second neoplasm (animal no. 222) consisted of a solid proliferation of clear and basophilic cells without tubular areas as were present in the renal cell tumors in the high-dose group. It also

had some areas with features consistent with the amphophilic-vacuolar spontaneous type of tumor. Therefore, both tumors observed in the mid-dose group were regarded as spontaneously occurring tumors that were not related to repeated dosing with JNJ-28431754.
The NOEL for renal proliferative lesions in this study is considered to be 30 mg/kg by the PWG for both male and female rats. At this dose level there was no evidence of increased renal tubule cell proliferation. Treatment-related atypical tubule cell hyperplasia was not observed in male or female rats at this dose level. The rat with renal tumor and atypical hyperplastic tubule was changed to spontaneous thus the atypical hyperplasia did not contribute to treatment-related renal tumors.   No renal tubule tumors were observed in female rats at this dose level. In male rats in the 30 mg/kg/day dose group, one neoplasm was considered to be a familial type of neoplasm and the second was characteristic of spontaneously occurring renal tubule adenomas. The morphologic characteristics of this neoplasm were distinguished from the tumors occurring in the HD group by its solid appearance and lack of a distinct tubular pattern."

Reviewer note

As noted earlier, the amphophilic vacuolated (A-V) appearance of renal tubule tumor in male #259 may have indeed been different than the renal tumors in the HD rat but the solid proliferation of clear and basophilic cells in rat #222 was not sufficiently different than the basophilic appearance of treatment related renal tumors in the HD rats. Even ▨ [(b) (4)] in his report states (Sept 13, 2011) that one of the MD males may have transitioned from amphophilic-alveolar to conventional basophilic tumor seen in the HD rats and did not classify the tumor in this rat as spontaneous. The reviewer considers renal tubule tumor in MD male rat #222 as possibly treatment related. Although a single incidence of a treatment-related renal tumor in the MD will not change the statistical significance, it does suggest a dose-response relationship between the dose of canagliflozin and the increased incidence of renal tubule tumors.

What non-neoplastic changes were observed in the kidney?
>   Other than renal tubule tumors, are there other treatment related histologic findings of possible significance (hyperplasia, etc.)?

"The study pathologist reported a wide range of non-proliferative treatment-related renal changes: a dose-related increase in severity of chronic progressive nephropathy (CPN), dilated tubules, pelvic dilatation, and increased mineralization in cortex or medulla. For CPN, a statistically significant increase in frequency was observed in all canagliflozin treatment groups, without a clear dose-relationship in males, but a possible dose-related increase in frequency in females. Dose-related increases in the severity grade of CPN were also reported by the study pathologist.
The majority of the non-neoplastic changes observed in the kidney were considered to represent exaggerated pharmacologic activity of JNJ-28431754. Canagliflozin is a sodium glucose co-transport 2 (SGLT2) inhibitor that selectively inhibits SGLT2 relative to SGLT1 (~167-fold) resulting in increased urinary glucose excretion and reduced blood glucose levels by an insulin-independent mechanism.
At all dose levels in the 6-month chronic toxicity study there were increases in urine volume, marked increases in urinary glucose and increases in urine calcium (Ca) and phosphorus (P) accompanied by a low urine pH. Serum chemistry changes in all doses included decreased Ca

(0.98x-0.96x); decreased glucose (0.88x-0.49x); and increased blood urea nitrogen (BUN) (1.2-2.3x). At necropsy, kidney weights were increased (1.1- 1.3x) in all doses which correlated microscopically with minimal to slight tubule dilation and minimal to slight hyperplasia without atypia of the lining of the papilla and pelvis were observed only in the 100 mg/kg/day treated animals.

In the 24-month carcinogenicity study, organ weights and urinary Ca and P levels were not measured. Increased urinary glucose excretion and decreased blood glucose levels were present at all dose levels at the 52-week and 105 - 106 week sampling intervals. Similar to the 6-month chronic toxicity level, the most prominent morphologic change consisted of minimal to mild dilation of distal tubules in the cortex and collecting tubules of the outer medulla.

Collectively, these changes reflect a marked perturbation of the normal renal physiology, especially at the high dose level. Although they are not considered to be directly related to the renal tubule tumors observed in the high dose group (100 mg/kg/day) they may indirectly contribute to their induction."

Reviewer note
In the initial safety report, the sponsor insisted that the dose-related increase in CPN was likely the cause of renal tumors in rats. The patient consent form further emphasized the role of CPN on canagliflozin induced increase in renal tumors. The Division rejected the role of CPN, as there was no data supporting such relationship and only severe end-stage CPN have been associated with renal tumors. The CPN in the rat carcinogenicity study did not reach the level of severity to contribute to renal tumors in this study. The PWG agreed that CPN did not contribute to renal tumors. Furthermore, they graded the severity by 1 to 2 grades lower than the study pathologist. Renal tumors in SD rats are rare, occurring less than 0.3% while CPN occurs in nearly every rat at old age. For CPN to play a role, one would expect significantly higher incidence of renal tumors than 0.3% to 1% reported in publications (Chandra et al, Appl. Toxicol, 1993, Hard el al, Toxicol Pathol, 2008 and Seely et al, Toxicol Pathol, 2002).

In the initial safety report, there was also a dose-dependent increase in the number of renal tubule hyperplasia in both male and female rats. Since renal tubule hyperplasia is a pre-neoplastic signal leading to neoplastic lesions, the increase in number of hyperplasia supported the increase in renal tumors. However, after PWG analysis, renal tubule hyperplasia was only slightly increased in HD males (6 vs. 3 in control), leading the PWG to conclude that there was absence of tubule hyperplasia as a general response to canagliflozin treatment thus there was no pre-neoplastic signal for renal tumors. The PWG appeared to have diagnosed most incidences of renal tubule hyperplasia as renal tubule hypertrophy since the initial report had no hypertrophy but the final report did. It should be noted that of all the data submitted in the final report, only renal hyperplasia was significantly changed. Although the rat carcinogenicity was peer reviewed, the name of the second pathologist was not provided. Since the original histopathology report and other SGLT2 inhibitors are known to increase renal tubule hyperplasia, the reviewer is not swayed by the new diagnosis.

Are there morphologic changes suggesting cell damage or tubular cell injury that would be relevant to the pathogenesis of cell proliferation?

"The PWG examined slides from high dose male rats from 13-Wk, 6-month and 24-month studies. There was no histologic evidence of cytotoxicity in any of the sections examined that suggested cell damage or tubular cell injury was present that would lead to increased tubule cell proliferation and renal tubule tumors in the high dose male and female rats. The most consistent histologic finding noted in all of the studies was minimal to slight dilation of the cortical distal tubules and collecting tubules of the outer stripe of the medulla (OSOM) in the high-dose (100 mg/kg). This histologic change was likely related to the increased urine volume, a pharmacologic effect of JNJ-28431754."

Reviewer note
The PWG concluded that there was no evidence of cytotoxicity such as tubule cell degeneration and regeneration in toxicology studies and by diagnosing only few hyperplasia in the 2-year rat study, they saw no evidence as what may have caused the renal tumors. It is not clear why there was no renal signal in the rat toxicology studies except for tubule dilation and mineralization (possibly due to high Ca excretion). There was however renal tubule degeneration and regeneration in the MD male and HD male and female dogs, suggesting that chronic canagliflozin treatment may indeed cause renal injury.

What is the dose response for non-neoplastic changes that could be of relevance to increased tubular cell proliferation?

"Non-neoplastic morphologic changes were not observed in the kidneys of high dose male rats during the PWG that were considered relevant to increased cell proliferation."

Is there a correlation between the incidence/severity of CPN and the renal tubule tumors?
    Is the CPN sufficient to explain the formation of renal tubule tumors?

"Chronic progressive nephropathy (CPN) is a spontaneous, age-related renal disease affecting rats. Male rats are more severely affected than females. In earlier stages, CPN is characterized microscopically as scattered, discrete foci of basophilic tubules with thickened basement membranes located in the renal cortex. As the disease progresses, more tubules become affected and are accompanied by glomerular pathology with some interstitial infiltration of mononuclear cells. Affected tubules display a range of changes including degeneration, atrophy, and regeneration. In very advanced stages of the disease, nearly all of the renal parenchyma is affected and occasional foci of atypical tubule hyperplasia may be present. CPN is not only a degenerative disease, but due to its regenerative aspects it has a high cell proliferation rate in affected tubules, particularly in advanced (end-stage) CPN and is a risk factor for increases in the background incidence of renal tubule tumors. Chemicals known to exacerbate the severity of CPN to an advanced stage can result in a small increase in the incidence of atypical tubule hyperplasia and renal tubule adenomas. It would be expected that renal tumor induction mediated through this pathway would be a minimal response with

respect to tumor incidence and tumor size or grade, probably occurring in male rats only (Hard, 1998). Because CPN is a rodent-specific entity, the finding of a small, statistically significant increase in renal tubule neoplasms, linked to exacerbation of CPN by test chemical in a preclinical study for carcinogenicity, can be regarded as having no relevance for extrapolation and human risk assessment (Hard and Khan, 2004; Hard et al, 2009).

In the current carcinogenicity study, both renal tubule adenoma and carcinoma were observed in high-dose (100 mg/kg/day) male and female rats. The incidence of renal tubule tumors in the high-dose groups was greater than historical control incidences. Although there was a slight increase in CPN in treated groups as compared to the control group, this increase was not dose-related in male rats. Additionally, the severity of CPN in treated groups as compared to the control group was not greatly increased. The renal tubule tumors which were observed did not consistently occur in animals with advanced, end-stage CPN."

	Incidence and Severity of Chronic Progressive Nephropathy (CPN) Reported by the Study Pathologist in the 24-Month Repeated Dose Oral Carcinogenicity Study in the Rat (Study TOX8986). Table 5.							
Sex	Male				Female			
Dose (mg/kg/day)	0	10	30	100	0	10	30	100
No. of Animals	65	65	65	65	65	65	65	65
CPN	43	60	59	56	23	37	47	50
Grade 1	22	28	17	9	19	31	34	10
Grade 2	10	23	31	25	3	4	7	19
Grade 3	10	7	8	16	1	2	4	17
Grade 4	1	2	3	5	-	-	1	2
Grade 5	-	-	-	1	-	-	1	2

"Although there was an increased incidence and severity of CPN in both sexes reported by the study pathologist, the increase in severity was mild to moderate with most animals given a score of Grade 2 or 3. During the PWG review of the kidney from male rats, in most instances the severity of CPN diagnosed by the PWG was often one grade lower than that reported by the study pathologist. The PWG considered the severity of CPN in this study to be relatively low as compared to their experience examining kidney sections from Sprague-Dawley rats used in 2-year carcinogenicity bioassays. In their experience, with renal tubule tumors that are associated with an increase in CPN, a severity score of 4 or 5 (end-stage nephropathy) would generally be expected. In the opinion of the PWG, the slight increase in incidence and severity of CPN in treated groups reported by the study pathologist was not sufficient to explain the formation of renal tubule tumors."

Reviewer note
As discussed earlier, the severity of CPN was not high enough to play a role in this study. Indeed, the associatetion between CPN and renal tumor is rather weak at best even in cases of severe CPN. CPN occurs in nearly all SD rats, especially in males with age while the incidence of renal tubule tumor is very low (0.3%). One would except significantly higher incidence of renal tumors in rats if CPN was a major contributor. The association between end-stage CPN and renal tumors does not prove a cause and effect since one can not predict an animal with severe CPN will develop renal tubule tumors. When nearly every animal has CPN at the end of life,

attributing 1 or 2 cases of renal tumors to one of the rats with a severe case of CPN is not a strong argument for a relashionship.

Was there any correlation between the increase of urinary volume and the incidence/severity of CPN/renal tubule tumors?

"The most consistent treatment related observation across all studies was the presence of minimal to slight dilation of the cortical distal tubules and collecting tubules of the outer stripe of the outer medulla (OSOM). This observation correlates very well with the increased urinary volume which was noted in treated rats in all studies reviewed. Although only slides from the control and high-dose (100 mg/kg/day) male rats were reviewed by the PWG, the data presented in the individual reports indicates that there was an increase in urinary volume in all treated groups and that it was consistently accompanied by tubule dilation in the renal cortex. The PWG did not feel that there was a direct correlation between the increased urinary volume and the incidence/severity of CPN or renal tubule tumors."

PWG Conclusion:

"The results of the PWG review of kidney histology sections from rats exposed to JNJ-28431754 by oral gavage in repeated dosing toxicity and carcinogenicity studies confirmed the presence of renal tubule tumors in the high dose (100 mg/kg/day) male and female rats in the 24-Month Repeated Dose Oral Carcinogenicity Study in the Rat (Study TOX8986). The two renal cell tumors present in the mid-dose (30 mg/kg/day) male rats were considered not related to treatment due to their distinctive morphology which distinguished them from the tumors in the high dose group and the fact that one of the two tumors was morphologically characteristic of a spontaneously occurring familial tumor that has been reported to occur in Sprague-Dawley rats.

The most consistent treatment-related observation across all studies was the presence of minimal to slight dilation of the cortical distal tubules and collecting tubules of the outer stripe of the outer medulla (OSOM) and papillae. This observation correlated very well with the increased urinary volume and glycosuria which was noted in treated rats in al studies reviewed. The PWG did not feel that there was a direct correlation between the increased urinary volume and the incidence/severity of CPN.

In the opinion of the PWG, the slight increase in incidence and severity of CPN in treated groups reported by the study pathologist was not sufficient to explain the formation of renal tubule tumors. No other non-neoplastic changes, including evidence of cytotoxicity, was observed that were considered by the PWG to be directly relevant to increased tubule cell proliferation leading to tumor formation."

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
02/01/2013
Approval recommended

TODD M BOURCIER
02/01/2013
Approval recommended

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 204042   **Applicant: Janssen Pharmaceuticals Inc.**   **Stamp Date: May 31, 2012**

Drug Name: Canagliflozin   **NDA/BLA Type: 505(b)(1)**

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		Per agreement, the ongoing juvenile rat study will be submitted at the 4-month safety update during the NDA review cycle (Sept 2012).
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 submitted a rationale to justify the alternative route?	x		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	x		All the pivotal toxicology and long term mechanistic studies (6-month high fructose and low calcium diet studies) were performed according to GLP regulations.
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	x		As noted earlier, the juvenile rat study will be submitted around Sept 2012. The sponsor has an ongoing 15-month mechanistic study that was initiated in Nov of 2011. The sponsor has provided no additional information as when the study will be available for review.

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

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/m2 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		The impurities in the product were less than the qualification threshold of (b) (4) thus requiring no qualification. The toxicology studies covered the potential impurities.
11	Has the applicant addressed any abuse potential issues in the submission?	x		Studies to examine canagliflozin abuse potential were not carried out. Canagliflozin which targets kidney tubules with poor brain distribution had no CNS effect in 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	July 30, 2012
Reviewing Pharmacologist	Date
Todd Bourcier / David Carlson (acting)	July 30, 2012
Team Leader/Supervisor	Date

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

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
07/31/2012
The canagliflozin NDA is fileable.

DAVID B CARLSON
08/01/2012
I concur (signing for Todd Bourcier)