CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

202293Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology/Toxicology Review

From: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 202293
Agency receipt date: 7/11/2013 (resubmission)
Drug: dapagliflozin
Sponsor: Bristol Myers Squibb/AstraZeneca
Indication: Type 2 Diabetes Mellitus treatment

Reviewing Division: Division of Metabolism and Endocrinology Products

Introductory Comments: During the previous review cycle, the pharm/tox reviewer and team leader concluded that the nonclinical data supported approval of dapagliflozin for the indication listed above. I concurred. Some additional nonclinical studies to further assess the potential for bladder tumor promotion were suggested but not required.

While carcinogenicity studies of dapagliflozin in rats and mice did not show evidence of drug-related neoplasms, an imbalance in bladder cancer was noted in clinical trials. The sponsor conducted several additional nonclinical studies to potentially explore whether dapagliflozin had activity that might contribute to bladder cancer development. These studies were included in the resubmission. The studies included in vitro studies and a study of a bladder tumor xenograft implanted in the flank of immunodeficient mice. Dapagliflozin did not enhance cell growth in vitro or tumor xenograft growth in vivo. However, the reviewer noted that because these studies were not conducted in urinary bladders in vivo, the bladder microenvironment, which could be important in neoplastic development, was not recapitulated. The division is, therefore, considering including such a tumor model as a post-marketing requirement in an action letter for this NDA.

Conclusions:
I agree with the division pharm/tox conclusion that dapagliflozin can be approved from the pharm/tox perspective.

Data from additional animal studies is not likely to supplant conclusions about the risk of bladder cancer derived from human clinical trial and postmarketing data. Therefore, such an animal study may not be essential. However, information from a study in animals in which the effect of dapagliflozin on neoplastic development in the urinary bladder was assessed could conceivably alter labeling recommendations on the use of the product. Therefore, while not needed for approval, such a study may be warranted as a postmarketing requirement.

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/s/

PAUL C BROWN
12/20/2013

Memorandum

SUPERVISOR MEMO

Date:	19 Dec 2013
RE:	NDA 202293 Complete Response
Sponsors:	Bristol Myers Squibb / AstraZeneca
Drug/Indication	Dapagliflozin (SGLT2 inhibitor) Type 2 diabetes
Author	Todd Bourcier, Ph.D.

Dr. Mukesh Summan (the primary toxicology reviewer) and I concluded in 2011 that the nonclinical profile of dapagliflozin supported approval of NDA 202293. The FDA issued a Complete Response based on unresolved issues related to cardiovascular, liver, and malignancy endpoints from the phase 3 clinical trials. This memo specifically addresses the steps taken and additional information submitted by the sponsor in the Complete Response that addressed the numerical imbalance in bladder cancers from the clinical trials. Note that our recommendation has not changed: the current nonclinical profile of dapagliflozin remains supportive of approval.

The non-clinical studies submitted in the original NDA did not identify a carcinogenic hazard for dapagliflozin. This was driven primarily by the lack of pre-neoplastic or neoplastic findings in the bladder from 2yr rat and mouse studies at high exposure multiples of drug (>90x clinical dose). In light of the numerical imbalance in bladder cancer from the clinical trials, the 'clean' result from the rodent carcinogenicity studies was interpreted by us as being either a false negative, or evidence for a lack of biological plausibility that dapagliflozin is causally related to the clinical cases of cancer. While both interpretations can be argued, it was my opinion that the latter argument held greater sway. I concluded that additional pre-market nonclinical studies would be exploratory and of limited practical use to refining the risk assessment for bladder cancer.

In the course of the ensuing Dispute Resolution, concern was again raised that dapagliflozin may act as a tumor promoter possibly as a consequence of changes in urinary volume, flow, and composition. It is feasible that a putative tumor promoting effect of dapagliflozin was inadequately addressed in the 2yr rodent studies due to the lack of spontaneous pre-neoplastic and neoplastic bladder lesions. As such, the Dispute Resolution letter included a recommendation that the sponsor conduct additional nonclinical studies focused on evaluating potential tumor promotion with dapagliflozin, and specifically a study in a rodent model of bladder tumor promotion.

Dr. Summan and I conveyed to the sponsor on five different occasions that the most appropriate study to conduct would involve the use of an orthotopic bladder tumor promotion model. These

models would best mimic the clinical situation: a change in urinary composition and renal function from dapagliflozin and transitional tumors in the bladder. Instead, the sponsor embarked on a number of *in vitro* and *in vivo* studies, none of which included an orthotopic model. Results from these studies confirmed but did not substantially extend what we already concluded: that dapagliflozin by itself does not act as a carcinogen, and that any putative promoting effect would be related to secondary changes in the microenvironment of the bladder *in vivo*. Dr. Summan's review and the FDA's nonclinical briefing document for the December Advisory Committee meeting provides a detailed critique of the sponsor's submitted studies.

The numerical imbalance of bladder cancer diagnoses from the clinical trials remains an unresolved clinical issue. This issue was cited by most advisory committee members as a cause for caution, but not as a cause for denying drug approval. Approaches using epidemiological methods and leveraging the cardiovascular outcomes trial to best address the bladder cancer risk is currently being explored by the review team. At the advisory committee, the sponsor stated that they now plan to conduct further nonclinical studies in an orthotopic model of bladder tumor promotion. There are several models that allow evaluation of dapagliflozin on transitional cell tumor growth within the bladder, such as transplanting human bladder tumor cells to mouse bladders (e.g., orthotopic models). Another is the hydroxybutyl nitrosamine (BBN) model wherein bladder tumors are selectively induced in rodents administered a genotoxic agent, followed by administration of test promoters of interest. Results from these additional nonclinical studies may impact the warnings and recommendations for use in the drug label; therefore, these studies are best conducted as a ***post-marketing requirement*** to ensure that they are completed in a timely fashion and in a manner acceptable to FDA reviewers.

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/s/

TODD M BOURCIER
12/19/2013
Pharm/tox recommends approval with a PMR

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:	202293
Supporting document/s:	0097
Applicant's letter date:	07.11.2013
CDER stamp date:	07.11.2013
Product:	Dapagliflozin
Indication:	Type 2 Diabetes Mellitus
Applicant:	Bristol-Myers Squibb (BMS)/AstraZeneca (AZ)
Review Division:	DMEP
Reviewer:	Mukesh Summan, PhD, DABT
Supervisor/Team Leader:	Todd Bourcier, PhD
Division Director:	Jean-Marc Guettier, MD
Project Manager:	Abolade (Bola) Adeolu

Disclaimer

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1 Executive Summary

1.1 Introduction

The proposed dapagliflozin film-coated tablet was submitted in accordance with 21 USC 505(b)(1) for the treatment of type 2 diabetes mellitus. The advisory committee for dapagliflozin in the first NDA review cycle was held July 19th 2011 and resulted in a three month extension of the PDUFA date. A Complete Response Letter (CRL) was sent to the sponsor on January 17th 2012. The sponsor submitted NDA 202293 for the second NDA review cycle on July 11th 2013.

Additional nonclinical studies were not required but were conducted nonetheless to address, in part, the CRL action with respect to the clinical safety concerns regarding the numerical imbalance of bladder tumors observed in the phase III clinical trials.

1.2 Brief Discussion of Nonclinical Findings

All pivotal nonclinical studies were conducted using oral administration of the drug, which is the clinical exposure route, and in accordance with US FDA GLP regulations (21CFR58) as stated by the sponsor. Safety margins to expected human exposure were estimated using C_{max} = 136 ng/mL and AUC_{0-24} = 465 ng.h/mL plasma exposure in healthy subjects at the proposed maximum recommended human dose (MRHD) of 10 mg dapagliflozin. This summary primarily addresses the new information provided in the resubmission.

Pharmacology
Dapagliflozin (BMS-512148 or Farxiga™) is a selective inhibitor of sodium glucose co-transporter (SGLT) 2. SGLT2 is selectively expressed in the kidney S1 proximal tubule and is responsible for the majority (90%) of the renal reabsorption of glucose. Inhibition of SGLT2 by dapagliflozin results in the excretion of glucose thereby producing glucosuria. In in vitro studies dapagliflozin was a potent and selective inhibitor of human (h) SGLT2 relative to the closely related hSGLT1 with a selectivity of 1242-1600-fold. Dapagliflozin was also found to selectively inhibit mouse (m) and dog (d) SGLT2 relative to mGLT1 and dSGLT1, respectively, but with lower selectivity compared to the human SGLT1/2 homologues (130-fold in the mouse and 436-fold in the dog, respectively), thus showing a greater likelihood of off-target SGLT1 inhibition at high exposures in the mouse and dog.

Off-target inhibition of mouse SGLT1 with a single dose of dapagliflozin at approximately 5x MRHD (10 mg) resulted in an increase in urinary glucose excretion in a mouse animal model where the mice lacked a functional renal SGLT2 transporter (SGLT2 knockout). The SGLT2 knockout mice were glucosuric and addition of dapagliflozin at 0.05x and 0.5x MRHD did not result in enhanced glucosuria as was also observed at these dapagliflozin concentrations, in the wild types cohorts. Although, SGLT1 is pivotal for intestinal glucose absorption, it is also expressed in the kidney in the S3 segment of the kidney proximal tubule and has a minor (10%) role in the renal

reabsorption of glucose. Nevertheless, inhibition of SGLT1 in the kidney of SGLT2 knockout mice is a likely explanation of enhanced glucosuria in the SGLT2 knockout mice. Although dapagliflozin exposure (AUC) in the wild-type or SGLT2 mice was not evaluated, it is assumed that the mouse SGLT1 EC_{50} (299 nM) was reached to produce the observed glucosuria. The clinical dose of dapagliflozin is expected to be within the selective range for SGLT2 inhibition, based on the results from the SGLT2 knockout mouse experiment.

PK/ADME
Treatment in the nude mouse xenograft study, with dapagliflozin for 2 weeks resulted in exposure (AUC) that was approximately dose proportional and the T_{max} ranged from 1-3 hours. The PK of the dapagliflozin glucuronide metabolite showed a similar pattern, but exposure and T_{max} were 39-58-fold lower relative to dapagliflozin. Dapagliflozin exposure in the xenograft study represents pharmacologically active doses ranging from 6-75x MRHD.

Metabolism
Saxagliptin and its major metabolite (BMS-510849) minimally inhibited dapagliflozin-mediated metabolism by UGTA19. In addition, dapagliflozin minimally inhibited UGT1A1. Drug-drug interactions for dapagliflozin with the DPPIV inhibitor saxagliptin and its metabolite BMS-510849 thus appear unlikely.

Dapagliflozin also minimally inhibited multiple cytochrome P450 enzymes with an IC_{50} at > 40 μM. Dapagliflozin is therefore unlikely to inhibit cytochrome P450 enzymes in vivo and thus also unlikely to interact with drugs that are metabolized by these enzymes.

Special Toxicology Studies
Mice with a glucosuric phenotype (SGLT2 knockout) were compared to their wild type cohorts with particular interest in evaluation of pre-neoplastic or neoplastic changes for a subset of tissues examined at necropsy or histopathologically and in particular for the bladder. The phenotype of these mice was examined from weaning (approx. 3 weeks) to when the mice were 15 months old.

Pre-neoplastic (hyperplasia) and neoplastic bladder histopathology was not observed in the mice lacking a functional SGLT2 transporter. This was consistent with the two-year rodent bioassays where bladder tumors were absent in dapagliflozin-treated and control (placebo) rats and mice, and only three control rats showed transitional epithelial hyperplasia from a total of 520 rodents combined. However, this phenotyping study did not address the potential of dapagliflozin to promote pre-existing bladder lesions in the in situ microenvironment of changes in urinary volume, flow and composition in the bladder.

In nonclinical rodent models of diabetes, dapagliflozin promoted glucose excretion, polyuria and lowered plasma glucose under conditions of hyperglycemia (oral glucose tolerance test). Transcriptional profiling for genes involved in cell cycle regulation and tumor promotion was carried out in a diabetic animal model for the liver, kidney, fat and

skeletal muscle in male ZDF rats exposed to a low dose of dapagliflozin (0.5 mg/kg) for 5 weeks. Exposure/PK was not evaluated in the transcriptional profiling study; however, 0.5 mg/kg dapagliflozin was used in the 2 year rat bioassay and approximates 7x MRHD (10 mg). Only one cell cycle regulating and tumor promoting gene (Stathmin 1) was up regulated (58%) in adipose tissue at the end of treatment. However, with regard to the observed numerical imbalance of bladder tumors observed in the clinic, tumor promoter transcriptional changes described by the sponsor were of limited value here, as the bladder tissue was not evaluated in this transcriptional profiling nonclinical study.

The sponsor also evaluated six human bladder transitional cell carcinoma (TCC) cell lines exposed to varying concentrations of dapagliflozin, dapaglizon-3-O-glucuronide or glucose in vitro. TCC cell line growth was not enhanced under these conditions and this is consistent with 2 year rodent carcinogenicity studies where bladder tumor formation with dapagliflozin was not observed in rats and mice at drug exposures reaching ~160x and ~90x MRHD, respectively. This adds to the weight of evidence that dapagliflozin is not a direct tumor promoter. However, evaluation of TCC cell lines with dapagliflozin and glucose in isolation fails to account for the bladder microenvironment changes in urine volume, flow and composition under dapagliflozin use in the clinic.

To mitigate this flaw, the sponsor used a tumor promotion xenograft model where human bladder TCC cell lines were subcutaneously implanted in the flank of immnuodeficient (nude) mice. The human bladder tumors were allowed to establish themselves prior to oral treatment at either low (6 - 12x MRHD) or high (30 - 75x MRHD) dapagliflozin or dapagliflozin-3-O-glucuronide (0.1 – 0.9x MRHD) exposures for 11-14 days. Treatment with dapagliflozin or dapagliflozin-3-O-glucuronide did not enhance tumor growth relative to untreated control mice. Again, however, the xenograft model did not address the potential impact of changes in urinary volume, flow and composition within the microenvironment of the bladder on tumor cell growth. Therefore, this study fails to address the potential promotion of pre-existing bladder tumors with exposure to dapagliflozin or its metabolites within the bladder, as would occur in the clinical population.

Numerous mouse models have been developed to study human cancer and are used to investigate malignancy and metastasis as well as response to therapy. A more appropriate animal model that would allow evaluation of dapagliflozin on transitional cell tumor growth within the bladder, would include orthotopic models where tumor cells are implanted into an organ of their origin. Another rodent model for bladder tumors uses 4-hydroxybutyl(butyl)nitrosamine (BBN) as the tumor initiator followed by the test promoter of interest. Both of these models under conditions of oral treatment with dapagliflozin at pharmacologically active doses would more closely resemble the clinical use of dapagliflozin.

In summary, the results of the new nonclinical studies submitted to address the clinical safety concern for the numerical imbalance of bladder tumors in the clinic add to the weight of evidence that dapagliflozin does not act as a carcinogen. However, any

putative human bladder risk from dapagliflozin would likely be related to tumor promotion secondary to changes in the microenvironment of the bladder in vivo.

1.3 Recommendations

1.3.1 Approvability

AP (Approval)
Pharmacology/Toxicology recommends approval of NDA 202293.

1.3.2 Additional Non Clinical Recommendations

The primary deficiency of the mouse xenograft model is that the transplanted human bladder cancer cells were not exposed to the microenvironment of the bladder where changes in urinary volume, flow and composition are taking place under conditions of dapagliflozin use *in vivo*. A more appropriate animal model that would allow evaluation of dapagliflozin on transitional cell tumor growth within the bladder is the orthotopic models where bladder tumor cells are implanted into the bladder. Another rodent model for bladder tumors uses 4-hydroxybutyl(butyl)nitrosamine (BBN) as the tumor initiator followed by the test promoter of interest. Both of these models under conditions of oral treatment with dapagliflozin at pharmacologically active doses would more closely resemble the clinical use of dapagliflozin.

The numerical imbalance of bladder tumors observed clinically in the phase III human trials remains an issue, regardless of the outcome of additional animal studies. Therefore, effort might be better spent developing a post-market pharmacovigilance plan rather than a PMR for an orthotopic tumor promotion model in rodents.

1.3.3 Labeling

Established Pharmacological Class (Highlights/Indications & Usage):
Dapagliflozin will be described as a "sodium-glucose co-transporter 2 (SGLT2) inhibitor". This is consistent with the EPC for the currently approved drug in the class, canagliflozin.

HIGHLIGHTS

----------USE IN SPECIFIC POPULATIONS----------
• Pregnancy: (b) (4). Use during pregnancy only if the potential benefit justifies the potential risk to the fetus (8.1)
• Nursing mothers: (b) (4)

Section 8.1
Pregnancy Category C

(b) (4)
Pregnancy Category C
There are no adequate and well-controlled studies of Farxiga™ in pregnant women. Based on results (b) (4)
dapagliflozin may affect renal development and maturation. (b) (4)

(b) (4)

(b) (4)

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.

8.3 Nursing Mothers

It is not known (b) (4) Farxiga™ is excreted in human milk. (b) (4) Data in juvenile rats directly exposed to (b) (4) showed risk to the developing kidney (renal pelvic and tubular dilatations) during maturation. Since human kidney maturation occurs *in utero* and during the first 2 years of life when lactational exposure may occur, there may be risk to the developing human kidney. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from Farxiga™, a decision should be made whether to discontinue nursing or to discontinue Farxiga™, taking into account the importance of the drug to the mother (b) (4)

8.4 Pediatric Use

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

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

(b) (4)

(b) (4)

2 Drug Information

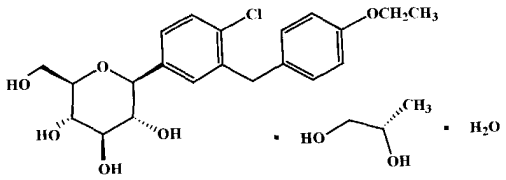
2.1 Drug

CAS Registry Number	960404-48-2
Generic Name	Farxiga™
Code Name	Dapagliflozin/BMS-512148
Chemical Name	1S)-1,5-Anhydro-1- C-[4-chloro-3-[(4-ethoxyphenyl)methyl]-pheny1]-D-g1ucitol, (S)-propylene glycol, monohydrate

Molecular Formula/Molecular Weight

$C_{21}H_{25}ClO_6.C_3H_8O_2.H_2O$ / MW = 502.98 (*S*-Propylene glycol monohydrate); = 408.87 ((b) (4) non-hydrated form)

Structure or Biochemical Description



Pharmacologic Class Sodium Glucose co-Transporter 2 (SGLT2) Inhibiror

2.2 Relevant INDs, NDAs, BLAs and DMFs

NDA 202293 was developed under IND 68652

2.3 Drug Formulation

Dapagliflozin film-coated tablets are manufactured as 5 mg or 10 mg strengths.

2.4 Comments on Novel Excipients

None.

2.5 Comment on Impurities/Degradants of Concern

None.

2.6 Proposed Clinical Population and Dosing Regimen

Dapagliflozin is indicated for treatment of Type 2 diabetes mellitus (T2DM). The recommended dose is 10 mg taken once daily. A lower starting dose of 5 mg is recommended for patients with volume depletion.

2.7 Regulatory Background

The sponsor submitted NDA 202293 to the Agency on December 27th 2010. The advisory committee for dapagliflozin in the first NDA review cycle was held July 19th 2011 and resulted in a three month extension of the PDUFA date. A CRL (Complete Response Letter) was sent to the sponsor on January 17th 2012. The sponsor submitted NDA 202293 for the second NDA review cycle on July 11th 2013.

3 Studies Submitted

3.1 Studies Reviewed

In Vitro Potency of Dapagliflozin (BMS-512148) Against Mouse Type 1 and Type 2 Sodium Dependent Glucose Transporters (Study# 930041858, non-GLP)

In Vitro Potency of Dapagliflozin (BMS-512148) Against Dog Sodium Glucose Co-transporters 1 and 2 (Study# 930052019, non-GLP)

Effects of Dapagliflozin and Its Primary 3-O-Glucuronide Metabolite on Human Bladder Tumor Cell Growth in Culture (Study #: 930066037, non-GLP)

Effect of Glucose Concentration on Growth of Bladder Cancer Cell Lines in Culture (Study# 930053120, non-GLP)

Oral Exposure of Dapagliflozin (BMS-512148) in Tumor Bearing Mice Studies: 1) Exposure in Nude Mice During A Preliminary Study And 2) Exposure During Studies In Nude Mice Bearing EJ1 or UMUC3 Tumors (Study #NCPK 39, non-GLP)

Effects of Dapagliflozin on The Growth Of Human Bladder Cancer Xenografts in Nude Mice (Study #: 930067105, non-GLP)

Phenotyping Study of 15-Month Old Homozygous SGLT2 Deficient Mice and Wild-Type Cohorts (Study# DT11112, non-GLP)

Effect of Dapagliflozin on Renal Excretion in Awake Non-Diabetic Wild-Type and SGLT2-/- Mice (Study# 930044592, non-GLP)

Transcriptional Profiling Analyses of Male ZDF Rat Tissues Following 1 Week and 5 Weeks of Dapagliflozin Treatments (Study# 930052593, non-GLP)

Evaluation of the UGT1A1 Inhibition Potential by Dapagliflozin (BMS-512148) in Human Liver Microsome Incubations (Study# 930054954, non-GLP)

Evaluation of the Potential for Saxagliptin (BMS-477118) and its Major Metabolite, BMS-510849, to Inhibit UGT1A9 in Human Liver Microsome Incubations Using Dapagliflozin or Propofol as Substrates (Study# 930059456, non-GLP)

Inhibition of CYP Enzymes Activity in Human Liver Microsomes (Study# 930053153, non-GLP)

In Vitro Assessment of the Role of Renal and Hepatic Uptake Transporters in Dapagliflozin (BMS-512148) Disposition (Study# 930053412, non-GLP)

3.2 Studies Not Reviewed

Addendum 03: SGLT2 Genomic Dossier (Study# 93005304)

Determination of the Ki Values for Phlorizin against Human SGLT1 and SGLT2 Co-transporters Using the LC-MS3 Method (Study# 930058576, non-GLP)

Quantitation of BMS-512148 and BMS-801576 in Mouse Plasma via HPLC with MS/MS Detection (Study# BMSR#4157)

Formation rate of Glucuronides of Dapagliflozin in Incubations with Individual Human Liver Microsomes Genotyped with UGT1A9 (Study# 930054146, non-GLP)

Dapagliflozin: Platelet Function Study using ADP-, TRAP-, Arachidonic Acid- and Collagen induced Platelet Aggregation in Human Platelet Rich Plasma, In Vitro (Study# AZM120523-01, non-GLP)

3.3 Previous Reviews Referenced

NDA 202293 review #1 (08.30.2011)
Study# 930059456 was fully reviewed by Dr. Alavi (03.05.2013) under IND 63,634.

4 Pharmacology

4.1 Primary Pharmacology

In Vitro Potency of Dapagliflozin (BMS-512148) Against Mouse Type 1 and Type 2 Sodium Dependent Glucose Transporters (Study# 930041858, non-GLP)

Method
CHO K1 cells, were stably transfected with cDNA for mouse (m) SGLT1 or mSGLT2, respectively, and were used to measure the uptake of ^{14}C-alpha-methyl-glucopyranoside ([^{14}C]-AMG) in the presence of dapagliflozin or phlorizin. For mSGLT1, dapagliflozin was used at 0.003 – 30 μM and phlorizin was used at 0.001 – 10 μM, respectively, measured in triplicate. For mSGLT2 dapagliflozin was used at 0.1 – 1000 nM and phlorizin was used at 0.3 – 3000 nM, respectively, measured in quadruplicate. Liquid scintillation counting (LSC) was used to measure the uptake of [^{14}C]-AMG

Results
Dapagliflozin was found to inhibit mSGLT1 and mSGLT2-mediated transport of [^{14}C]-AMG with an EC_{50} of 299 and 2.3 nM, respectively, thus showing a 130-fold selectivity for the inhibition of mSGLT2 (see sponsor's table below).

Phlorizin was found to inhibit mSGLT1 and mSGLT2-mediated transport of [^{14}C]-AMG with an EC_{50} of 364 and 60 nM, respectively, thus showing a 6-fold selectivity for the inhibition of mSGLT2 (see sponsor's table below).

Table 1. Dapagliflozin Inhibition of Mouse (m) SGLT1/2 Mediated Transport of [^{14}C]-AMG in CHO Cells (sponsor's table)

Compound	mSGLT2 Mean EC_{50} (nM) ± SE	mSGLT1 Mean EC_{50} (nM) ± SE	Selectivity for mSGLT2 vs. mSGLT1 (fold)
BMS-512148	2.3 ± 0.6	299 ± 166	125
Phlorizin	60 ± 22	364 ± 239	6.5

Source: E-Notebook 78363 Exp.8-14

In Vitro Potency of Dapagliflozin (BMS-512148) Against Dog Sodium Glucose Co-transporters 1 and 2 (Study# 930052019, non-GLP)

Method

CHO K1 cells, were stably transfected with cDNA for dog (d) SGLT1 or dSGLT2, respectively, and were used to measure the uptake of ^{14}C-alpha-methyl-glucopyranoside ([^{14}C]-AMG) in the presence of dapagliflozin or phlorizin. For dSGLT1, dapagliflozin was used at 0.003 – 30 μM and phlorizin was used at 0.001 – 10 μM, respectively, measured in triplicate. For dSGLT2 dapagliflozin was used at 0.01 – 300 nM and phlorizin was used at 0.003 – 3 μM, respectively, measured in triplicate. Liquid scintillation counting (LSC) or LC-MS-MS-MS (LC-MS3) was used to measure the uptake of [^{14}C]-AMG. As the radioactivity measurement using LSC was the basis for evaluating mouse and rat SGLT1/2 transporter activity using [^{14}C]-AMG uptake, this method was used as the basis for reporting dSGLT1/2 activity in the current report.

Results

Dapagliflozin was found to inhibit dSGLT1 and dSGLT2-mediated transport of [^{14}C]-AMG with an EC_{50} of 698 and 1.6 nM, respectively, thus showing a 436-fold selectivity for the inhibition of dSGLT2 (see sponsor's table below). Phlorizin was found to inhibit dSGLT1 and dSGLT2-mediated transport of [^{14}C]-AMG with an EC_{50} of 357 and 51 nM, respectively, thus showing a 7-fold selectivity for the inhibition of dSGLT2 (see sponsor's table below).

Table 2. Dapagliflozin Inhibition of Dog (d) SGLT1/2 Mediated Transport of [^{14}C]-AMG in CHO Cells (sponsor's table)

Summary: Inhibition dog SGLT1 and SGLT2 activity by dapagliflozin and phlorizin

Accumulation of [^{14}C]-alpha-methyl glucopyranoside (AMG)

Compound	dSGLT2 Mean EC_{50} (nM) ± SEM	dSGLT1 Mean EC_{50} (nM) ± SEM	Selectivity for dSGLT2 vs. dSGLT1 (fold)
Dapagliflozin	1.6 ± 1.0	698 ± 203	436
Phorizin	51 ± 19	357 ± 95	7

Accumulation of AMG measured by LCMS3

Compound	dSGLT2 Mean EC_{50} (nM) ± SEM	dSGLT1 Mean EC_{50} (nM) ± SEM	Selectivity for dSGLT2 vs. dSGLT1 (fold)
Dapagliflozin	1 ± 0.5	609 ± 209	609
Phorizin	42 ± 10	276 ± 128	7

Using the LC-MS3 method to measure [^{14}C]-AMG transport, showed dapagliflozin inhibited dSGLT1 and 2 with an EC_{50} of 609 and 1 nM, respectively, thus showing a 609-fold selectivity for the inhibition of dSGLT2 (see sponsor's table above). Similarly, using the LC-MS3 method to measure [^{14}C]-AMG transport, phlorizin inhibited dSGLT1 and 2 with an EC_{50} of 276 and 42 nM, respectively, thus showing a 7-fold selectivity for the inhibition of dSGLT2 (see sponsor's table above).

4.2 Secondary Pharmacology

Effect of Dapagliflozin on Renal Excretion in Awake Non-Diabetic Wild-Type and SGLT2-/- Mice (Study# 930044592, non-GLP)

Key Findings

Glucosuria above baseline was observed in SGLT2 knockout mice with dapagliflozin at both 1 and 10 mg/kg. This suggests dapagliflozin inhibited SGLT1 in the kidney and is responsible for the additional glucosuria. This is consistent with the glucose transport (reabsorption) capacity of renal SGLT1 which approximates 10% of the filtered glucose load.

Method

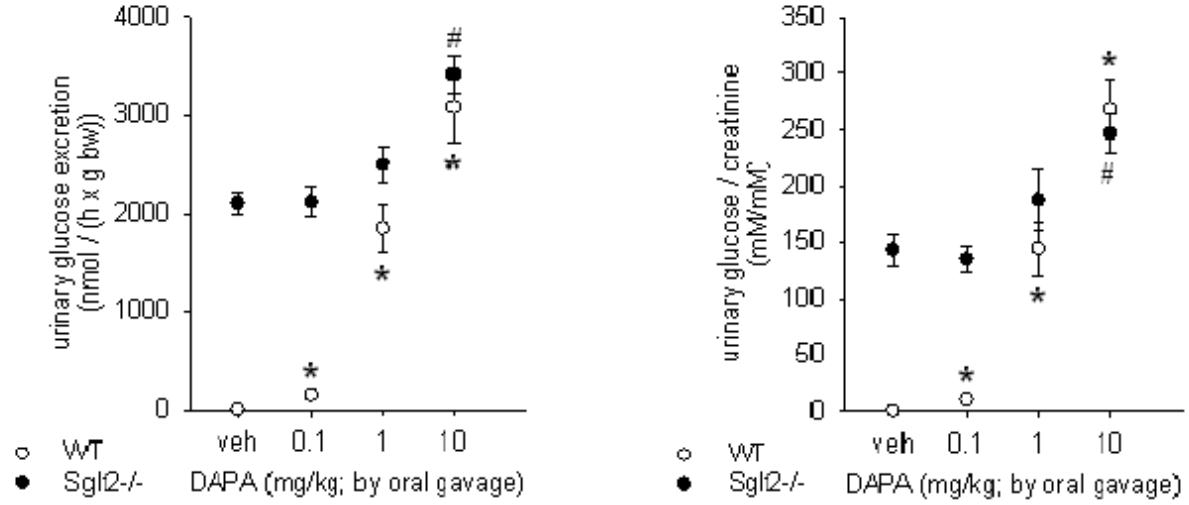
Male wild type (WT) C57BL/6 mice and SGLT2 knockout (KO or -/-) mice (n = 6-9/group) were administered dapagliflozin via oral gavage at 0, 0.1, 1 and 10 mg/kg in 1% ethanol in water, together with an oral water load of 30 μL/g bodyweight. Following

treatment gentle abdominal compression was applied to empty the mouse bladder, prior to placing the mice in metabolism cages and urine was collected for up to 3 hours post-dose without access to food and water. Standard urinalysis was conducted to measure urinary glucose, calcium (Ca), creatinine, sodium (Na^+) and potassium (K^+). The mice used in the present study were 3-5 months of age.

Results

Dose-dependent glucosuria was observed in WT mice (see sponsor's figure below). Dapagliflozin at greater than or equal to 1 mg/kg had an additional SGLT2-independent glucosuric effect (see sponsor's figure below). Normalizing for creatinine produced similar results (see sponsor's figure below).

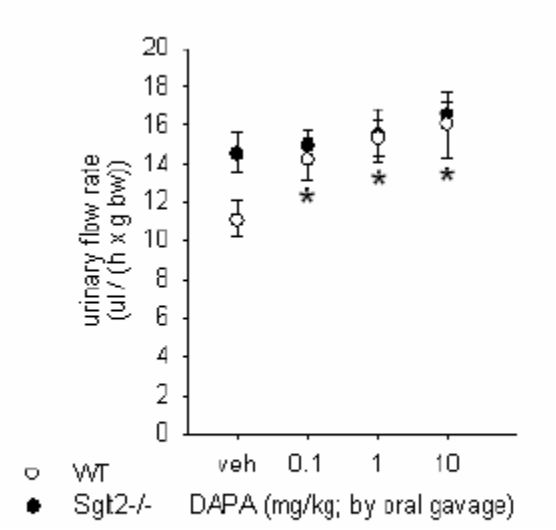
Figure 1. Dapagliflozin-induced Glucosuria in WT and SGLT2-/- Mice (sponsor's figure)



DAPA at doses >1 mg/kg have additional SGLT2-independent glucosuric effects. N=6-9; WT: * P<0.05 vs. veh; SGLT2-/-: # P<0.05 vs. veh.

Urine flow rate was also dose-dependently increased in the WT mice but not in the SGLT2-/- mice (see sponsor's figure below)

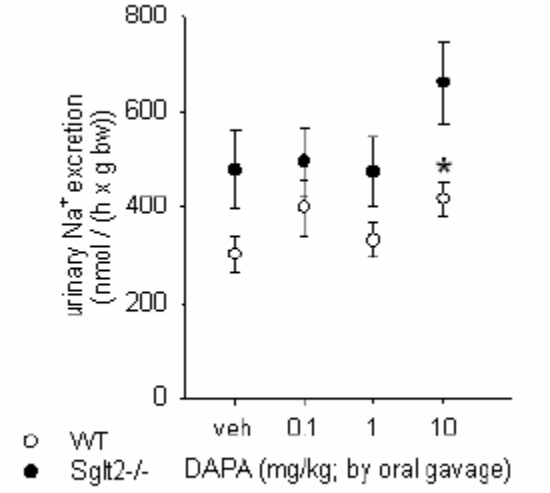
Figure 2. Urinary Flow Rate in WT and SGLT2-/- Mice Treated with Dapagliflozin (sponsor's figure)



N=6-9; WT: * P<0.05 vs. veh; SGLT2-/-: # P<0.05 vs. veh.

Urinary sodium excretion was increased at 10 mg/kg in WT mice (see sponsor's figure below)

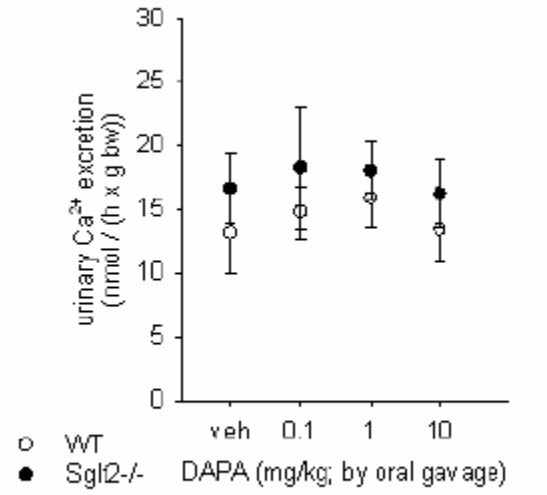
Figure 3. Urinary Sodium Excretion in WT and SGLT2-/- Mice Treated with Dapagliflozin (sponsor's figure)



N=6-9; WT: * P<0.05 vs. veh; SGLT2-/-: # P<0.05 vs. veh.

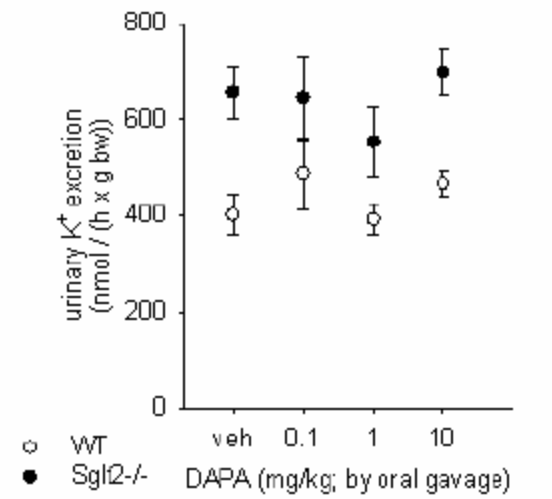
Urinary excretion of Ca^{+} and K^{+} was highly variable (see sponsor's figures below)

Figure 4. Urinary Calcium Excretion in WT and SGLT2-/- Mice Treated with Dapagliflozin (sponsor's figure)



N=6-9; WT: * P<0.05 vs. veh; SGLT2-/-: # P<0.05 vs. veh.

Figure 5. Urinary Potassium Excretion in WT and SGLT2-/- Mice Treated with Dapagliflozin (sponsor's figure)



N=6-9; WT: * P<0.05 vs. veh; SGLT2-/-: # P<0.05 vs. veh.

Reviewer note: Plasma levels (exposure) of dapagliflozin were not assessed in the present study. On a body surface area basis 0.1, 1 and 10 mg/kg in the SGLT2

knockout and wild type mouse represent 0.05x, 0.5x and 5x MRHD (10 mg). In the mouse carcinogenicity study 5 and 15 mg/kg were used as the low and mid dose and represent 4x and 14x MRHD, respectively, on an exposure (AUC) basis. Assuming linear exposure and single dose exposure is similar to multiple dose exposure, 10 mg/kg in the mouse could be 8-10x MRHD based on AUC. In the mouse the EC_{50} for SGLT1 and SGLT2 inhibition is 299 and 2.3 nM, thus showing 130-fold selectivity for SGLT2. At the maximum clinical dose (10 mg) the C_{max} is 0.2 μM. Thus the clinical dose of dapagliflozin is expected to be within the selective range of human SGLT2 inhibition where the EC_{50} for human SGLT2 inhibition is 1 nM, but not human SGLT1 inhibition where the EC_{50} for human SGLT1 inhibition is 1600 nM. In the present mouse SGLT2 knockout study, dapagliflozin at 0.05x and 0.5x MRHD did not result in additional glucosuria and thus are unlikely to inhibit SGLT1 in humans.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Oral Exposure of Dapagliflozin (BMS-512148) in Tumor Bearing Mice Studies: 1) Exposure in Nude Mice During A Preliminary Study And 2) Exposure During Studies In Nude Mice Bearing EJ1 or UMUC3 Tumors (Study# NCPK 39, non-GLP)

Method

The effects of dapagliflozin on the in vivo growth of two human bladder transitional cell carcinoma (TCC) cell lines (EJ-1 and UMUC3, respectively) was examined in male and female nude mice (Nu/Nu). In a preliminary study male Nu/Nu mice (n = 3/sex/group) were exposed to a single oral dose of dapagliflozin as follows at 0, 4, 20 or 150 mg/kg, respectively. Blood was collected at 1, 3, 6, 16 and 24 hr for plasma PK analysis of dapagliflozin (BMS-521148) or its metabolite BMS-801576 (3-O-glucuronide) using LC/MS.

In the definitive study the male and female Nu/Nu mice were treated to daily oral dose of dapagliflozin (in 90 PEG400 and 10% water, 10 mL/kg) for 2 weeks (EJ-1 tumor) or until the average group tumor burden reached approximately 1000 mg (UMUC3 tumor) as follows (sponsor's tables):

Table 3. Treatment Schedule for Nu/Nu Mice + EJ-1 Tumor (sponsor's table)

Group	No. of animals / Sex	Treatment	Dose (mg/kg)	Schedule
1	8 / F	Vehicle Control	0	QDx14
2	8 / F	dapagliflozin	4	QDx14
3	8 / F	dapagliflozin	20	QDx14
4	8 / M	Vehicle Control	0	QDx14
5	8 / M	dapagliflozin	12	QDx14
6	8 / M	dapagliflozin	60	QDx14

Table 4. Treatment Schedule for Nu/Nu Mice + UMUC3 Tumor (sponsor's table)

Group	No. of animals / Sex	Treatment	Dose (mg/kg)	Schedule
1	8 / F	Vehicle Control	0	QDx11
2	8 / F	dapagliflozin	4	QDx11
3	8 / F	dapagliflozin	20	QDx11
4	8 / M	Vehicle Control	0	QDx11
5	8 / M	dapagliflozin	12	QDx11
6	8 / M	dapagliflozin	60	QDx11

Blood was collected at 1, 3 and 6 hr post-dose on day 4 post-dose for the UMUC3 tumor bearing mice and at day 5 post-dose for the EJ-1 tumor bearing mice, respectively, for plasma PK analysis of dapagliflozin (BMS-521148) or its metabolite BMS-801576 (3-O-glucuronide) using LC/MS.

Results

Preliminary Study

For dapagliflozin (BMS-512148) C_{max} and $AUC_{(0-T)}$ (0-16 hr) was less than dose proportional for males and greater than dose proportional for females. $AUC_{(0-T)}$ was 1-3-fold lower in males compared to females (see sponsor's table below). T_{max} for dapagliflozin ranged from 1-3 hr. Similar PK results were obtained for the dapagliflozin 3-0-glucuronide metabolite (BMS-801576). However, both C_{max} and $AUC_{(0-T)}$ for BMS-801576 were 26-55-fold lower relative to dapagliflozin (see sponsor's table below). T_{max} for BMS-801576 ranged from 1-4 hr.

Table 5. PK in Nu/Nu Mice following a Single Oral Dapagliflozin Dose – DRF Study (sponsor's table)

Parameter	Day	BMS-512148 Dose Sex					
		4 mg/kg/day		20 mg /kg/day		150 mg/kg/day	
		M	F	M	F	M	F
		BMS-512148					
Cmax (ng/ml)	1	544	781	2370	4883	37867	45233
AUC(0-T)[a] (hr*ng/ml)		1591	3154	5968	19398	165209	234360
Tmax(hr)		1	3	1	3	1.7	2.3
		BMS-801576					
Cmax (ng/ml)	1	19	27	55	182	685	852
AUC(0-T)[a] (hr*ng/ml)		60	106	180	651	3503	4951
Tmax (hr)		1.0	2.3	1.0	4.0	1.7	2.3

[a]AUC(0-16h) was calculated due to less than LLOQ values for most of the data points at 24 h

Definitive Study

Dapagliflozin and its metabolite BMS-801576 were found in the vehicle control plasma at the 1, 3 and 6 hr collection time points for the UMUC3 tumor bearing males and females (results not shown). The sponsor states that the source of the drug and metabolites in these samples is due to 'post-collection cross-contamination. Glucosuria was not observed in the vehicle control animals (see study report# 930067105 in the Special Toxicology Studies section below) and the presence of dapagliflozin and its metabolites in the vehicle control plasma is unlikely to be due to inadvertent dapagliflozin treatment of the vehicle control animals. **Reviewer note**: this reviewer concurs with the sponsor's assessment that the low level contamination has minimal impact on the study outcome.

In male and female Nu/Nu mice carrying the EJ-1 tumor C_{max} and $AUC_{(0-T)}$ was approximately dose proportional for dapagliflozin-treated mice. T_{max} for dapagliflozin ranged from 1.7-3.3 hr (see sponsor's table below). PK parameters for the dapagliflozin glucuronide metabolite BMS-8051576 were similar to dapagliflozin, however both C_{max} and $AUC_{(0-T)}$ for BMS-8051576 were 39-58-fold lower relative to dapagliflozin (see sponsor's table below).

In male and female Nu/Nu mice carrying the UMUC3 tumor C_{max} and $AUC_{(0-T)}$ was less than dose proportional in males and females. T_{max} for dapagliflozin ranged from 1.7-3 hr (see sponsor's table below). PK parameters for the dapagliflozin glucuronide metabolite BMS-8051576 were similar to dapagliflozin, however both C_{max} and $AUC_{(0-T)}$ for BMS-8051576 were 38-61-fold lower relative to dapagliflozin (see sponsor's table below). T_{max} for BMS-801576 ranged from 1.7-3 hr.

Table 6. PK in Nu/Nu Mice following Multiple Oral Dapagliflozin Doses – Definitive Study (sponsor's table)

Tumor Model	EJ1[a]				UMUC3[b]			
Sex	F	M	F	M	F	M	F	M
Daily Dose (mg/kg)	4	12	20	60	4	12	20	60
	BMS-512148				BMS-512148			
Cmax (ng/ml)	928	2124	5437	10160	1253	1175	3623	9847
AUC(0-T) (hr*ng/ml)	2830	5545	17241	34799	3336	3918	14068	22884
Tmax (hr)	2.7	1.7	1.7	3.3	1.7	1.7	3.0	2.3
	BMS-801576				BMS-801576			
Cmax (ng/ml)	18	55	110	223	28	27	83	160
AUC(0-T) (hr*ng/ml)	49	133	323	719	87	85	328	371
Tmax (hr)	1.7	1.7	1.7	2.3	1.7	1.7	3.0	2.3

[a]The plasma samples from mice in this group were collected after 5 days of daily dosing; b the plasma samples from mice in this group were collected after 4 days of daily dosing

Metabolism
Evaluation of the UGT1A1 Inhibition Potential by Dapagliflozin (BMS-512148) in Human Liver Microsome Incubations (Study# 930054954, non-GLP)

Method
UGT1A1 is important in the metabolism of several medications including statins. Dapagliflozin is a substrate of UGT1A9 but it's interaction with UGT1A1 is unknown. Pooled human liver microsomes were used to determine the inhibitory effect of dapagliflozin (0 – 50 μM) on UGT1A1. Estradiol (30 μM) was used as the UGT1A1 substrate, forming estradiol 3-O-glucuronide, which was measured using HPLC Atazanavir (0-50 μM), a known UGT1A1 inhibitor, was used as a positive control. . Metabolites were quantified using LC/MS/MS.

Results
Atazanavir inhibited UGT1A1-mediated formation of estradiol 3-O-glucuronide with an IC_{50} of 0.98 μM. Dapagliflozin inhibited -mediated formation of estradiol 3-O-glucuronide with an IC_{50} of >50 μM (see sponsor's table below). Therefore dapagliflozin minimally inhibits UGT1A1.

Table 7. IC_{50} for Dapagliflozin-mediated inhibition of UGT1A1 (sponsor's table)

Compound	Conc. Range (μM)	Run #1 IC_{50} (μM)	Run#2 IC_{50} (μM)	Mean IC_{50} (μM)
Atazanavir (UGT1A1 inhibitor)	0-50	1.25	0.7	0.98
Dapagliflozin (BMS-512148)	0-50	>50	>50	>50

Evaluation of the Potential for Saxagliptin (BMS-477118) and its Major Metabolite, BMS-510849, to Inhibit UGT1A9 in Human Liver Microsome Incubations Using Dapagliflozin or Propofol as Substrates (Study# 930059456, non-GLP)

Reviewer note: Study# 930059456 was also submitted to the IND for saxagliptin (IND 63,634) and was fully reviewed by Dr. Alavi (03.05.2013).

Summary
The anti-diabetic medication saxagliptin (BMS-477118) is an approved dipeptidyl peptidase 4 (DPP4) inhibitor that is metabolized by CYP3A4 to form the pharmacologically active metabolite BMS-510849. The potential for saxagliptin and BMS-510849 (0 - 50 μM) to interact with the metabolism of dapagliflozin (0.5 or 5 μM) was evaluated in human liver microsomes. Dapagliflozin is glucuronidated by UGT1A9

to form dapagliflozin 3-0-glucuronide, which is the predominant human metabolite and is pharmacologically inactive. Dapagliflozin and propofol (144 μM) were used as the UGT1A9 substrates and niflumic acid (0 - 5 μM) was used as the positive control inhibitor of UGT1A9.

With dapagliflozin at 0.5 and 5 μM, the IC_{50} for niflumic acid was 0.21 and 0.185 μM. In contrast, saxagliptin and BMS-510849 inhibited dapagliflozin-mediated UGT1A9 metabolism with IC50's of >50 μM, respectively. Therefore drug-drug interactions of dapagliflozin and saxagliptin or BMS-510849 seem unlikely.

Inhibition of CYP Enzymes Activity in Human Liver Microsomes (Study# 930053153, non-GLP)

Summary

The sponsor has determined the IC_{50}s for inhibition of CYP450 enzyme metabolism of probe specific substrates in human liver microsomes (HLM) with dapagliflozin at up to 40 μM. Dapagliflozin was found to have IC_{50} at greater than 40 μM in each case as shown in the sponsor's table below:

Table 8. Dapagliflozin IC_{50} Against CYP450 Enzymes (sponsor's table)

CYP Enzyme	CYP Activity	IC50 (μM) [a]	
		No Preincubation	After 30-minute Preincubation
CYP1A2	Phenacetin *O*-deethylation	> 40 (-1)	> 40 (1)
CYP2B6	Bupropion hydroxylation	> 40 (20)	> 40 (18)
CYP2C8	Amodiaquine *N*-deethylation	> 40 (10)	> 40 (9)
CYP2C9	Diclofenac 4'-hydroxylation	> 40 (6)	> 40 (5)
CYP2C19	S-Mephenytoin 4'-hydroxylation	> 40 (10)	> 40 (10)
CYP2D6	Dextromethorphan *O*-demethylation	> 40 (0)	> 40 (8)
CYP3A4	Midazolam 1'-hydroxylation	> 40 (-5)	> 40 (-14)
CYP3A4	Testosterone 6β-hydroxylation	> 40 (3)	> 40 (4)

a When IC50 is > 40 μM, mean value of percent inhibition observed at 40 μM is provided in parenthesis.

The C_{max} of dapagliflozin is 0.27 μM, therefore dapagliflozin is likely to minimally inhibit CYP450 enzymes in vivo.

In Vitro Assessment of the Role of Renal and Hepatic Uptake Transporters in Dapagliflozin (BMS-512148) Disposition (Study# 930053412, non-GLP)

Summary
HEK-293 cells or MDCK cells were stably transfected with cDNA coding for human (h) OCT2, OAT1, OAT3, OATP1B1, OATP1B3 or mock constructs to determine if [^{3}H]dapagliflozin (5 nM or 1 μM) is a substrate for these transporters or an inhibitor of probe substrates for each transporter. Known inhibitors of each transporter were used as positive controls.

Dapagliflozin was not a substrate for human OCT2, OAT1, OATP1B1 or OATP1B3; but was a substrate of human OAT3 (results not shown). Dapagliflozin also only inhibited hepatic OATP1B1 and OATP1B3 with IC_{50} values of 69 and 8 μM, respectively (see sponsor's table below). The C_{max} of dapagliflozin is 0.27 μM, therefore dapagliflozin is likely to minimally inhibit these transporters.

Table 9. IC_{50} for Hepatic OATP1B1 and OATP1B3 Inhibition by Dapagliflozin (sponsor's table).

Transporter	IC50 (μM)	Substrate
hOATP1B1	69.3 ± 7.5	[^{3}H]E17βG
hOATP1B3	8.0 ± 5.2	[^{3}H]CCK-8

10 Special Toxicology Studies

Phenotyping Study of 15-Month Old Homozygous SGLT2 Deficient Mice and Wild-Type Cohorts (Study# DT11112, non-GLP)

Key Findings
Hyperplastic bladder or bladder neoplasia was not observed in SGLT2 knockout (KO) mice that were evaluated without dapagliflozin treatment, from weaning to 15 months of age. This is consistent with the two year nonclinical carcinogenicity bioassays in mice and rats where pre-neoplastic alterations were also not observed in the bladder. However the current study does not address the role of dapagliflozin in the bladder where pre-existing pre-neoplastic or neoplastic bladder tissue is present.

Method
Thirty-one wild type (WT) (15 male and 16 female C57BL/6J) mice and 35 (23 male and 12 female) SGLT2 knock-out (SGLT2 KO or SGLT2 -/-) mice were weaned at approximately 3 weeks of age and had free access to water and feed (18% protein) for up to 15 months of age. Genotypes were confirmed by qPCR of DNA isolated from toe snips/clipping. Mice were housed at 1-4 animals/cage.

No pharmacologic treatment or intervention was carried out throughout the study. Daily morbidity and mortality checks were carried out with "periodic" body weight measurements and urine glucose measurements (via dipstick). Necropsy was not carried out on found dead mice and cause of death was not established. At 15 months of age, blood was obtained for hematocrit and serum blood chemistry evaluation. Upon euthanasia, gross necropsy and a limited histopathological examination (bladder, kidneys, liver, heart, pancreas, adrenal gland, thyroid, spleen, male and female reproductive glands, skin, brain and skull) was carried out.

Results

Mortality

The sponsor reports that of the 23 male and 12 SGLT2 KO mice, only 20 males and 10 females survived to 15 months of age. However, the sponsor's 15 month terminal body weight table shows 20 male and 11 female SGLT2 KO mice at this time point.

The sponsor reports that of the 15 male and 16 female WT mice, only 14 males and 12 females survived to 15 months of age. The sponsor however shows 15 male and 13 female WT animals at this time point in the 15 month body weight table. Three WT female were euthanized at approximately 13 months age due to ulcerative dermatitis.

Body Weight

At 15 months of age male WT and SGLT2 KO mice body weights were unremarkable (see sponsor's table below). Mean body weight was reduced 10% in the SGLT2 KO females.

Table 10. 15 Month Terminal Body Weight in Untreated SGLT2 KO and WT Mice (sponsor's table)

Genotype	Number of Animals		Body Weight (g)[b]	
	Male	Female	Male	Female
SGLT2 KO[c]	20	11	40.5 ± 6.6[d]	33.1 ± 5.7
SGLT2 WT[e]	15	13	41.1 ± 7.8	36.7 ± 8.2

[a] Animals were weighed live in the non-fasted state immediately prior to terminal anesthesia.

[b] Mean ± standard deviation.

[c] SGLT2 KO animals were glycosuric.

[d] Includes animal no. 1220 with a low body weight (23.5 g) attributed to renal insufficiency resulting fr chronic polycystic kidney disease.

[e] SGLT2 WT animals were non-glycosuric.

Hematology
At 15 months of age hematocrit was unremarkable in male and female WT and SGLT2 KO mice (see sponsor's table below)

Table 11. Fifteen Month Hematocrit in Untreated SGLT2 KO and WT Mice (sponsor's table)

Genotype	Number of Animals		Hematocrit (%)[b]	
	Male	Female	Male	Female
SGLT2 KO	20	11	44.3 ± 3.9[c]	47.8 ± 3.6
SGLT2 WT	13[d]	13	45.5 ± 3.3	46.2 ± 2.6

[a] Orbital sinus blood samples.
[b] Mean ± standard deviation.
[c] Includes animal no. 1220 with a low hematocrit (32%) attributed to renal insufficiency resulting from chronic polycystic kidney disease.
[d] Blood samples were missing from animal nos. 1150 and 1213.

Clinical Blood Chemistry
Glucose
The sponsor states that serum glucose was statistically significantly increased (10%) in the male SGLT2 KO mice. However, statistical analysis was not submitted by the sponsor. Serum glucose was unremarkable in female WT and SGLT2 KO mice (see sponsor's table below)

Table 12. Fifteen Month Glucose in Untreated SGLT2 KO and WT Mice (sponsor's table)

Genotype	Number of Animals		Serum Glucose (mg/dL)[b]	
	Male	Female	Male	Female
SGLT2 KO	20	10	261 ± 39	262 ± 36
SGLT2 WT	14	12	237 ± 40	264 ± 50

[a] Heart blood samples from non-fasted animals.
[b] Mean ± standard deviation.

Serum Creatinine and Phosphorus
Serum creatinine was statistically significantly increased (19%) in the male SGLT2 KO mice (see sponsor's table below). Serum phosphorus was statistically significantly increased (23%) in the female SGLT2 KO mice (see sponsor's table below). However, statistical analysis was not provided by the sponsor. Serum creatinine was unremarkable in the female SGLT2 KO mice. Serum phosphorus was unremarkable in

the SGLT2 KO males. Creatinine and phosphorus were, however, within the standard deviation for each variable and are considered within biological variability

Table 13. Fifteen Month Urea Nitrogen and Creatinine in Untreated SGLT2 KO and WT Mice (sponsor's table)

Genotype	Number of Animals		Urea Nitrogen (mg/dL)[b]		Creatinine (mg/dL)[b]	
	Male	Female	Male	Female	Male	Female
SGLT2 KO	20	10	31 ± 25[c]	24 ± 4	0.19 ± 0.06[d]	0.16 ± 0.04
SGLT2 WT	14	12	27 ± 4	26 ± 5	0.16 ± 0.03	0.16 ± 0.03

[a] Heart blood samples from non-fasted animals.

[b] Mean ± standard deviation.

[c] Includes animal no. 1220 with a high urea nitrogen level (134 mg/dL) attributed to renal insufficiency resulting from chronic polycystic kidney disease.

[d] Includes animal no. 1220 with a high creatinine level (0.42 mg/dL) attributed to renal insufficiency resulting from chronic polycystic kidney disease.

Table 14. Fifteen Month Phosphorus and FGF23 in Untreated SGLT2 KO and WT Mice (sponsor's table)

Genotype	Number of Animals (P/FGF23)		Phosphorous (mg/dL)[b]		FGF23 (pg/mL)[b]	
	Male	Female	Male	Female	Male	Female
SGLT2 KO	20/19	10/11	7.8 ± 0.8	8.8 ± 1.4	424 ± 323[c]	746± 745
SGLT2 WT	14/15	12/13	7.6 ± 1.7	7.2 ± 1.0	527 ± 413	370 ± 170

[a] Heart blood samples from non-fasted animals.

[b] Mean ± standard deviation.

[c] Excludes animal no. 1220 with a high FGF level (> 4724 pg/mL) attributed to renal insufficiency resulting from chronic polycystic kidney disease.

Serum Urea Nitrogen/FGF23/AST and ALT

Serum urea nitrogen and FGF23 were unremarkable in male and female WT and SGLT2 KO mice (see sponsor's tables above). AST and ALT were unremarkable in male and female WT and SGLT2 KO mice (results not shown).

Serum Calcium and Albumin

Serum calcium and albumin were unremarkable in male and female WT and SGLT2 KO mice (see sponsor's table below).

Table 15. Fifteen Month Serum Calcium and Albumin in Untreated SGLT2 KO and WT Mice (sponsor's table)

Genotype	Number of Animals		Calcium (mg/dL)[b]		Albumin (g/dL)[b]	
	Male	Female	Male	Female	Male	Female
SGLT2 KO	20	10	9.8 ± 0.4[c]	9.5 ± 0.3	3.2 ± 0.2	3.0 ± 0.2
SGLT2 WT	14	12	9.7 ± 0.2	9.5 ± 0.2	3.2 ± 0.2	3.1 ± 0.2

[a] Heart blood samples from non-fasted animals.

[b] Mean ± standard deviation.

[c] Includes animal no. 1220 with a high calcium level (11.2 mg/dL) attributed to renal insufficiency resulting from chronic polycystic kidney disease.

Urinary Glucose

As expected all SGLT2 KO mice had marked glucosuria (2000 mg/dL) except male #1220 which was found to with congenital polycystic kidney disease (urinary glucose at 500 mg/dL) (results not shown). All WT mice did not show glucosuria (results not shown)

Histopathology

The sponsor states that tissue histopathology was unremarkable. However, a pathology report was absent from the study report.

Urinary Bladder Histopathology

Hyperplasia and neoplasia were not observed in the urinary bladder of SGLT2 KO mice (see sponsor's table below)

Table 16. Fifteen Month Urinary Bladder Histopathology (sponsor's table)

Genotype	Number of Animals		Histopathologic Finding[a]			
	Male	Female	Lymphoid cell aggregates		Inflammation	
			Male	Female	Male	Female
SGLT2 KO	20	11	1	3	1[b]	0
SGLT2 WT	15	12	1	7	0	0

[a] Number of animals observed with histopathologic finding. Proliferative changes, i.e. hyperplasia or neoplasia, were not observed in the mucosa. Original histopathology findings are retained BMS electronic lab notebook no. 77643-30.

[b] Animal 1220 also had chronic polycystic kidney disease and renal inflammation.

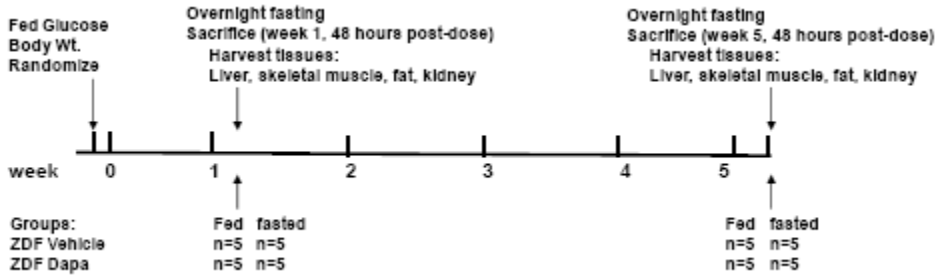
Transcriptional Profiling Analyses of Male ZDF Rat Tissues Following 1 Week and 5 Weeks of Dapagliflozin Treatments (Study# 930052593, non-GLP)

Reviewer note: In this study, dapagliflozin at 0.5 mg/kg for a maximum duration of 5 weeks is a low dose and a very short duration in comparison to a lifetime exposure (carcinogenicity) studies, carried out in mice and rats. Therefore the tumor promoter gene expression changes in the present study are of limited value for assessment of tumor formation. More importantly an imbalance of bladder cancer was observed in the clinical trials. Unfortunately, the bladder was not one of the tissues harvested in the present study and this study offers marginal value to the role of gene expression changes involved in tumor promotion. Consequently, only a summary of pertinent results are reviewed here.

Method

Seven week old male ZDF rats were treated daily with 0 (vehicle) or 0.5 mg/kg dapagliflozin for 5 weeks (20 males/group). At one week of treatment and 48 hours post-dose, 5 fed males/group were sacrificed and 5 males/group were fasted overnight prior to sacrifice. A similar fed/fasted and sacrifice regimen was conducted at the end of 5 weeks of treatment (see sponsor's figure below). At sacrifice the liver, skeletal muscle, fat and kidney were harvested from each animal for RNA isolation and transcriptional profiling using microarrays or qRT-PCR.

Figure 6. Male ZDF Rat Study Design (sponsor's figure)



Results

Very small changes and numbers of genes (probesets) were observed in male ZDF rats following dapagliflozin treatment for 1 and 5 weeks (0.3% and 1%, respectively), with the liver showing the most response to dapagliflozin treatment under fed or fasted conditions (see sponsor's tables below).

Table 17. Number of Significantly Changed (Gene) Probesets Following One Week of Dapagliflozin Treatment (sponsor's table)

	Fasted		Fed		
	Up-regulated	Down-regulated	Up-regulated	Down-regulated	Total probesets [a]
Liver	3 [b] (0.015%) [c]	10 (0.05%)	13(0.06%)	2 (0.001%)	20271
Kidney	12 (0.05%)	4 (0.016%)	61 (0.25%)	15 (0.06%)	24527
Skeletal Muscle	8 (0.036%)	12 (0.05%)	28 (0.13%)	2 (0.01%)	22079
Adipose	26 (0.08%)	33 (0.11%)	23 (0.075%)	58 (0.19%)	30625

[a] Total number of eligible probesets that have a maximum signal intensity of no less than 12 across experimental groups

[b] Number significantly changed probesets (defined as having a p-value of less than 0.005 and a fold change of greater than 1.5 or less than -1.5 when comparing dapagliflozin treatment group over vehicle group

[c] Percentage is based on number of significantly changed probesets over the number of total eligible probesets

Table 18. Number of Significantly Changed (Gene) Probesets Following Five Weeks of Dapagliflozin Treatment (sponsor's table)

	Fasted		Fed		
	Up-regulated	Down-regulated	Up-regulated	Down-regulated	Total probesets [a]
Liver	71[b](0.36%)[c]	111 (0.56%)	161 (0.81%)	113 (0.57%)	19916
Kidney	26 (0.11%)	21 (0.09%)	27 (0.11%)	31 (0.13%)	24069
Skeletal Muscle	37 (0.16%)	4 (0.02%)	37 (0.16%)	13 (0.06%)	22447
Adipose	45 (0.15%)	4 (0.01%)	45 (0.15%)	55 (0.18%)	30303

5 weeks of dapagliflozin treatment compared to vehicle treatment

[a] Total number of eligible probesets that have a maximum signal intensity of no less than 12 across experimental groups

[b] Number significantly changed probesets (defined as having a p-value of less than 0.005 and a fold change of greater than 1.5 or less than -1.5 when comparing dapagliflozin treatment group over vehicle group

[c] Percentage is based on number of significantly changed probesets over the number of total eligible probesets

The sponsor evaluated the liver, kidney, fat and skeletal muscle for genes involved in cell cycle regulation and tumor promotion. Only one tumor promoting gene (stathmin 1)[#] was upregulated (58%) in adipose tissue following 5 weeks of dapagliflozin treatment (results not shown). Therefore treatment of male ZDF rats with dapagliflozin at 0.5 mg/kg for 5 weeks, minimally changes tumor promoting genes in the tissues examined.

[#]Maeshima H et al,: Toxicology In Vitro: 23: 148-157 (2009)
[#]Maeshima H et al,: Toxicology In Vitro: 24: 995-1001 (2010)

Effects of Dapagliflozin and its Primary 3-O-Glucuronide Metabolite on Human Bladder Tumor Cell Growth in Culture (Study# 930066037, non-GLP)

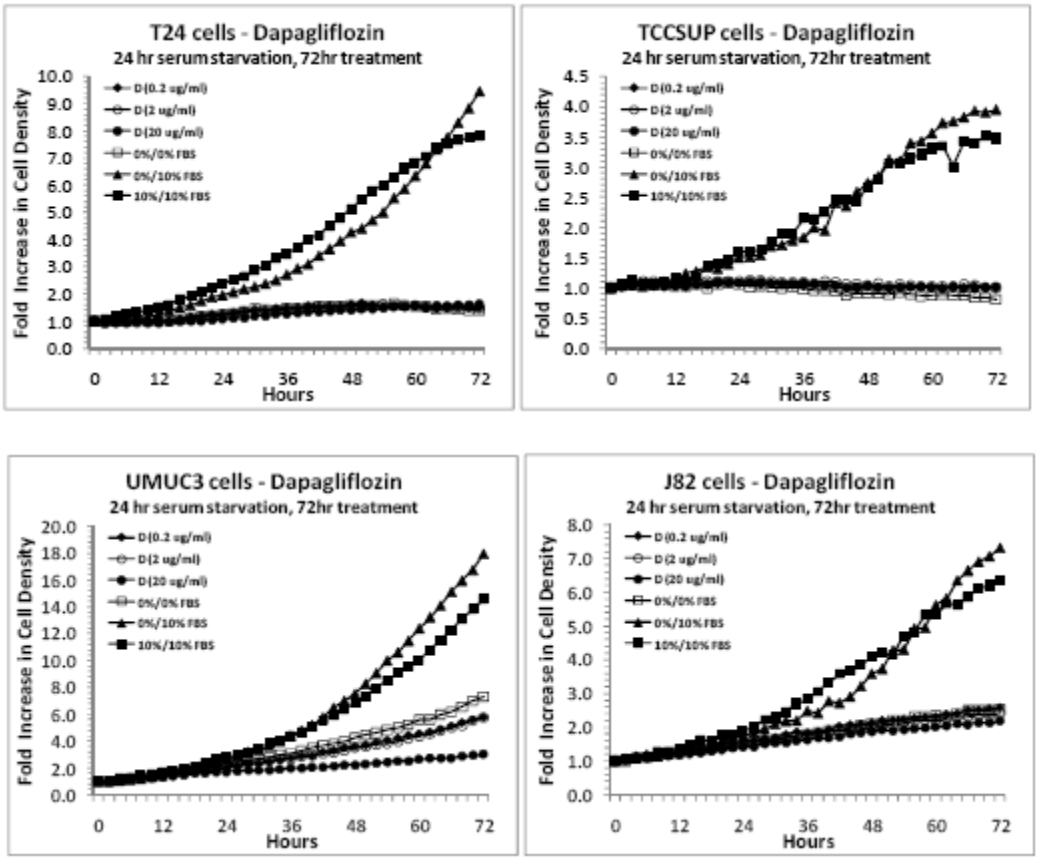
Method

Six human bladder transitional cell carcinoma (TCC) cell lines (T24, TCCSUP, UM-UC-3, J82, SW780 and EJ-1) at 20, 000 or 40, 000 cells/well were treated with dapagliflozin or dapagliflozin 3-O-glucuronide (0, 0.2, 2 or 20 μg/mL) in vitro for 72 hr in 12 well plates. The cell lines were allowed to attach for 24 hr and then cultured in serum-free media for an additional 24 hr, prior to treatment with dapagliflozin or dapagliflozin 3-O-glucuronide. As a positive control some cell lines were cultured in medium alone with 10% FBS. TCC cell line growth was analyzed in real time using an IncuCyte™ live cell imaging system (microscope with digital image scanner) every 2 hr for 72 hr without removing the culture plate from the incubator. The IncuCyte™ cell proliferation assay software was used to determine TCC cell line growth. The EJ-1 cell line was evaluated in duplicate and all other TCC cell lines were evaluated in triplicate. **Reviewer note:** this analysis presumes that the correct TCC cell density was used.

Results

Dapagliflozin or dapagliflozin 3 O-glucuronide at 0.2-20 μg/mL did not promote the growth the human bladder TCC cell lines (see sponsor's figures below).

Figure 7. Effect of Dapagliflozin on Human Bladder TCC Growth In Vitro (sponsor's figure)



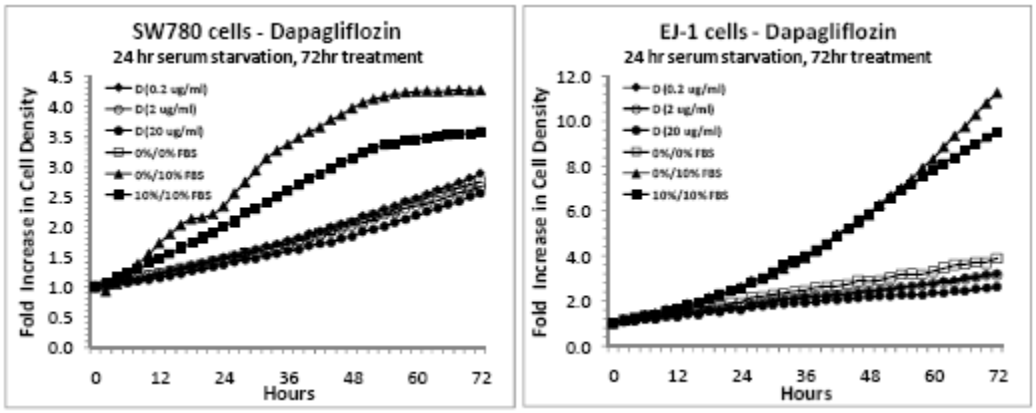
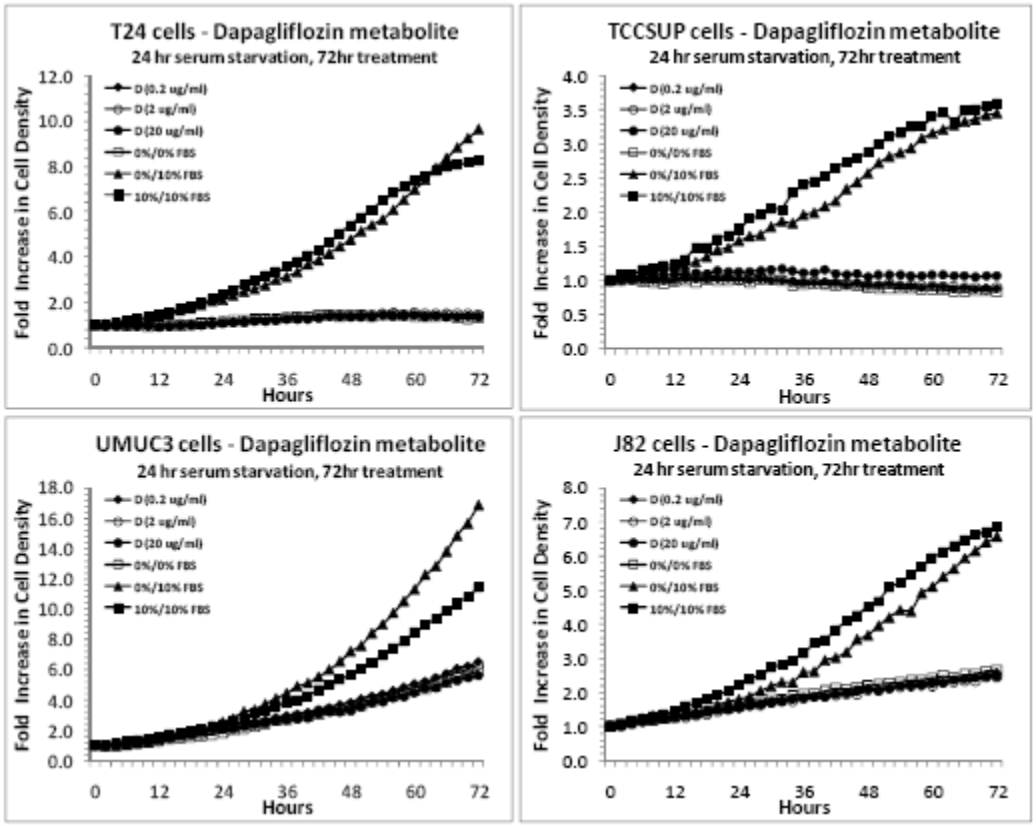
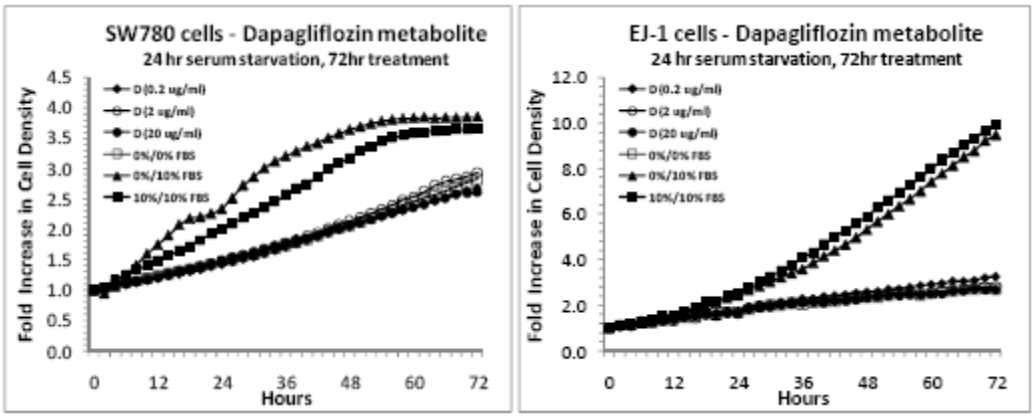


Figure 8. Effect of Dapagliflozin-3-O-glucuronide on Human Bladder TCC Growth In Vitro (sponsor's figure)





Effect of Glucose Concentration on Growth of Bladder Cancer Cell Lines in Culture (Study# 930053120, non-GLP)

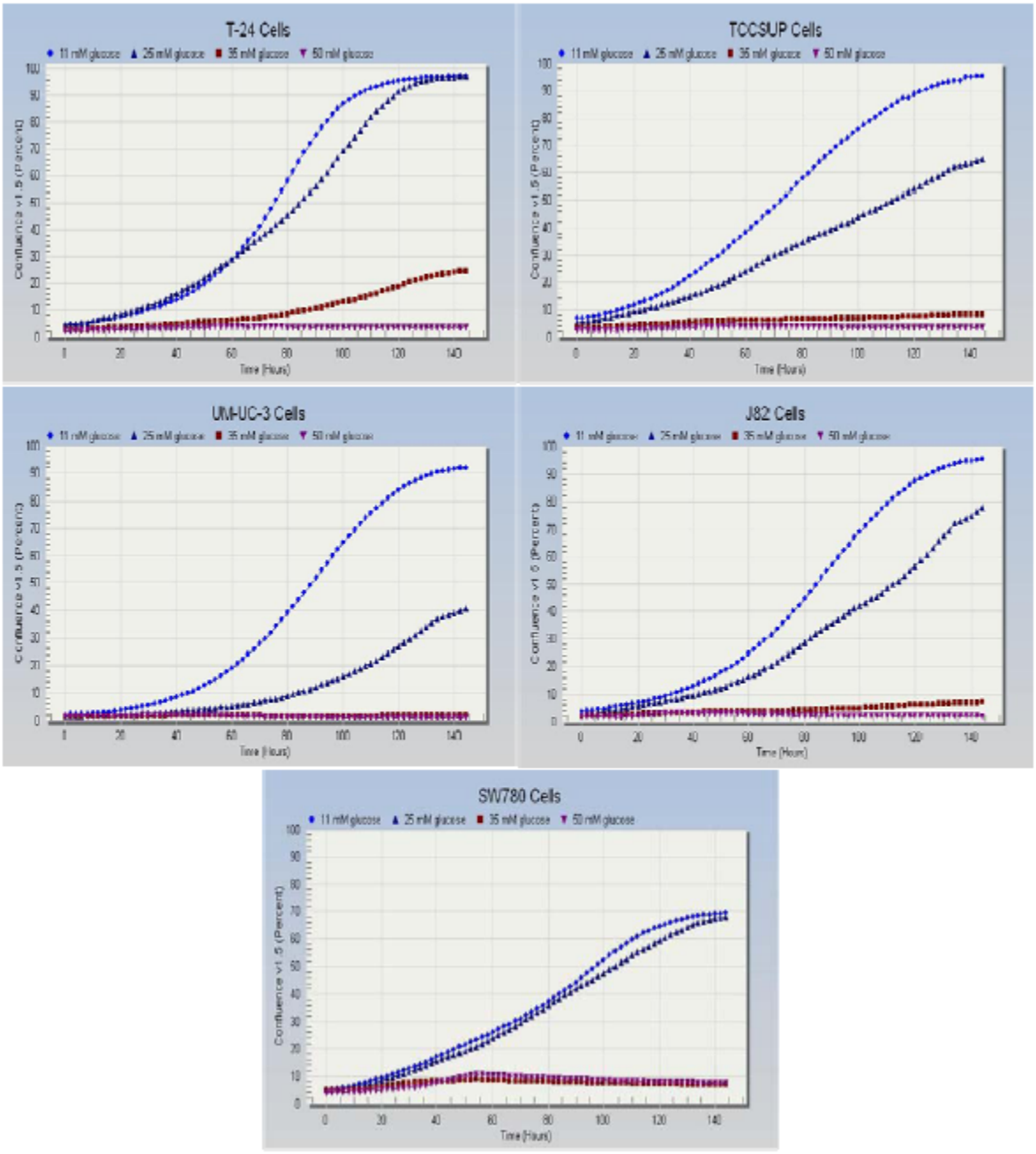
Method

Bladder transitional cell carcinoma (TCC) cell lines (T-24, TCCSUP, UM-UC-3, J82, SW780 and RT4) were seeded at 40,000 or 80, cells per well in 12 well plates using RPMI 1640 medium with glucose concentration adjusted to 11, 25, 35 or 50 mM, respectively. TCC cell line growth was analyzed in real time using an IncuCyte™ live cell imaging system (microscope with digital image scanner) every 2 hr for 145 hr. The IncuCyte™ cell proliferation assay software was used to determine TCC cell line growth.

Results

Each TCC cell line grew well with a glucose concentration of 11 mM reaching 70% confluence by 145 hr. Increasing the glucose concentration to 25 mM had no appreciable effect on growth in the T-24 and SW780 TCC cell lines and reduced growth in all other TCC cell lines (see sponsor's figure below). Increasing the glucose concentration to 35 or 50 mM visibly reduced growth in all TCC cell lines (see sponsor's figure below).

Figure 9. Effect of Glucose Concentration on Human Bladder TCC Growth In Vitro



Effects of Dapagliflozin on the Growth of Human Bladder Cancer Xenografts in Nude Mice (Study# 930067105, non-GLP)

Method

The effects of dapagliflozin on the in vivo growth of two human bladder transitional cell carcinoma (TCC) cell lines (EJ-1 and UMUC3, respectively) was examined in male and female nude mice (Nu/Nu) (8/sex/group). Nu/Nu mice were given a subcutaneous implant of tumor fragment (approx. 20-30 mg) with a 13-gauge trocar. Tumors were allowed to grow to approximately 100 mg prior to dapagliflozin treatment. Tumors were measured using a caliper twice weekly until a "target" size of 1 g was reached. Each animal was weighed prior to treatment and again following the last dapagliflozin treatment. The sponsor estimated tumor weight (mg) with the following formula:

$$\text{Tumor weight} = (\text{length} \times \text{width}^2) \div 2$$

The sponsor calculated tumor growth inhibition was calculated using the following formula:

$$\%\ \text{Tumor Growth Inhibition} = \frac{\left(1 - \frac{T_t}{T_0} * \frac{C_0}{C_t}\right)}{\left(1 - \frac{C_0}{C_t}\right)} \quad \text{where,}$$

C_t = Median control tumor size at end of treatment

C_0 = Median control tumor size at treatment initiation

T_t = Median tumor size of treated group at end of treatment

T_0 = Median tumor size of treated group at treatment initiation

Male and female Nu/Nu mice were treated to daily oral dose of dapagliflozin (in 90% PEG400 and 10% water, 10 mL/kg) for 2 weeks (EJ-1 tumor) or until the average group tumor burden reached approximately 1 g (UMUC3) (11 days) as follows (sponsor's tables):

Table 19. Treatment Schedule for Nu/Nu Mice + EJ-1 Tumor

Group	No. of animals / Sex	Treatment	Dose (mg/kg)	Schedule
1	8 / F	Vehicle Control	0	QDx14
2	8 / F	dapagliflozin	4	QDx14
3	8 / F	dapagliflozin	20	QDx14
4	8 / M	Vehicle Control	0	QDx14
5	8 / M	dapagliflozin	12	QDx14
6	8 / M	dapagliflozin	60	QDx14

Table 20. Treatment Schedule for Nu/Nu Mice + UMUC3 Tumor

Group	No. of animals / Sex	Treatment	Dose (mg/kg)	Schedule
1	8 / F	Vehicle Control	0	QDx11
2	8 / F	dapagliflozin	4	QDx11
3	8 / F	dapagliflozin	20	QDx11
4	8 / M	Vehicle Control	0	QDx11
5	8 / M	dapagliflozin	12	QDx11
6	8 / M	dapagliflozin	60	QDx11

Blood was collected at 1, 3 and 6 hr post-dose on day 4 for the UMUC3 tumor bearing mice and at day 5 for the EJ-1 tumor bearing mice, respectively, for plasma PK analysis of dapagliflozin (BMS-521148) or its metabolite BMS-801576 (3-O-glucuronide) using LC/MS **(reported in study# NCPK 39 in PK/ADME section 5.1 above).**

The presence of glucose in the urine using urine dipsticks, was determined at day 4 post-dose for the UMUC3 tumor bearing mice and at day 5 post-dose for the EJ-1 tumor bearing mice, respectively, as a measure of dapagliflozin pharmacodynamic activity. The caudal abdomen of each mouse was depressed until a drop of urine was expressed and then dropped on to the urine test strip.

Results

Dapagliflozin treatment in the female Nu/Nu mice at 4 and 20 mg/kg represents exposure multiples of 6x and 37x MRHD in the EJ-1 tumor bearing mice and 7x and 30x MRHD in the UMUC3 tumor bearing mice, respectively (see sponsor's table below). In addition, dapagliflozin treatment in the male Nu/Nu mice at 12 and 60 mg/kg represents exposure multiples of 12x and 75x MRHD in the EJ-1 tumor bearing mice and 8x and 49x MRHD in the UMUC3 tumor bearing mice, respectively (see sponsor's table below).

Table 21. Exposure PK Following Dapagliflozin Treatment in Tumor-bearing Mice (sponsor's table)

Summary of exposures to dapagliflozin and its primary human 3-O-Glucuronide Metabolite achieved in tumor-bearing mice								
	EJ-1 Model				UMUC3 Model			
	Low Dose		High Dose		Low Dose		High Dose	
	Female	Male	Female	Male	Female	Male	Female	Male
Dose (mg/kg/day)	4	12	20	60	4	12	20	60
Dapagliflozin AUC0-24hr (ng·h/mL)	2830	5545	17241	34799	3386	3918	14068	22884
Dapagliflozin Margin*	6.1x	12x	37x	75x	7.2x	8.4x	30x	49x
3-O-Gluc AUC0-24hr (ng·h/mL)	49	133	323	719	87	85	328	371
3-O-Gluc Margin*	0.1x	0.2x	0.4x	0.9x	0.1x	0.1x	0.4x	0.4x

* Exposure margins were calculated in relation to the human AUC values at the maximum recommended human dose (10 mg): dapagliflozin, 465 ng·h/mL; 3-O-Gluc metabolite, 837 ng·h/mL.

Exposure to the dapagliflozin 3-O-glucuronide was 38-61-fold lower relative to dapagliflozin exposure (see sponsor's table above).

Glucosuria was present in all dapagliflozin-treated xenograft tumor bearing mice, except control animals (see sponsor's tables below)

Table 22. Glucosuria in EJ-1 Tumor-bearing Nu/Nu Mice (sponsor's table)

EJ-1 (Female Mice)

Control	Neg - 0	100mg/dL	250mg/dL	500mg/dL	1000mg/dL	2000+mg/dL
Mouse #1	x					
Mouse #2	x					
Mouse #3	x					
Mouse #4	x					
Mouse #5	x					
Mouse #6	x					
Mouse #7	x					
Mouse #8	x					

20 mg/kg	Neg - 0	100mg/dL	250mg/dL	500mg/dL	1000mg/dL	2000+mg/dL
Mouse #1						x
Mouse #2						x
Mouse #3						x
Mouse #4						x
Mouse #5						x
Mouse #6						x
Mouse #7						x
Mouse #8						x

4 mg/kg	Neg - 0	100mg/dL	250mg/dL	500mg/dL	1000mg/dL	2000+mg/dL
Mouse #1						x
Mouse #2						x
Mouse #3						x
Mouse #4						x
Mouse #5						x
Mouse #6						x
Mouse #7						x
Mouse #8						x

EJ-1 (Male Mice)

Control	Neg - 0	100mg/dL	250mg/dL	500mg/dL	1000mg/d	2000+mg/dL
Mouse #1	ND					
Mouse #2	x					
Mouse #3	ND					
Mouse #4	x					
Mouse #5	x					
Mouse #6	x					
Mouse #7	x					
Mouse #8	ND					

60 mg/kg	Neg - 0	100mg/dL	250mg/dL	500mg/dL	1000mg/d	2000+mg/dL
Mouse #1						x
Mouse #2						x
Mouse #3						x
Mouse #4						x
Mouse #5						x
Mouse #6						x
Mouse #7						x
Mouse #8						x

12 mg/kg	Neg - 0	100mg/dL	250mg/dL	500mg/dL	1000mg/d	2000+mg/dL
Mouse #1						x
Mouse #2						x
Mouse #3					x	
Mouse #4						x
Mouse #5						x
Mouse #6					x	
Mouse #7						x
Mouse #8					x	

ND=not done

Table 23. Glucosuria in UMUC3 Tumor-bearing Nu/Nu Mice (sponsor's table)

UMUC3 (Female Mice)

Control	Neg - 0	100mg/dL	250mg/dL	500mg/dL	1000mg/dL	2000+mg/dL
Mouse #1	x					
Mouse #2	x					
Mouse #3	x					
Mouse #4	x					
Mouse #5	x					
Mouse #6	x					
Mouse #7	x					
Mouse #8	x					

UMUC3 (Male Mice)

Control	Neg - 0	100mg/dL	250mg/dL	500mg/dL	1000mg/dL	2000+mg/dL
Mouse #1	x					
Mouse #2	x					
Mouse #3	x					
Mouse #4	x					
Mouse #5	x					
Mouse #6	x					
Mouse #7	x					
Mouse #8	x					

20 mg/kg	Neg - 0	100mg/dL	250mg/dL	500mg/dL	1000mg/dL	2000+mg/dL
Mouse #1						x
Mouse #2						x
Mouse #3						x
Mouse #4						x
Mouse #5						x
Mouse #6						x
Mouse #7						x
Mouse #8						x

60 mg/kg	Neg - 0	100mg/dL	250mg/dL	500mg/dL	1000mg/dL	2000+mg/dL
Mouse #1					x	
Mouse #2					x	
Mouse #3						x
Mouse #4						x
Mouse #5						x
Mouse #6						x
Mouse #7						x
Mouse #8						x

4 mg/kg	Neg - 0	100mg/dL	250mg/dL	500mg/dL	1000mg/dL	2000+mg/dL
Mouse #1						x
Mouse #2						x
Mouse #3						x
Mouse #4						x
Mouse #5						x
Mouse #6						x
Mouse #7						x
Mouse #8						x

12 mg/kg	Neg - 0	100mg/dL	250mg/dL	500mg/dL	1000mg/dL	2000+mg/dL
Mouse #1						x
Mouse #2						x
Mouse #3						x
Mouse #4						x
Mouse #5						x
Mouse #6						x
Mouse #7						x
Mouse #8						x

Treatment of EJ-1 tumor bearing Nu/NU mice with dapagliflozin at 4-60 mg/kg/day for 14-days did not result in enhanced tumor growth relative to untreated control mice (see sponsor's figure and table below). Increasing the dose of dapagliflozin increased the calculated tumor growth inhibition and resulted in a reduction in body weight change, except for the EJ-1 tumor bearing females (see sponsor's table below). **Reviewer note:** reduced body weight/body weight gain due to glucosuria is a known pharmacodynamic effect of the SGLT2 inhibitor class. It is likely that reduced body weight may also have inhibited xenograft tumor growth.

Figure 10. Effect of Dapagliflozin on EJ-1 Human Bladder TCC Xenograft Growth in Female (A) and Male (B) Nu/Nu Mice (sponsor's figure)

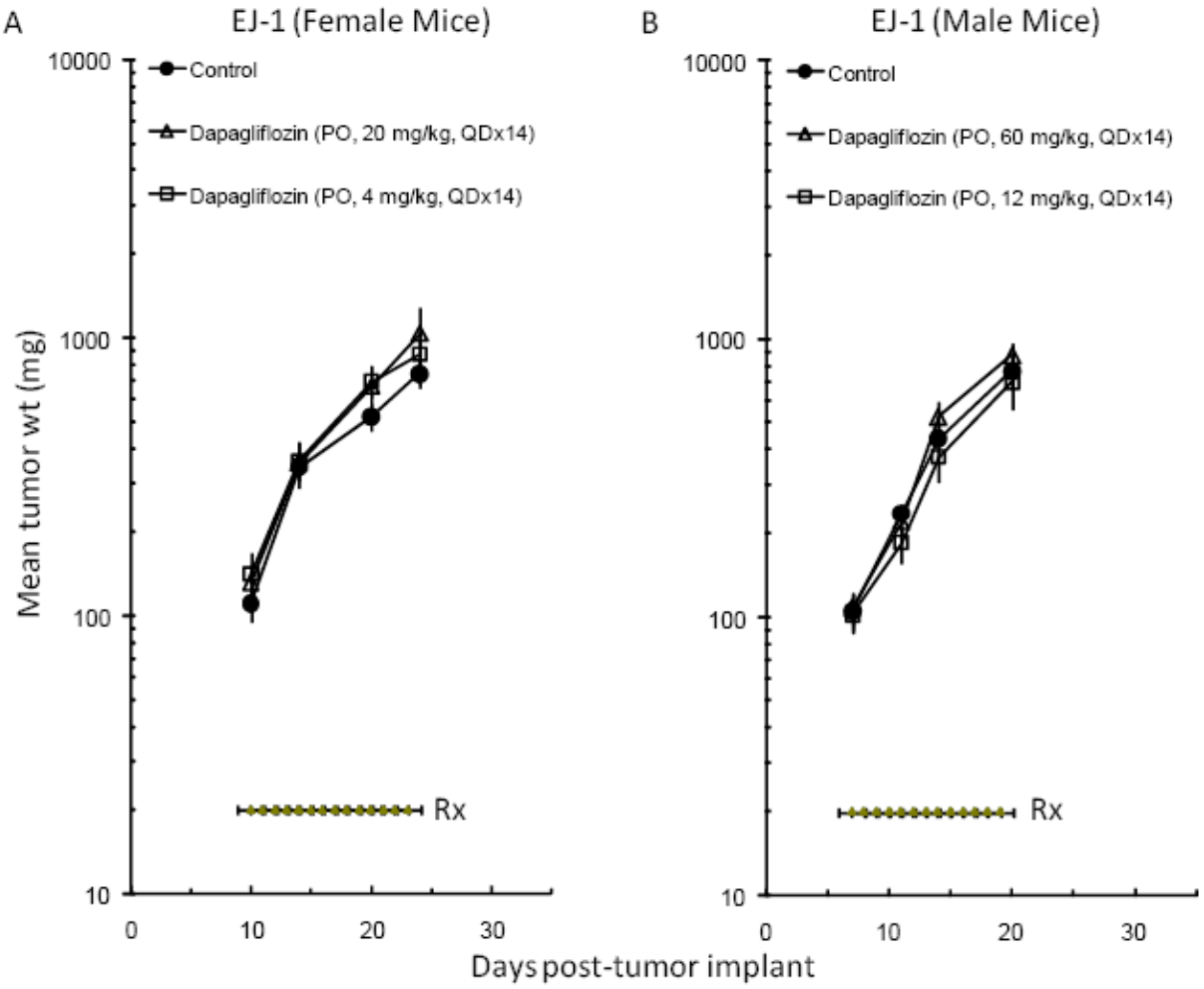


Table 24. Tumor Growth and Body Weight Change in Tumor Bearing Nu/Nu Mice (sponsor's table)

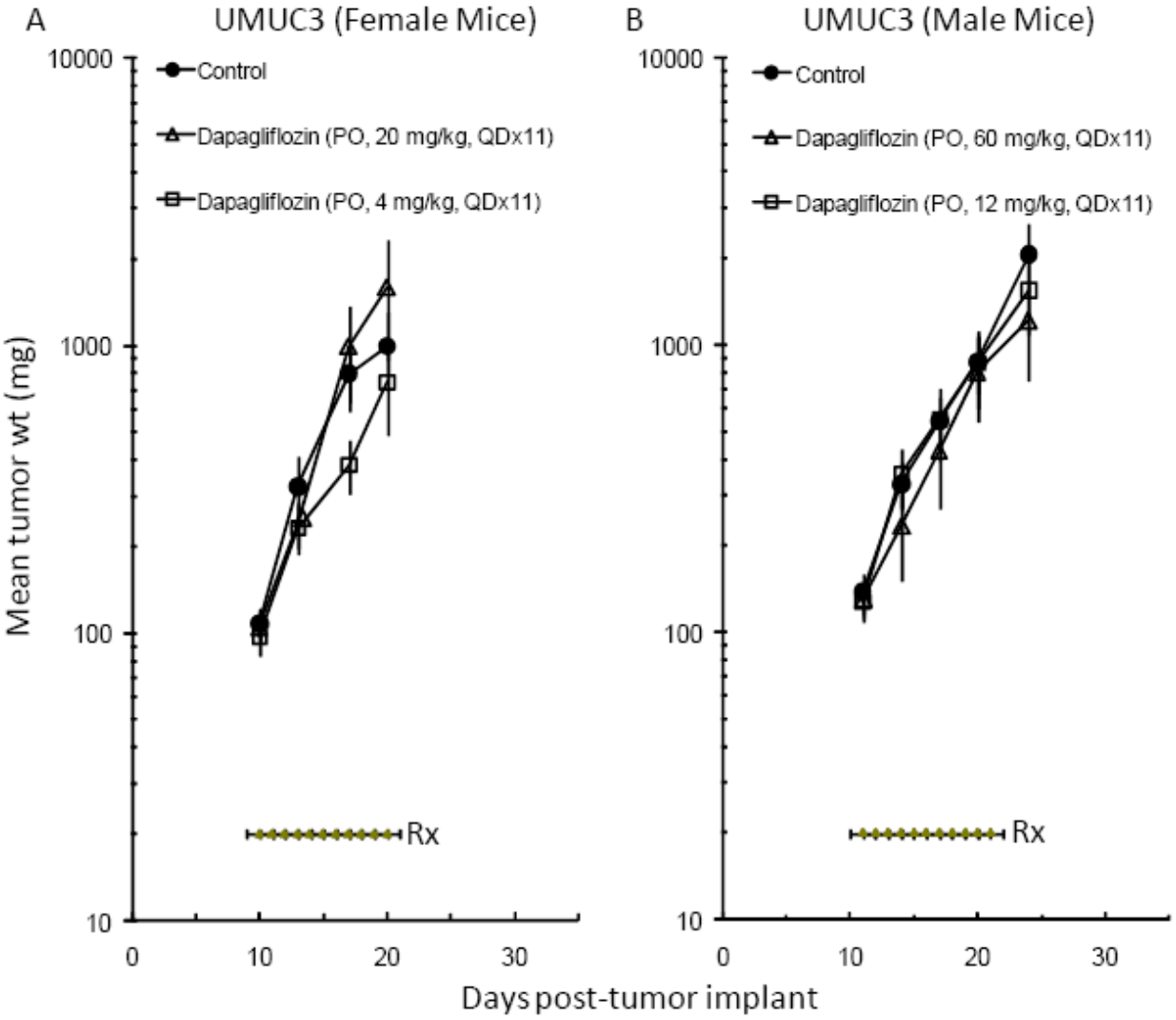
Effects of dapagliflozin on the growth of 2 human bladder transitional cell carcinoma xenografts in male and female nude mice

		Dapagliflozin Treatment		Effects of Dapagliflozin on		
			Dose	Tumor growth*		Body wt.
Tumor	Sex	Route, Schedule	(mg/kg)	%TGI	P values	change (%)
EJ-1	Female	PO, QDx14	20	2	0.7789	2.3
			4	-4	0.2319	1.7
	Male	PO, QDx14	60	-9	0.5994	2
			12	-2	0.6454	4
UMUC3	Female	PO, QDx11	20	10	0.5737	1.4
			4	-23	0.8785	1.8
	Male	PO, QDx11	60	22	0.6454	0.4
			12	-18	0.9591	2.4

* % TGI = % tumor growth inhibition (see Materials and Methods for more details). P values referred to comparison of tumor weight between control and treatment groups

Treatment of UMUC3 tumor bearing Nu/NU mice with dapagliflozin at 4-60 mg/kg/day for 14-days did not result in enhanced tumor growth relative to untreated control mice (see sponsor's figure and table above). Increasing the dose of dapagliflozin increased the calculated tumor growth inhibition and resulted in a reduction in body weight change (see sponsor's table above). **Reviewer note:** reduced body weight/body weight gain due to glucosuria is a known pharmacodynamic effect of the SGLT2 inhibitor class. It is likely that reduced body weight may also have inhibited xenograft tumor growth.

Figure 11. Effect of Dapagliflozin on UMUC3 Human Bladder TCC Xenograft Growth in Female (A) and Male (B) Nu/Nu Mice (sponsor's figure)



11 Integrated Summary and Safety Evaluation

The proposed dapagliflozin film-coated tablet was submitted in accordance with 21 USC 505(b)(1) for the treatment of type 2 diabetes mellitus. The advisory committee for dapagliflozin in the first NDA review cycle was held July 19th 2011 and resulted in a three month extension of the PDUFA date. A Complete Response Letter (CRL) was sent to the sponsor on January 17th 2012. The sponsor submitted NDA 202293 for the second NDA review cycle on July 11th 2013. In the first NDA review cycle there were no nonclinical deficiencies and we recommended approval of NDA 202293. The additional nonclinical data submitted in the sponsor's complete response does not change our recommendation for approval.

Nonclinical studies were primarily conducted to address the CRL action with respect to the clinical safety concerns regarding the numerical imbalance of bladder tumors observed in the phase III clinical trials.

All pivotal nonclinical studies were conducted using oral administration of the drug, which is the clinical exposure route, and in accordance with US FDA GLP regulations (21CFR58) as stated by the sponsor.

Safety margins to expected human exposure were estimated using C_{max} = 136 ng/mL and AUC_{0-24} = 465 ng.h/mL plasma exposure in healthy subjects at the proposed maximum recommended human dose (MRHD) of 10 mg dapagliflozin.

Pharmacology
Dapagliflozin (BMS-512148 or Farxiga™) is a selective inhibitor of sodium glucose co-transporter (SGLT) 2. SGLT2 is selectively expressed in the kidney S1 proximal tubule and is responsible for the renal reabsorption of glucose. Inhibition of SGLT2 by dapagliflozin results in the excretion of glucose thereby producing glucosuria. In *in vitro* studies, dapagliflozin was a potent and selective inhibitor of human (h) SGLT2 relative to the closely related hSGLT1 with a selectivity of 1242-1600-fold. Selectivity for SGLT2 relative to the closely related SGLT1 was also observed for mice (130-fold) and dogs (436-fold), but the selectivity in these species and the rat (207-fold) is much lower compared to humans increasing the likelihood of off-target SGLT1 inhibition in these non-human species at high dapagliflozin exposures.

Treatment of mice lacking a functional renal SGLT2 transporter (SGLT2 knockout) with a single dose of dapagliflozin at approximately 5x MRHD (10 mg) resulted in a SGLT2-independent increase in urinary glucose excretion, suggesting off-target inhibition of SGLT1 in these mice. As expected wild-type (SGLT2 intact) comparator mice produced dose-dependent glucosuria at 0.05 – 5x MRHD. Although, dapagliflozin exposure (AUC) in the wild-type or SGLT2 knockout mice was not evaluated, it is assumed plasma exposure reached the SGLT1 EC_{50} (299 nM) to produce the observed glucosuria in the SGLT2-deficient mice.

PK/ADME
In the definitive study to assess the pharmacokinetics (PK) of 2 weeks of dapagliflozin treatment in the nude mouse xenograft study, exposure (AUC) was approximately dose proportional and the T_{max} ranged from 1-3 hours. The PK of the dapagliflozin glucuronide metabolite showed a similar pattern, but exposure and T_{max} were 39-58-fold lower relative to dapagliflozin. Dapagliflozin exposure in the xenograft study represents pharmacologically active doses ranging from 6-75x MRHD.

Metabolism
Dapagliflozin minimally inhibited UGT1A1. Saxagliptin and its major metabolite (BMS-510849) minimally inhibited dapagliflozin-mediated metabolism by UGTA19. Drug-drug interactions for dapagliflozin with the DPPIV inhibitor saxagliptin and its metabolite BMS-510849 thus appear unlikely.

Dapagliflozin also minimally inhibited multiple cytochrome P450 enzymes with an IC_{50} at > 40 μM. Dapagliflozin is therefore unlikely to inhibit cytochrome P450 enzymes in vivo and thus also unlikely to interact with drugs that are metabolized by these enzymes.

Special Toxicology Studies
Phenotyping of untreated SGLT2 knockout mice and wild type cohorts was carried out from weaning to when the mice were 15 months of age. Mice with the glucosuric phenotype (SGLT2 knockout) were compared to their wild type cohorts with particular interest, in this reviewer's opinion, in evaluation of pre-neoplastic or neoplastic changes for a subset of tissues examined at necropsy or histopathologically and in particular for the bladder. The SGLT2 knockout mice had marked glucosuria and a pharmacodyanmic changes in some clinical chemistry parameters (e.g. creatinine) that were expected due to a lack of glucose. Of interest, pre-neoplastic (hyperplasia) and neoplastic bladder histopathology was not observed in the mice lacking a functional SGLT2 transporter. This was consistent with the two-year rodent bioassays where bladder tumors were absent in dapagliflozin-treated and control (placebo) rats and mice, and only three control rats showed transitional epithelial hyperplasia from a total of 520 rodents combined. However, this phenotyping study did not address the potential of dapagliflozin to promote pre-existing bladder lesions in the in situ microenvironment of changes in urinary volume, flow and composition in the bladder.

Transcriptional profiling of a limited subset of tissues (liver, kidney, fat and skeletal muscle) for genes involved in cell cycle regulation and tumor promotion, in a diabetic animal model (ZDF rats) exposed to a low dose of dapagliflozin (0.5 mg/kg) for 5 weeks, only identified one up-regulated tumor promoter gene in the adipose tissue. Stathmin 1 was increased by 58% and this gene has a critical role in cellular signal transduction in the modulation and control of microtubule polymerization; but has also been found to be highly expressed in a variety of human malignancies[#].

[#]Neumantis J: Expert. Opin. Ther. Targets: 16(7), 631-634 (2012)

However, with regard to the observed numerical imbalance of bladder tumors observed in the clinic, tumor promoter transcriptional changes described by the sponsor were of limited value here, as the bladder tissue was not evaluated in this transcriptional profiling nonclinical study.

Six human bladder transitional cell carcinoma (TCC) cell lines exposed to varying concentrations of dapagliflozin and dapagliflozin-3-O-glucuronide in vitro, did not result in TCC cell line growth. This is consistent with the results from 2-year rodent carcinogenicity studies where bladder tumor formation with dapagliflozin was not observed and adds to the weight of evidence that dapagliflozin is not a direct tumor promoter. A similar in vitro study with TCC cell lines and varying glucose concentrations resulted in TCC cell line growth at glucose concentrations of 11 mM, but inhibition of TCC cell lines at higher glucose concentrations. Tissue culture media is typically supplemented with glucose to mimic normoglycemic conditions observed in vivo (5.5 mM) and glucose concentrations above 10 mM are considered diabetic (Sigma-Aldrich: Glucose in Cell Culture: http://www.sigmaaldrich.com/life-science/cell-culture/learning-center/media-expert/glucose.html). Evaluation of TCC cell lines with dapagliflozin and glucose in isolation, fails to account for the in situ changes in the bladder microenvironment under conditions of dapagliflozin use in nonclinical studies in vivo, or in human clinical trials.

To mitigate this flaw, the sponsor used a tumor promotion xenograft model where human bladder TCC cell lines were subcutaneously implanted in the flank of immnuodeficient (nude) mice. The human bladder tumors were allowed to establish themselves prior to oral treatment at either low (6 - 12x MRHD) or high (30 - 75x MRHD) dapagliflozin or dapagliflozin-3-O-glucuronide (0.1 – 0.9x MRHD) exposures for 11-14 days. Treatment with dapagliflozin or dapagliflozin-3-O-glucuronide did not enhance tumor growth relative to untreated control mice. Again, however, the xenograft model, did not evaluate changes in urinary volume, flow and composition within the microenvironment of the bladder, and therefore cannot assess the promotion of pre-existing bladder tumors with exposure to dapagliflozin or its metabolites.

Two-year rodent bioassays remain the contemporary standard by which the carcinogenic potential of most investigational pharmaceuticals intended for chronic use is addressed, though these studies are not perfect predictors of human cancer risk. An investigational compound that tests negative for neoplasms in the rat and mouse two-year bioassays, particularly at the multiples of clinical exposure achieved with dapagliflozin, is typically viewed as having low or negligible carcinogenic potential for human subjects. The studies conducted by the sponsor add to the weight of evidence that dapagliflozin is not a direct tumor promoter or inducer and that if tumor promotion occurs, it is secondary to changes in urinary volume, flow and composition within the bladder.

The primary deficiency of the xenograft model is that the transplanted human bladder cancer cells were not exposed to the microenvironment of the bladder where changes in urinary volume, flow and composition are taking place under conditions of dapagliflozin

use. Numerous mouse models have been developed to study human cancer and are used to investigate malignancy and metastasis as well as response to therapy. In the human tumor xenograft model in mice, human tumor cells are transplanted (inoculated) under the skin or into the organ type in which the tumor originated into immunocompromised mice, that do not reject human cells. A more appropriate animal model that would allow evaluation of dapagliflozin on transitional cell tumor growth within the bladder would include orthotopic models where tumor cells are implanted into an organ of their origin. Another rodent model for bladder tumors uses 4-hydroxybutyl(butyl)nitrosamine (BBN) as the tumor initiator followed by the test promoter of interest. Both of these models under conditions of oral treatment with dapagliflozin at pharmacologically active doses would more closely resemble the clinical use of dapagliflozin.

In summary, the results of the new nonclinical studies submitted to address the clinical safety concern for the numerical imbalance of bladder tumors in the clinic add to the weight of evidence that dapagliflozin does not act as a carcinogen. However, any putative human bladder risk from dapagliflozin would likely be related to tumor promotion secondary to changes in the microenvironment of the bladder in vivo.

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/s/

MUKESH SUMMAN
12/09/2013

TODD M BOURCIER
12/09/2013
pharm/tox supports approval

Tertiary Pharmacology/Toxicology Review

From: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 202293
Agency receipt date: December 28, 2010
Drug: dapagliflozin
Sponsor: Bristol Myers Squibb/Astrazenca
Indication: Type 2 Diabetes Mellitus treatment

Reviewing Division: Division of Metabolism and Endocrinology Products

Introductory Comments: The pharm/tox reviewer and team leader concluded that the nonclinical data support approval of dapagliflozin for the indication listed above.

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

Dapagliflozin was not teratogenic in rats or rabbits at doses providing large margins of exposure compared to humans. Renal cortical 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, 1 mg/kg, which produced an AUC in the animals about 15 times higher than in humans at the maximum recommended human dose. Dapagliflozin was also found to be present in the milk of lactating rats at identical exposures in a peri- and postnatal study. Consequently, the pharm/tox reviewer and supervisor recommend against using dapagliflozin in pregnant women during the 2nd and 3rd trimester and in nursing women. The pharm/tox supervisor notes in his review that the nonclinical data could support either a Pregnancy category C or X depending on whether or not the risk of the renal developmental effects would be acceptable in some clinical situations.

Carcinogenicity studies of dapagliflozin were conducted in rats and mice. The Executive Carcinogenicity Assessment Committee concurred that both of these studies were adequate and that there were no drug-related neoplasms.

Conclusions:
I agree with the division pharm/tox conclusion that dapagliflozin can be approved from the pharm/tox perspective. Labeling will be finalized separately. I agree that dapagliflozin may carry some risk to developing fetuses and newborns. The final wording for the pregnancy section to address this risk will be developed in conjunction with other disciplines.

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/s/

PAUL C BROWN
01/13/2012

SUPERVISOR MEMO

Date:	22 Aug 2011
RE:	NDA 202293
Sponsors:	Bristol Myers Squibb / AstraZeneca
Drug/Indication	Dapagliflozin (SGLT2 inhibitor) Type 2 diabetes

Bristol Myers Squibb is seeking marketing approval for dapagliflozin, proposed trade name (b) (4) as a treatment option for Type 2 diabetes. Dapagliflozin is a small molecule inhibitor of the Sodium Glucose Co-Transporter 2, a protein preferentially 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 dapagliflozin results in the loss of most filtered glucose to the urine in an amount proportional to the glomerular filtration rate (GFR) and the 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.

Because dapagliflozin would be a first-in-class SGLT2 inhibitor for the treatment of diabetes, certain aspects of its pharmacology merit comment. SGLT2 mediates the active transport of filtered glucose into the proximal epithelium which is 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, dapagliflozin results in the loss of filtered glucose and sodium. This is manifest clinically and in animals by glucosuria/polyuria, dehydration, and caloric loss commensurate with modest loss of body weight. Because efficacy with dapagliflozin 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 dapagliflozin 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. In this regard, SGLT2 inhibition with dapagliflozin distinctly differs from therapeutic targets of other oral anti-diabetic drugs.

Dr. Mukesh Summan, the primary nonclinical reviewer, concludes that the pharmacology and toxicology data support approval of dapagliflozin (10mg q.d.). *I concur with Dr. Summan's assessment.*

Dr. Summan's decision is based on large exposure margins between animal toxicities and clinical exposure at the proposed maximum dose of 10mg/day. Also, the effects in animals observed at clinically relevant drug exposures are reasonably related to the pharmacodynamics of

SGLT2 inhibition and were not considered indicative of tissue toxicity with long term use of dapagliflozin.

The chronic 6 month rat and 12 month dog toxicology studies identified no adverse target organ toxicity at drug exposure exceeding 300-fold the clinical dose. As drug exposure increased still further, the severity of the pharmacodynamic changes resulted in adverse effects including mineralization of multiple tissues, hyperplastic/degenerative histological changes in the kidney, accretion of trabecular bone, and occasional morbidity and mortality. As these adverse effects occur at very high multiples of clinical drug exposure, they are not considered indicative of clinical risk in an adult patient population.

The following issues were identified during review of the dapagliflozin NDA. These issues were included in the FDA's background package for the advisory committee meeting on dapagliflozin held in July 2011, and are summarized here.

Bone Health

Dapagliflozin increased trabecular bone in rats resulting in greater bone mass, density, and strength. This effect occurred at a drug exposure approximately 129-times higher than clinical exposure in the two-year study in rats. The same finding was observed in shorter term toxicology studies but at substantially higher drug levels, suggesting that emergence of this phenomenon is both dose- and time-dependent. Structural changes to the bone were not observed in the dog after one year of dosing at high exposure, though there was evidence of reduced serum (1,25)-dihydroxy vitamin D and reduced urinary deoxypyridinoline at the highest exposure and increased urinary calcium excretion at all doses. Regardless of the change in calcium and bone biomarkers in animals, the safety margin remains quite high to the final clinical dose for the structural change in bone in the most sensitive toxicology species (rats).

Neoplasms/Malignancies

A safety issue discussed at the dapagliflozin public advisory committee was the apparent imbalance of certain malignancies in the clinical trials, specifically an increased incidence of breast and bladder cancers in patients on dapagliflozin compared to placebo. A convincing genotoxic or carcinogenic signal was not observed with dapagliflozin, however, in a series of preclinical studies. Dapagliflozin was not genotoxic in several *in vitro* and *in vivo* studies, though structural damage to chromosomes was observed at high concentrations (≥150ug/ml) in clastogenicity assays. The high drug concentration needed to generate the signal and other issues discussed in Dr. Summan's review support the view that dapagliflozin is unlikely to be genotoxic at clinically relevant doses. Dapagliflozin also did not increase the incidence of any tumor in rats or mice at drug exposures reaching 129x and 70x the clinical dose, respectively, in the 2-year carcinogenicity studies.

Rodent carcinogenicity studies are sensitive assays but are not perfect predictors of human risk. However, an investigational compound that tests 'negative' for neoplasms in the rat and mouse 2-year bioassays, particularly at the multiples of clinical exposure achieved with dapagliflozin, is typically viewed as having low or negligible carcinogenic potential in human subjects. Factors that could confound the adequacy of the studies to detect a tumor signal are not present in this case: notably, rats and mice generate all the metabolites identified in human subjects and in sufficient quantities for an adequate evaluation, expression and function of rodent and human SGLTs 1 and 2 is similar, dapagliflozin is pharmacodynamically active in rodents, and exposure to dapagliflozin in rodents reached 129x and 70x clinical exposure.

The preclinical assessment of carcinogenicity was, at worst, uninformative to understanding the cause of the cancer imbalance in the clinical trials. BMS has argued that the lack of a signal in preclinical studies demonstrates the lack of biological plausibility that dapagliflozin is causally related to the clinical cases of cancer. There are valid points to both views, though in my opinion the latter argument holds greater sway. Given the clarity of the 'clean' tumor outcome in the 2yr animal studies, additional pre-market nonclinical studies would be exploratory and of limited practical use to refining the current risk assessment.

Pregnancy and Lactation

BMS argues that dapagliflozin must not be used during the second and third trimesters of pregnancy or during nursing. Their recommendation is based on adverse findings following drug exposure during the peri/post-natal and juvenile periods in rats. Exposure to dapagliflozin in rats from birth to approximately 13 weeks of age, and especially from post-natal weeks 3-6, results in dilatation of the renal pelvis and tubules and a lower rate of body growth at exposure less than 15-times the clinical dose. A 'no-effect dose' was not identified, so it is likely that exposure causing this adverse effect in rats occurs very near clinical exposure. This susceptible period 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. Dapagliflozin is present in the milk of lactating rats at a ~0.5:1 ratio to plasma and is transferred to weaning pups in sufficient quantities to elicit pharmacodynamic effects (glucosuria/polyuria).

Dr. Summan and I agree that dapagliflozin 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 if the label allows for the use of dapagliflozin during pregnancy based on the risk/benefit for the individual patient and developing fetus. If exposure to dapagliflozin is not acceptable under any risk/benefit scenario, then a contraindication for pregnancy and a Category X are also supported by the preclinical data. Category D is typically used when human experience with a drug indicates fetal risk (e.g., ACE inhibitors), but this is not applicable to dapagliflozin.

A final pregnancy category designation and accompanying language will be settled once comments are received from the medical and maternal health teams on whether dapagliflozin offers sufficient clinical benefits to offset the renal developmental risk identified in the animals.

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/s/

TODD M BOURCIER
08/31/2011
Pharm/tox supports AP

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:	202293
Supporting document/s:	000
Applicant's letter date:	12/27/20101
CDER stamp date:	12/28/2010
Product:	Dapagliflozin (b) (4)
Indication:	Type 2 Diabetes Mellitus
Applicant:	Bristol Myers Squibb/AstraZeneca
Review Division:	Division of Metabolic and Endocrine Products
Reviewer:	Mukesh Summan, PhD, DABT
Supervisor/Team Leader:	Todd Bourcier, PhD
Division Director:	Mary Parks, MD
Project Manager:	Mehreen Hai, PhD

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 202293 are owned by Bristol Myers Squibb/AstraZeneca (BMS/AZ) or are data for which BMS/AZ has obtained a written right of reference. Any information or data necessary for approval of NDA 202293 that BMS/AZ does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 202293.

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1 Executive Summary

1.1 Introduction

1.2 Brief Discussion of Nonclinical Findings

All pivotal nonclinical studies were conducted using oral administration of the drug, which is the clinical exposure route, and in accordance with US FDA GLP regulations (21CFR58) as stated by the sponsor. Safety margins to expected human exposure were estimated using C_{max} = 136 ng/mL and AUC_{0-24} = 465 ng.h/mL plasma exposure in healthy subjects at the proposed maximum recommended human dose (MRHD) of 10 mg dapagliflozin.

Pharmacology
Dapagliflozin (BMS-512148 (b) (4)) is a selective inhibitor of sodium glucose co-transporter (SGLT) 2. SGLT2 is selectively expressed in the kidney S1 proximal tubule and is responsible for the majority (90%) of the renal reabsorption of glucose. Inhibition of SGLT2 by dapagliflozin results in the excretion of glucose thereby producing glucosuria. In in vitro studies dapagliflozin was a potent and selective inhibitor of human (h) SGLT2 relative to the closely related hSGLT1 with a selectivity of 1242-1600-fold. In nonclinical models of diabetes dapagliflozin promoted glucose excretion, polyuria and lowered plasma glucose in diabetic and non-diabetic animal models under conditions of hyperglycemia (oral glucose tolerance test).

Safety pharmacology assessment of cardiovascular, neurological and pulmonary effects of dapagliflozin did not identify significant liabilities.

Absorption, Distribution, Metabolism and Excretion
An oral dose of dapagliflozin was rapidly absorbed and is approximately 80% bioavailable in the rat and dog but only 25% bioavailable in the non-human primate. Dapagliflozin distributes rapidly to most rat tissues with low amounts distributing to the brain and bone. The steady state volume of distribution for dapagliflozin was greater than that of plasma suggestive of extravascular distribution. Dapagliflozin has a longer half-life in humans (13 hours) than in the rat, monkey or dog (3-7 hours), suggestive of different rates of renal elimination. Plasma protein binding was high (91-95%) in humans and in all nonclinical species.

Dapagliflozin undergoes low (10%) oxidative metabolism in vitro by numerous human cytochrome P450 enzymes. The major metabolites in hepatocyte preparations were the glucuronide conjugates and dapagliflozin 3-O-glucuronide was the major human metabolite formed in the kidney and also the liver. UGT1A9 was the major human UDP-glucuronosyltransferase responsible for the formation of the inactive dapagliflozin 3-O-glucuronide and this enzyme is predominantly found in the kidney. No unique dapagliflozin human metabolites were identified. Dapagliflozin excretion is predominately via metabolism to the dapagliflozin 3-O-glucuronide followed by excretion

in the urine. The parent is also found to a much lower extent in the urine, feces and bile. Dapagliflozin was also found to be excreted in the milk of lactating rats.

Dapagliflozin was a weak substrate for p-glycoprotein and did not inhibit OAT1 or OCT2 and was weak inhibitor of hOAT3. These results suggest a low probability for drug-drug interactions except for potential inducers/inhibitors of UGT1A9 which may effect the elimination of dapagliflozin.

General Toxicology
Pivotal repeat dose studies were conducted in the Sprague-Dawley rat and Beagle dogs up to 6 and 12 months duration, respectively. In the rat the dapagliflozin exposure was 85-3097x MRHD and in the dog the exposure was 128x-3312x MRHD. Findings in the pivotal rat and dog studies were generally consistent with pharmacodynamic activity of dapagliflozin, including dose-dependent increases in urinary glucose. The toxicological profile of dapagliflozin supported the doses and duration of clinical studies conducted throughout drug development. At clinically relevant drug exposure, pharmacodynamic action also resulted in reduced body weight (BW), increased food consumption (FC), increased urinary volume, calcium (Ca), phosphorus, protein and decreased urinary osmolality.

In the 6 month rat study major target organs with toxicity included the kidney (chronic progressive nephropathy (CPN), mineral deposits, tubule epithelial hyperplasia and urothelial hyperplasia), sternum and femur (increased trabecular bone), heart, vessels and trachea (mineralization), adrenal glands (vacuolation/hypertrophy) and spleen and liver (extramedullary hematopoiesis). The target organs identified were likely the result of exaggerated pharmacology due to inhibition of SGLT2 (e.g. glucosuria) or were the result of off-target effects or were due to the osmotic and/or diuretic effect of enhanced glucose excretion (e.g. polyuria). Adrenal gland vacuolation could be a compensatory response of aldosterone production due to increased sodium excretion. Off-target effects include the increased trabecular bone and tissue mineralization, likely due to modulation of calcium homeostasis and increased urinary calcium excretion. The propensity for dapagliflozin to cause off-target inhibition of SGLT1 in humans is reduced due to the lower affinity of dapagliflozin for human SGLT1 compared to the rat. Overall, target organ toxicities in adult rats occurred at high exposure multiples (≥3097x MRHD) and the safety margins to the final clinical dose are high suggesting low clinical risk.

Reproductive Toxicology
Reproductive and developmental toxicity were assessed in fertility, early embryonic development, pre- and post-natal development and juvenile animal studies. In the fertility study no effects were seen on mating and fertility indices in the females and the males, except for altered spermatogenesis at 1707x MRHD. Resorptions were increased in females at the mid dose (188x MRHD) and above, resulting in a NOAEL for this finding of 39x MRHD. No effects were seen on mating and fertility indices in the females and a NOAEL for female fertility was 188x MRHD due to reduced weight gain at higher doses.

Dapagliflozin was not teratogenic at up to 75 mg/kg (1441x MRHD) in the rat. Higher exposures resulted in late gestational fetal deaths and malformations and skeletal variations at ≥ 1441x MRHD. Dapagliflozin was also not teratogenic in the rabbit at up to 1191x MRHD and effects on the litters, malformations and variations were not observed.

Exposure to dapagliflozin at 19-1415x MRHD in a pre- and post-natal development study in the rat had no pathological effects in the dams, yet showed renal pelvic dilatation at the high dose in the in utero and lactationally exposed pups (1415x MRHD). Due to reduced growth in the pups the NOAEL was <19x MRHD. In the dams the NOAEL was 249x MRHD due to reduced body weight gain at the high dose. Treatment of juvenile rat pups until maturity replicated the renal pelvic dilatation pathology but at drug exposure that is potentially clinically relevant, and also showed irreversibility in recovery animals, suggesting dapagliflozin is a renal pelvic development toxicant.

Genetic Toxicology
Dapagliflozin was not mutagenic or clastogenic in an in vitro Ames assay or in the in vivo assays: rat bone marrow micronucleus assay or peripheral blood lymphocyte chromosomal aberration assay or the hepatocyte unscheduled DNA synthesis (UDS) assay. However, dapagliflozin was clastogenic in the presence of S9 in multiple in vitro chromosomal aberration assays. The necessity of S9 (rat liver microsomes) to elicit clastogenicity indicates that an unidentified metabolite or metabolites of dapagliflozin were causative, not the intact parent molecule. However, all metabolites of dapagliflozin identified in human subjects have also been identified in mice and rats *in vivo*, and have been evaluated for genotoxic potential in nonclinical studies. The weight of evidence supports the view that dapagliflozin and its identified metabolites are unlikely to be clastogenic in human subjects.

Carcinogenicity
Dapagliflozin was assessed for its potential to induce tumors in two-year bioassays conducted in rats and mice. The two-year bioassays are intended to detect drug-induced tumors that arise from genotoxic as well as non-genotoxic mechanisms of action after approximately life-time exposure to an investigational drug. Dapagliflozin did not increase the incidence of any tumor in rats and mice at drug exposures reaching 131x and 72x the clinical dose, respectively.

Special Toxicology Studies
Exposure of rats in utero and during lactation to dapagliflozin at 16x to 918x MRHD resulted in excretion of dapagliflozin in the breast milk of lactating rats at a milk to plasma ratio of 0.49x. The fetal exposure to dapagliflozin was 2-142x MRHD, suggesting that pups were exposed to pharmacologically and toxicologically relevant levels of the drug.

A. Nonclinical Issues Relevant to Clinical Use

Pregnancy and Lactation

The sponsor has recommended against the use of dapagliflozin during the second and third trimesters of pregnancy, which is compatible with a contraindication for pregnant women or women who may become pregnant. The sponsor also recommends that women not take dapagliflozin during nursing. Their recommendations are based on adverse findings from exposure to dapagliflozin during the peri/post-natal and juvenile periods in rats. Exposure to dapagliflozin in rats from birth to approximately 13 weeks of age, and especially from post-natal weeks 3-6, results in dilatation of the renal pelvis and tubules and a lower rate of body growth at exposure less than 15-times the clinical dose. A 'no-effect dose' was not identified, so it is likely that exposure causing this adverse effect in rats occurs very near clinical exposure. This susceptible period 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 gestation, with functional renal development continuing until ~2yrs of age. The cause of renal pelvis and tubular dilation is not known. The FDA agrees with the sponsor that dapagliflozin should not be used during pregnancy or nursing.

1.3 Recommendations

1.3.1 Approvability

AP (Approval)

Pharmacology/Toxicology recommends approval of NDA 202293.

1.3.2 Additional Non Clinical Recommendations

None.

1.3.3 Labeling

(b) (4)

8.1 Pregnancy

(b) (4)

Note that pregnancy labeling language is subject to change pending further discussion within the clinical and maternal health review teams. The category and language may

be modified to recognize that the risk to fetal renal development appears to be restricted to the 2nd/3rd and not the 1st trimesters of pregnancy.

2 Drug Information

2.1 Drug

CAS Registry Number: **960404-48-2**

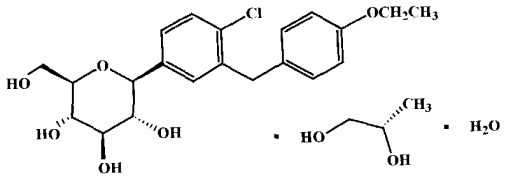
Generic Name (b) (4)

Code Name **Dapagliflozin/BMS-512148**

Chemical Name
(2*S*,3*R*,4*R*,5*S*,6*R*)-2-[4-Chloro-3-(4-ethoxybenzyl)phenyl]-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triol, (2*S*)-propane-1,2-diol (1:1) monohydrate

Molecular Formula/ Molecular Weight
$C_{21}H_{25}ClO_6.C_3H_8O_2.H_2O$ / MW = 502.98 (*S*-Propylene glycol monohydrate); = 408.87 ((b) (4) non-hydrated form)

Structure or Biochemical Description



Pharmacologic Class Sodium Glucose co-Transporter 2 (SGLT2) Inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 68,652 (dapagliflozin, BMS)

2.3 Drug Formulation

Dapagliflozin film-coated tablets are manufactured as 5 mg or 10 mg strengths.

2.4 Comments on Novel Excipients

None.

2.5 Comments on Impurities/Degradants of Concern

None.

2.6 Proposed Clinical Population and Dosing Regimen

Dapagliflozin is indicated for treatment of Type 2 diabetes mellitus (T2DM). The recommended dose is 10 mg taken once daily. A lower starting dose of 5 mg is recommended for patients with volume depletion.

2.7 Regulatory Background

NDA 202293 is the original submission of dapagliflozin as a new molecular entity under 505(b)1.

3 Studies Submitted

3.1 Studies Reviewed

Most studies were previously submitted and reviewed during the IND phase for dapagliflozin. Summaries of nonclinical reviews from the IND are included in this NDA review. Nonclinical studies reviewed/summarized in this submission include:

Primary Pharmacodynamics

In Vitro Inhibition of Type 1 and Type 2 Sodium-Dependent Glucose Transporters and Facilitative Glucose Transporters 1 and 4 by BMS-512148 and its Oxidative Metabolites BMS-511926 and BMS-639432: Comparison to Previous Lead Candidates (b) (4)

Secondary Pharmacodynamics

The Effect of BMS-512148 on Urinary Glucose Output and Plasma Glucose Lowering in Normal Male Sprague Dawley Rats
The Acute Blood Glucose Lowering Effect of BMS-512148 in Streptozotocin-Treated Sprague Dawley Rats
Acute Plasma Glucose Lowering Following Single Oral Doses of (b) (4) or BMS-512148 in Zucker Diabetic Fatty (ZDF) Rats
The Subchronic Antidiabetic Effect of BMS-512148 in Zucker Diabetic Fatty Rats

Safety Pharmacology

Effect on HERG/Ikr Currents and Rabbit Purkinje Fiber Action Potentials
One Month Oral Toxicity in Dogs
One Month Oral Toxicity in Dogs II
Three Month Oral Toxicity in Dogs

PK/ADME

Preclinical evaluation of the pharmacokinetics and metabolism of BMS-512148
Tissue Distribution of Radioactivity in Male Long-Evans rats Following Oral Administration of [^{14}C]BMS-512148
In Vitro Determination of Protein Binding of BMS-512148 and BMS-801576 in Human, Rat, Dog, Mouse and Rabbit Plasma
Evaluation of the inhibitory effects of BMS-512148 on the activity of cytochrome P450 enzymes CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 in human liver microsomes
Comparative in vitro biotransformation of [^{14}C]BMS-512148 in hepatocyte and liver microsomal preparations of mouse, rat, dog, monkey and human
Biotransformation of [^{14}C]BMS-512148 after Oral Administration to Bile Duct-Cannulated Rats
Comparative Biotransformation of [^{14}C]Dapagliflozin after Oral Administration to Intact Rats, Dogs, Mice, and Humans
Glucuronidation of BMS-512148 in incubations with human kidney, liver and intestinal microsomes

In Vitro Pharmacology of BMS-801576 and BMS-805525, Metabolites of Dapagliflozin in Humans
Dapagliflozin (BMS-512148): Oral Study of Toxicokinetics in Lactating Rat Dams and Their Nursing Pups
Lacteal Excretion of [14C]BMS-512148 Following Administration of a Single Oral Dose to Rats
Single-Dose Pharmacokinetics and Safety of 10 mg Dapagliflozin in Subjects with Hepatic Impairment Compared to Healthy Adult Subjects
Dapagliflozin and BMS-801576: Renal Transporter Interaction Studies

General Toxicology
Single-Dose Oral Toxicity Study in Mice
Single-Dose Oral Toxicity Study in Rats
Single-Dose Oral Toxicity Study in Dogs
Three Month Oral Range-Finding Toxicity Study in Mice
Six-Month Oral Toxicity in Rats
One Year Oral Toxicity in Dogs

DART
BMS-512148: Oral Study of Fertility and Early Embryonic Development in Rats
BMS-512148: Oral Study of Embryo-Fetal Development in the Rat
BMS-512148 Ten Day Oral Toxicokinetic Study in Pregnant Rats
BMS-512148: Oral Study of Embryo-Fetal Development in the Rabbit.
BMS-512148 Thirteen-Day Oral Toxicokinetics Study in Pregnant Rabbits
Dapagliflozin (BMS-512148): Oral Study of Pre- and Postnatal Development in Rats.
Dapagliflozin (BMS-512148): Oral Study of Toxicokinetics in Lactating Rat Dams and Their Nursing Pups.
Dapagliflozin (BMS-512148): Two Month Oral Developmental Study in Juvenile Rats with a One Month Recovery.

Genetic Toxicology
BMS-512148: Investigative reverse mutation study in *Salmonella typhimurium* and *Escherichia coli*
BMS-512148: Cytogenetic study in Chinese Hamster Ovary Cells
BMS-512148: Comparative Cytogenetics Study in Chinese Hamster Ovary Cells
BMS-512148 Investigative Cytogenetics and Cytoxicity Study in Chinese Hamster Ovary Cells
BMS-512148 Two-week Oral Investigative Study in Rats with Micronucleus Evaluation
BMS-512148: One Month Oral In Vivo/In Vitro Cytogenetics Study in Rat Peripheral Blood Lymphocytes

Carcinogenicity
BMS-512148 Oral Carcinogenicity Study in Rats
BMS-512148 Oral Carcinogenicity Study in Mice

3.2 Studies Not Reviewed

None.

3.3 Previous Reviews Referenced

None.

4 Pharmacology

4.1 Primary Pharmacology

Dapagliflozin is a competitive inhibitor of sodium glucose co-transporter (SGLT) 2, which is the major transporter involved in the reabsorption of glucose in the kidney.

Human (h) and rat (r) SGLT2 and the closely related SGLT1 were stably expressed in Chinese Hamster Ovary (CHO) cells and the role of dapagliflozin in inhibiting the transport of glucose analog [^{14}C]-alpha-methyl glucopyanoside (AMG) was determined. Dapagliflozin inhibited hSGLT2-mediated transport of AMG in CHO cells with high potency (IC_{50} 1 nM) relative to the closely related hSGLT1 (IC_{50} 1600 nM). Similarly, dapagliflozin inhibited rSGLT2-mediated transport of AMG in CHO cells with an IC_{50} 3 nM giving a 207-fold selectivity over the rSGLT1 transporter (IC_{50} 620 nM). Additional assay replicates revised the hSGLT2 and hSGLT1 IC_{50} to 1.12 nM and 1391 nM, respectively.

Table 1. Inhibition of human SGLT2 and SGLT1 EC_{50}

Compound	hSGLT2 Mean IC_{50} (nM) ± SEM	hSGLT1 Mean IC_{50} (nM) ± SEM	Selectivity for hSGLT2 vs. hSGLT1 (fold)
BMS-512148	1.0 ± 0.08	1600 ± 140	1600
BMS-511926	1.0 ± 0.1	1500 ± 100	1500
BMS-639432	1800[b]	> 8000	> 4
(b) (4)	10 ± 1.1	19,000 ± 3200	1900
	1.6 ± 0.17	230 ± 30	144
Phlorizin	34 ± 6	270 ± 22	8

Table 2. Inhibition of rat SGLT2 and SGLT1 EC_{50}

Compound	rSGLT2 Mean IC50 (nM) ± SEM	rSGLT1 Mean IC50 (nM) ± SEM	Selectivity for rSGLT2 vs. rSGLT1 (fold)
BMS-512148	3.0 ± 0.5	620 ± 70	207
BMS-511926	2.4 ± 0.4	260 ± 30	108
BMS-639432	ND	ND	ND
(b) (4)	43 ± 4	2900 ± 400	67
	2.6 ± 0.3	210 ± 10	81
Phlorizin	75 ± 8	302 ± 30	4

Dapagliflozin was also not an appreciable inhibitor of human or mouse adipocyte glucose transporter (GLUT) GLUT 1 and GLUT4 activity when tested at 20 μM in human and mouse 3T3-L1 adipocytes (see tables below). Low activity against the GLUT

transporters is desired as these transporters play a critical role in tissue glucose uptake in tissues such as the skeletal muscle and adipose tissue.

Table 3. Inhibition of human adipocyte GLUT1 and GLUT4

Compound	No BSA[b]		With 4% BSA	
	Basal	Insulin Stimulated	Basal	Insulin Stimulated
BMS-512148	9 ± 1	8 ± 3	0	0
BMS-511926	7	11	2	0
BMS-639432	0	0	ND	ND
(b) (4)	ND	ND	ND	ND
	3 ± 3	10 ± 6	1	0
Cytochalasin B	88 ± 2	89 ± 0.3	86 ± 1	88 ± 1

Table 4. Inhibition of rat 3T3-L1 Adipocyte GLUT1 and GLUT4 activity

	No BSA		With 4% BSA	
Compound	Basal	Insulin Stimulated	Basal	Insulin Stimulated
BMS-512148	20 ± 4	19 ± 7	0	3
BMS-511926	15 ± 2	6 ± 4	0	0
BMS-639432	0	0	ND	ND
(b) (4)	12 ± 1	11 ± 1	ND	ND
	9 ± 4	19 ± 4	0	0
Phloretin	71 ± 3	72 ± 4	ND	ND
Cytochalasin B	93 ± 3	92 ± 0.3	91 ± 0	94 ± 1

4.2 Secondary Pharmacology

Non-clinical efficacy of dapagliflozin was assessed in normal rats, streptozotocin-induced (STZ) diabetic rats and the Zucker diabetic fatty rat (ZDF) using the secondary pharmacology/pharmacodynamic markers of urinary glucose excretion (glucosuria), polyuria and plasma glucose lowering.

In the normal Sprague-Dawley (SD) rats exposed to dapagliflozin between 0.01-10 mg/kg and subject to an oral glucose tolerance test (OGTT), dapagliflozin reduced plasma glucose at 1 and 10 mg/kg. The reduction of plasma glucose was statistically significant (ss) at 1 and 10 mg/kg at 1 hour following OGTT. In addition, dapagliflozin

was shown to ss increase urinary volume and urinary glucose concentration and excretion.

Figure 1. Mean Plasma Glucose During an OGTT in Sprague-Dawley (SD) Rats

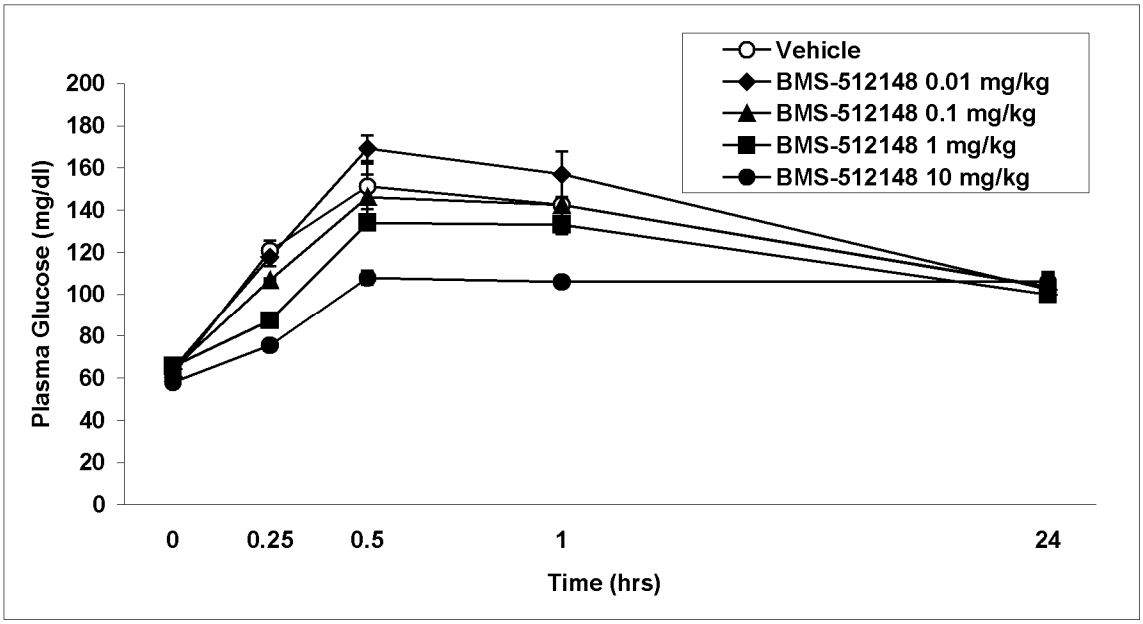


Figure 2. Mean Plasma Glucose AUC at 1 hour following the Glucose Load in an OGTT in the Normal SD Rat

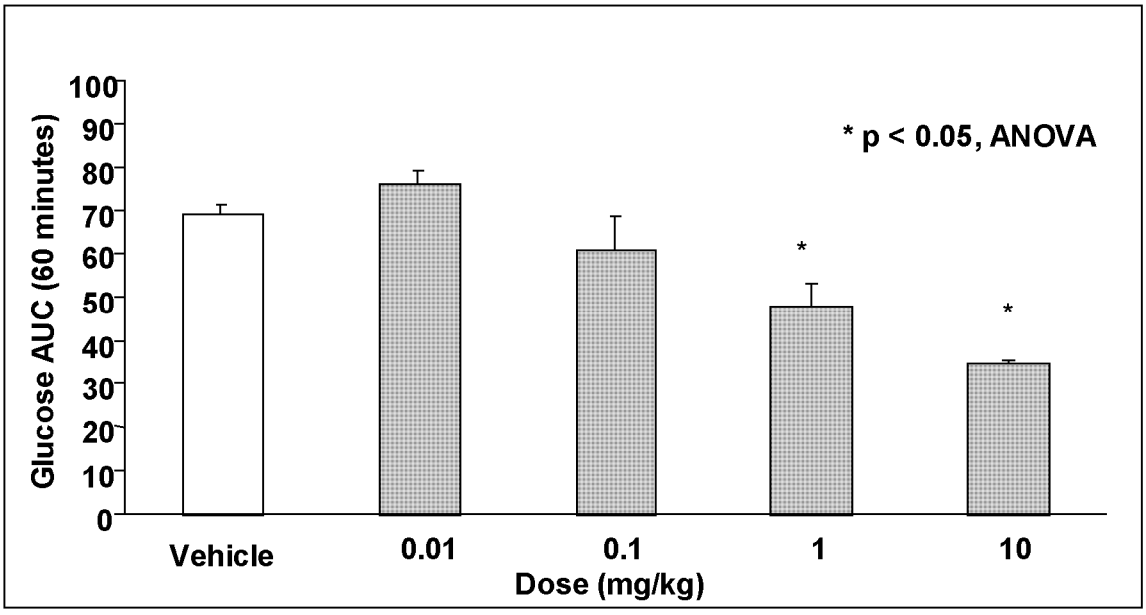
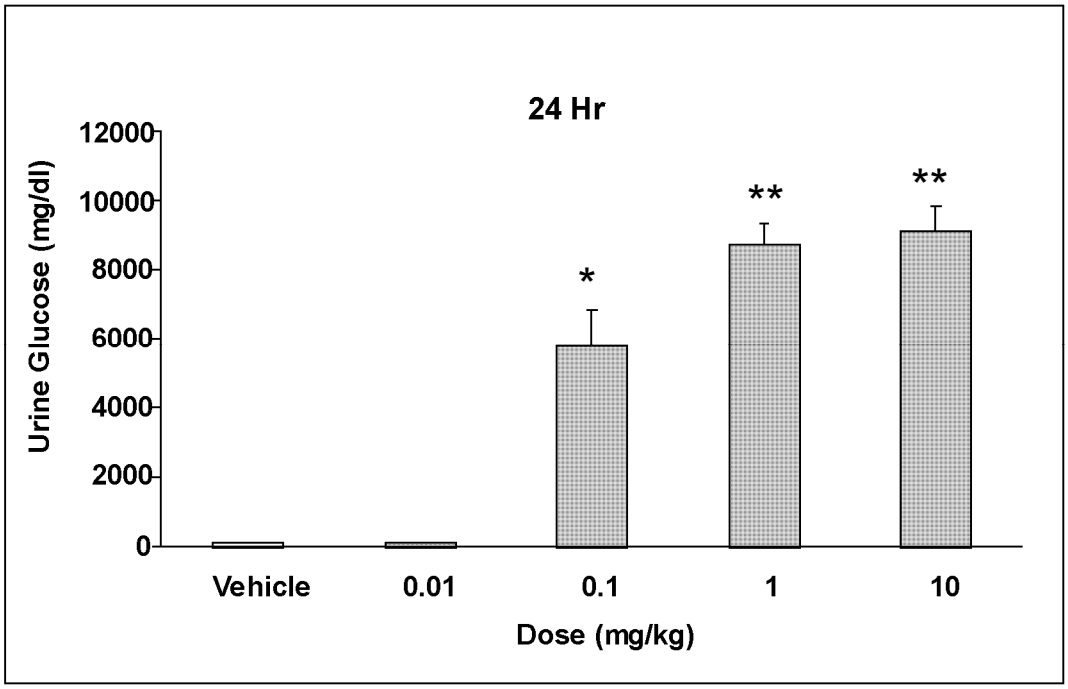
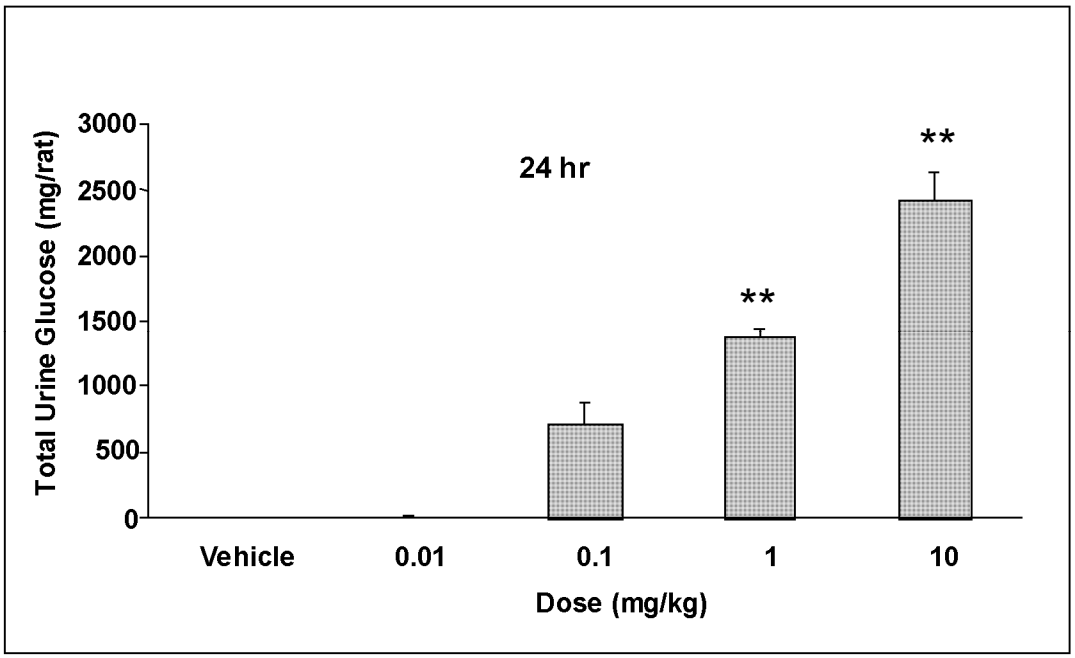


Figure 3. Mean 24 hour Urinary Glucose Concentration in Normal SD rat Following an OGTT.



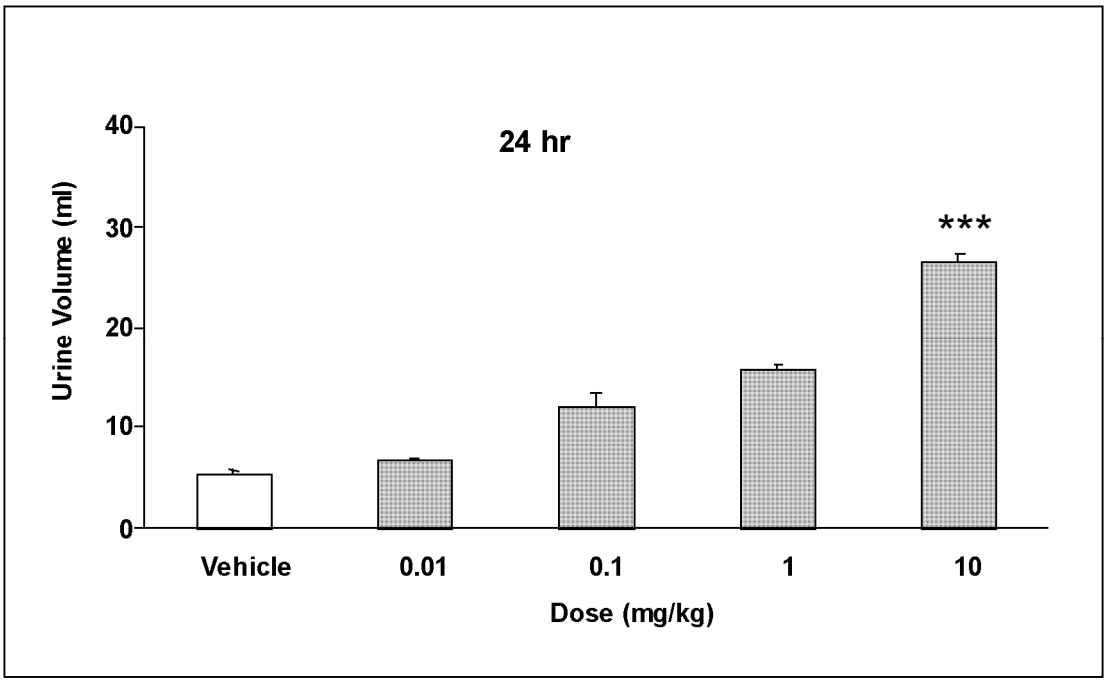
* p<0.05, ** p<0.01

Figure 4. Mean Glucose Excretion over 24 hours in the Normal SD Rat Following an OGTT.



** p<0.01

Figure 5. Mean Urine Volume Over 24 hours in the Normal SD Rat Following an OGTT.



*** p<0.001

Table 5. Mean Urinary Glucose and Urinary Volume Parameters in the SD Rat Following an OGTT

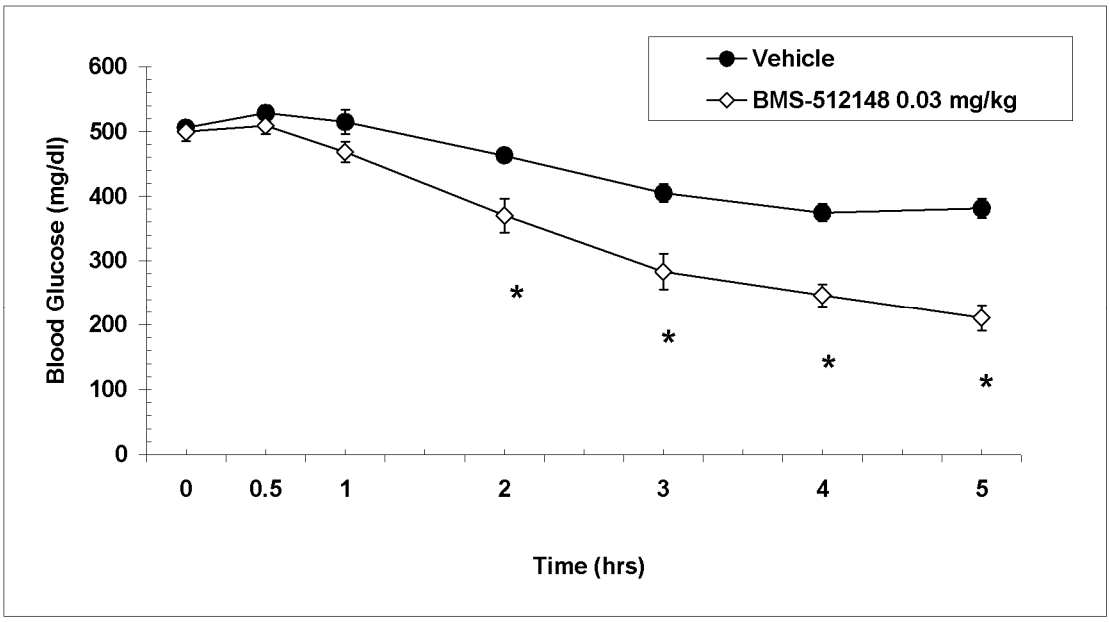
		Baseline (18 hr collection)			24 hours following treatment[a] (% change vs. baseline)		
Group	**Body Weight (grams) ± SEM**	**Mean Urine Volume (ml) ± SEM**	**Mean Urine Glucose (mg/dl) ± SEM**	**Mean Total Urine Glucose (mg) ± SEM**	**Mean Urine Volume (ml) ± SEM**	**Mean Urine Glucose (mg/dl) ± SEM**	**Mean Urine Glucose Total (mg) ± SEM**
Vehicle	247 ± 3	8 ± 2	10 ± 4	0.72 ± 0.1	5 ± 0.6	60 ± 3	3 ± 0.4
0.01	246 ± 3	6 ± 1	10 ± 1	0.6 ± 0.1	7 ± 0.1 (16.27)	66 ± 16 (589.6)	5 ± 1 (727.0)
0.1	249 ± 1	5 ± 1	14 ± 2	0.7 ± 0.1	12 ±1* (130.51)	5778 ± 1023* (40,025)	707 ± 165[b] (99,389)
1.0	249 ± 1	7 ± 3	14 ±7	0.6 ± 0.3	16 ± 0.4 (121.9)	8693 ±629** (61116)	1369 ±74** (214338)
10	253 ± 2	7 ± 1	7 ±1	0.44 ± 0.14	27 ± 0.8*** (298.9)	9102 ± 718** (137809)	2417 ± 205** (548760)

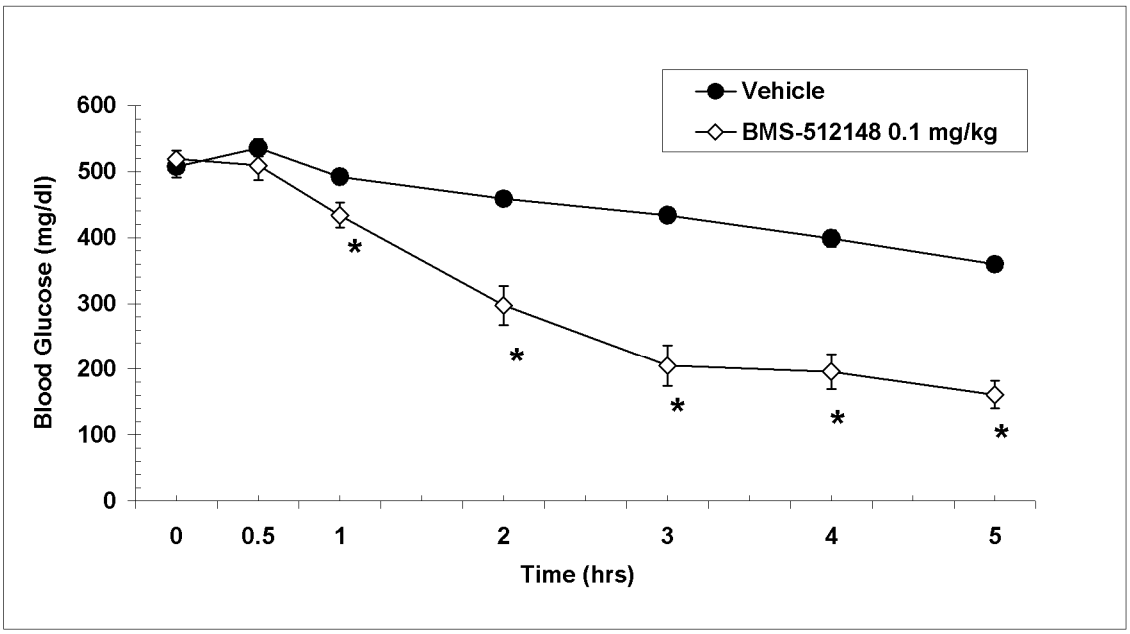
[a] Comparison to baseline values done using paired Student T-test. * p<0.05. ** p<0.01 *** P<0.001

[b] p = 0.05

The STZ-treated rat is a model of insulin deficient diabetes and STZ produces cytotoxicity in the pancreatic islet beta cells resulting in reduced insulin production and subsequent hyperglycemia, polyuria and polydipsia. Following a single oral dose (0.01-0.1 mg/kg) dapagliflozin produced a dose-dependent reduction in blood glucose which was statistically significant at 0.03 and 0.1 mg/kg, respectively.

Figure 6. Blood Glucose in the STZ-induced Diabetic Rat Following Dapagliflozin Treatment.

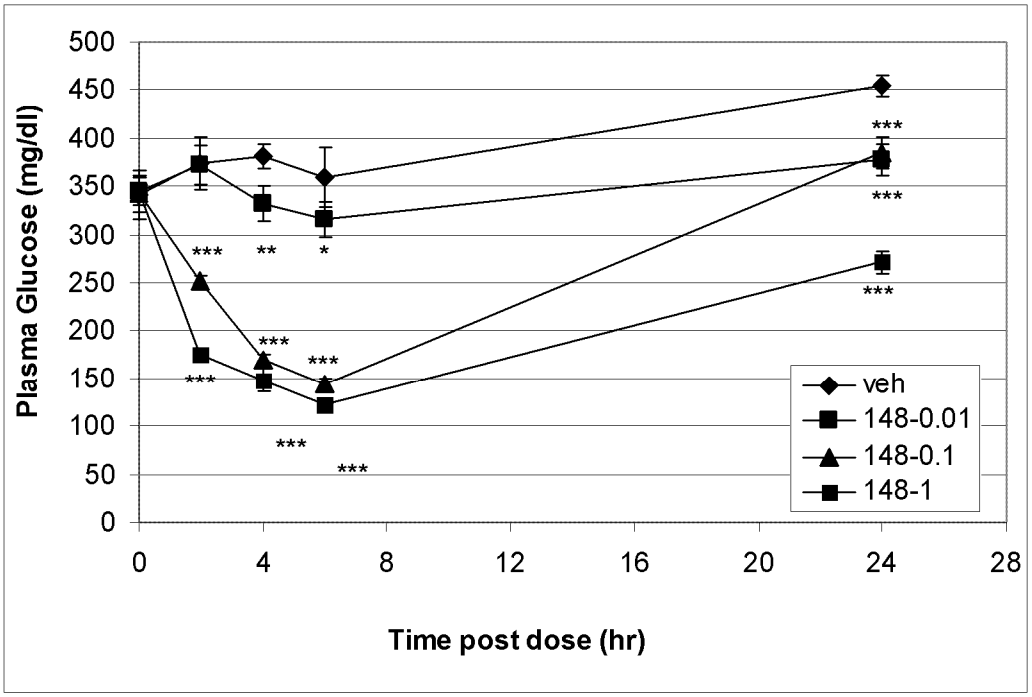




[a] N=6 per group. *: $p < 0.05$ vs. control group using a paired student *t*-test.

In the ZDF diabetic rat model a single oral dose of dapagliflozin dose-dependently and statistically reduced blood glucose (26-37%) over 6 hours and the blood glucose remained lower (12-17%) at 24 hours post-treatment:

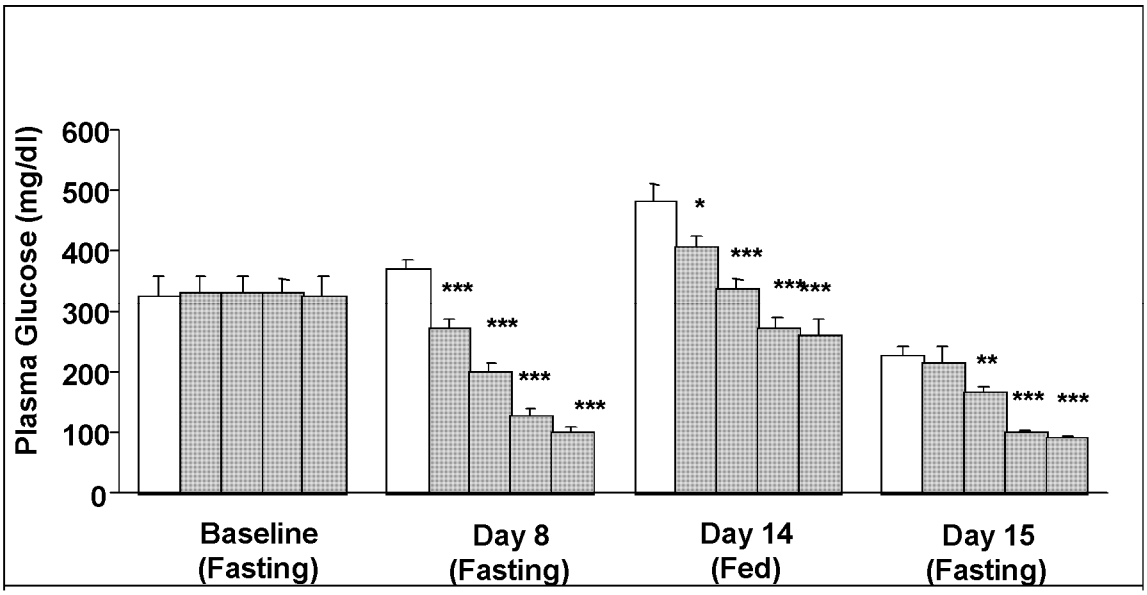
Figure 7. Mean Plasma Glucose in Male ZDF Rats Treated With a Single Oral Dose of Dapagliflozin



*: p<0.05, **: p< 0.01, ***: p<0.001 vs. vehicle

Sub-chronic daily oral treatment with dapagliflozin (0.01, 0.1, 1 and 10 mg/kg) in fed male diabetic ZDF rats for 2 weeks resulted in a statistically significant (ss) and dose-dependent reduction in fasting plasma glucose by day 8. Fed and fasting plasma glucose were also statistically significantly and dose-dependently reduced by day 14 and day 15 respectively.

Figure 8. Mean Fasting and Fed Plasma Glucose in Male ZDF Rats Treated Once Daily With Dapagliflozin



*: p<0.05, **: p< 0.01, ***: p< 0.001 vs. vehicle using ANOVA followed by a Fisher post hoc test.

4.3 Safety Pharmacology

Brief Summary

As part of the development program safety assessment for cardiovascular, neurological and pulmonary effects of dapagliflozin were conducted. Dapagliflozin minimally inhibited hERG potassium current and had no significant effect on action potential in the rabbit Purkinje fiber assay. Respiratory parameters evaluated in one month rat and dog studies show no effect with treatment with dapagliflozin. Axonopathy was observed in CNS tissues in a one month dog study but was not reproduced in a repeated dog study. The lack of reproducibility in this and subsequent toxicology studies in dogs indicates that the axonopathy was unrelated to drug treatment. Cardiovascular parameters were unchanged in a one-month repeat dose dog study at up to 250 mg/kg. However, in a 3 month dog study there was a minimal increase (18 msec) in QTc at week 13 in males treated with dapagliflozin at 180 mg/kg. This prompted a single dose “thorough QT study” in healthy human males at 20 and 150 mg that had no effect on QTc. The weight of evidence indicates that dapagliflozin does not prolong QTc in human subjects at clinically relevant exposure.

Neurological Effects

One Month Oral Toxicity Study in Dogs

Dogs were treated with dapagliflozin at 0, 5, 25 and 200 mg/kg once daily for one month. In the primary review cerebellum and spinal cord axonal degeneration were observed in 1/3 and 2/3 high dose females, respectively, relative to 0/3 in the control

females. The sponsor conducted an independent peer review with a neuropathologist who found evidence of axonopathy and spheroid lesions in all treatment groups in both the CNS and PNS with a slightly higher incidence in the high dose group. The NOAEL for neurological lesions was <5 mg/kg. A repeat dose study in the dog failed to replicate these findings. The lack of reproducibility indicates that axonopathy was unrelated to dapagliflozin treatment.

Table 6. Distribution of Neuroatomic Individual Axonopathies and Spheroid Lesions in Dogs Receiving Dapagliflozin for One Month

Neuroanatomic Lesion site		Group 1 Control	Group 2 5 mg/kg	Group 3 25 mg/kg	Group 4 200 mg/kg
Number of Animals in Group		6	6	6	6
Frontoparietal Ctx		0(6)	0(6)	0(6)	1(6)1M*
Centrum Semiovale		1(6)1F	0(6)	3(6)2M1F	1(6)1M
Corpus Callosum		1(6)1F	0(6)	0(6)	0(6)
Caudate Nucleus		2(6)1M1F	1(6)1F	0(6)	0(6)
Optic Chiasma		1(6)1F	2(5)2M	0(4)	2(4)2M
Optic radiation		3(6)2M1F	4(6)2M1F	1(6)1F	0(6)
Internal capsule		0(6)	4(6)2M1F	1(6)1F	2(6)1M1F
Piriform Ctx		1(6)1F	0(6)	1(6)1M	0(6)
Fornix		0(6)	0(4)	0(6)	1(6)1F
Thalamus		2(6)1M	0(6)	0(6)	0(6)
Basis pedunculi		0(6)	1(6)1F	1(6)1F	1(6)1F
Anterior colliculus		0(5)	0(6)	2(5)1M	1(4)1F
Periaqueductal gray		1(6)1M	1(6)1F	0(6)	1(6)1M
Med Long Fasciculus		1(4)1F	0(4)	1(6)1M	0(5)
Cerebellar white		2(6)1M	4(6)2M1F	0(6)	7(6)1M2F
Cerebellar Ctx (Torpedos)		8(6)1M	0(6)	0(6)	0(6)
Cerebellar Peduncle		0(6)	1(6)1M	0(5)	0(6)
Cerv Crd Funiculi		8(6)3M2F	2(6)2F	2(6)2M	5(6)1M1F
Cerv Crd Gray		0(6)	0(6)	0(6)	1(6)1M
Lumb Crd Funiculi		3(6)1M1F	0(6)	4(6)1M1F	9(6)2M3F
Medulla (Obex)		1(6)1F	0(5)	0(6)	1(6)1M
Cochlear Nuc		0(0)	0(0)	0(0)	3(1)1M
Hypoglossal Nuc		0(6)	0(5)	0(6)	1(5)1M
Gracile Nuc		0(6)	2(6)1M	1(6)1M	2(6)2F
Pyramids		0(6)	1(5)1F	0(6)	0(5)
Posterior Olive (Vacuolation)		0(6)	0(5)	2(6)1M1F	0(5)
Trigeminal Tract		1(6)1M	0(6)	0(6)	0(6)
Number of the Group with Lesions		6	6	6	6
Distribution of Lesions in Groups by Sex	M	12	10	7	14
	F	11	9	6	12
Total # of Lesions in the Group		36	23	19	39

*Number of individual lesions observed in the group; number of animals in the group with anatomic site available for examination is in parentheses; followed by the number and sex of animals in the group with the lesion.

Cardiovascular Effects
hERG activity
Dapagliflozin weakly inhibited hERG potassium current by 3.7% and 15% at 10 and 30 μM (see figure below) and had no effect on Purkinje fiber action potential at 2, 10 and 30 μM (see figure below).

Figure 9. Effect of Dapagliflozin on hERG Currents Expressed in a HEK293 cell line

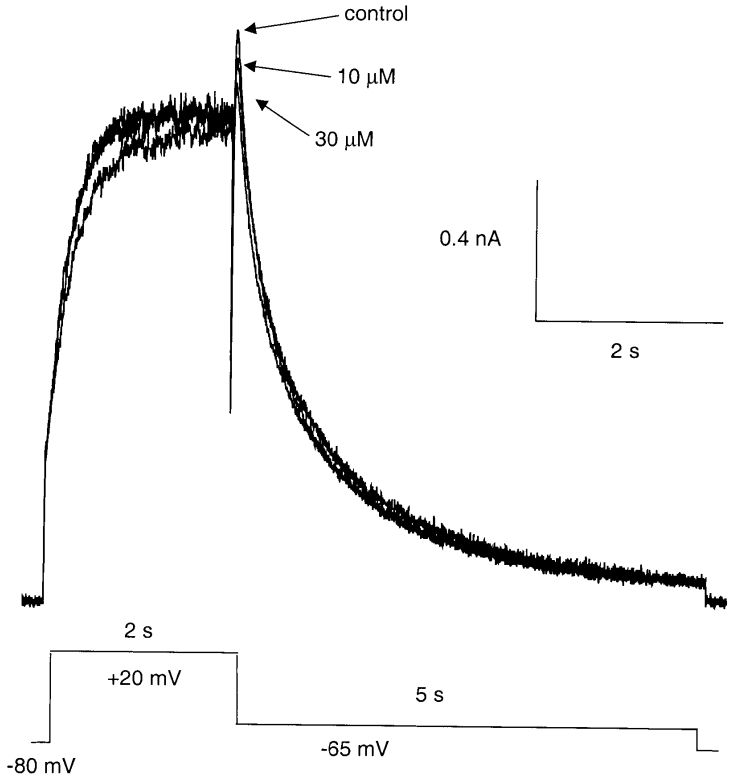
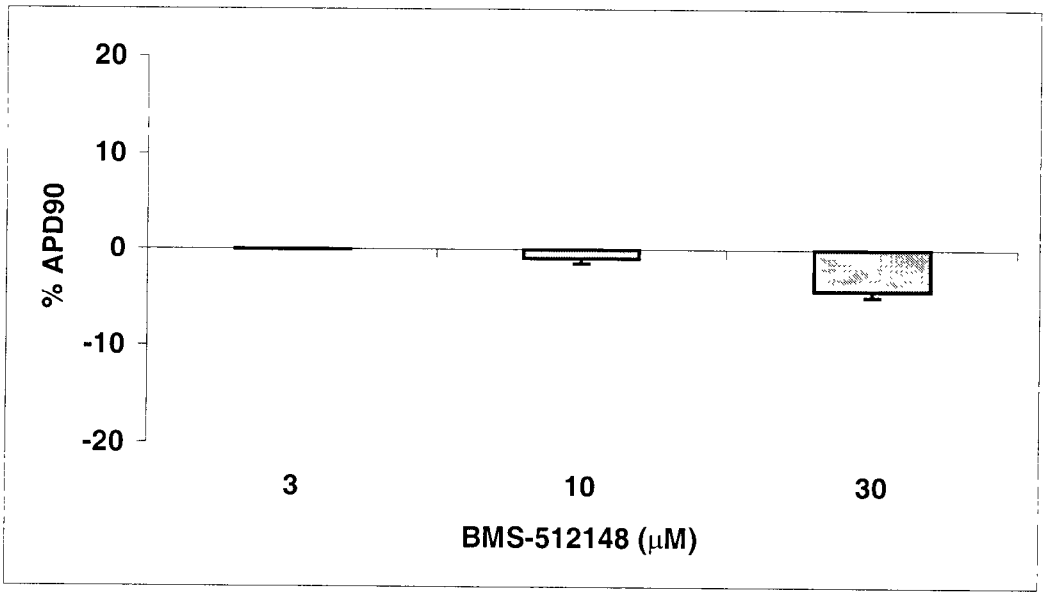


Figure 10. Effect of Dapagliflozin on Rabbit Purkinje Fiber Action Potential



Three Month Oral Toxicity Study in Dogs

Dogs were treated with dapagliflozin at 0, 5, 30 and 200 mg/kg once daily for three months. Mean QTc was increased 15 and 18 msec, respectively, at week 4 and 13 in the high dose males. Mean QTc was also minimally increased by 8 msec in the high dose recovery males, showing reversibility.

Table 7. Group Mean QTc Intervals in a 3 Month Dog Study

5 dogs/sex/group

Group/Sex	Pretest	Week 1	ΔQT[a]	Week 4	ΔQT[a]	Week 13	ΔQT[a]	Week 17[b]	ΔQT[a]
1M	209.80	213.66	+3.86	228.43	+18.63	223.88	+14.08	225.10	+15.30
2M	203.10	212.00	+ 8.9	198.26	- 4.84	220.30	+17.20	206.74	+ 3.64
3M	204.79	209.05	+ 4.26	227.08	+22.29	221.10	+16.31	204.57	- 0.22
4M	207.10	202.79	- 4.31	240.26	+33.16	240.07	+32.97	230.54	+23.44

Group/Sex	Pretest	Week 1	ΔQT[a]	Week 4	ΔQT[a]	Week 13	ΔQT[a]	Week 17[b]	ΔQT[a]
1F	206.66	207.43	+0.77	211.92	+5.26	212.97	+6.31	207.98	+ 1.32
2F	202.37	213.32	+10.95	232.63	+30.26	225.85	+23.48	217.48	+15.11
3F	214.44	221.20	+6.76	229.28	+14.84	229.04	+14.60	222.94	+ 8.50
4F	210.98	213.43	+2.45	224.74	+13.76	222.47	+11.49	199.14	-11.84

a- ΔQTc = mean QTc value – Pretest mean QTc value
b- Two (2) dogs/sex/group

Pulmonary Effects
One month rat and dog studies were conducted and dapagliflozin was assessed for overt respiratory signs post-treatment. There were no dapagliflozin-related changes to respiratory parameters.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Methods of Analysis
Plasma concentration of dapagliflozin: LC/MS/MS assay with a lower limit of quantification of 5, 20 and 50 ng/mL in rat, mouse, rabbit and dog plasma, respectively.

Radioactivity measurement: Liquid scintillation counting of relevant samples and Quantitative whole-body autoradiography (QWBA).

Metabolite identification: LC-MS or NMR-MS and authentic standards.

Fetal homogenate concentration of dapagliflozin: LC/MS/MS assay with a lower limit of quantification of 1 ng/mL in rat.

Absorption

The in vitro Caco-2 cell model was used to determine the rate of passage of dapagliflozin. The permeability coefficient of dapagliflozin was >150 nm/sec at a concentration of 50 μM at pH 6.5. Therefore the intrinsic permeability of dapagliflozin is comparable to compounds that exhibit good absorption (>50%) in humans. The oral bioavailability of dapagliflozin is 84% in the rat, 83% in the dog but only 25% in monkeys.

Absorption of dapagliflozin is rapid following oral administration with C_{max} being achieved at (T_{max}) 0.6, 1.7 and 1.9 hours for the dog, rat and monkey respectively. Following IV administration dapagliflozin was eliminated with a half-life (t ½) of 4.6, 3.5 and 7.4 hours in the rat, monkey and dog, respectively. In healthy humans (n=6) a dose of 1.22 mg/kg resulted in a t ½ dose of 12.9 hours (following a single oral dose of 10 mg). This t ½ resembles that of the dog and the C_{max} of 0.136 μg/mL is one quarter of that of the 1 mg/kg p.o. treated rat (sponsor's tables below).

Table 8. Species PK Parameters (sponsor's table)

Species	Route	Dose (mg/kg)	Cmax (μg/mL)	Tmax (h)	AUCtot (μg•h/mL)	$t_{1/2}$ (h)	Cl (mL/min/kg)	Vss (L/kg)	F (%)
Rat	IA	1	-	-	3.55±0.42	4.6±0.8	4.8±0.6	1.6±0.1	
	PO	1	0.60±0.46	1.7±2.0	2.96±0.73	-	-	-	84±21
Dog	IV	6.6	-	-	76.4±10.1	7.4±1.2	1.5±0.2	0.8±0.1	
	PO	6.6	10.7±1.6	0.6±0.4	63.6±7.3	-	-	-	83±2
Monkey	IV	6	-	-	17.1±6.8	3.5±1.9	6.4±2.3	0.8±0.2	
	PO	6	1.54±0.40	1.9±1.8	4.27±2.17	-	-	-	25±2

Dosing vehicle used in all pharmacokinetic studies was Polyethylene glycol-400/water/ethanol (45/45/10)

Table 9. Dapagliflozin Pharmacokinetic Parameters in Healthy Humans (sponsor's table)

	Dapagliflozin Pharmacokinetic Parameters							
	Cmax (ng/mL) Geom. Mean (CV%)	AUC(INF) (ng·h/mL) Geom. Mean (CV%)	AUC(0-T) (ng·h/mL) Geom. Mean (CV%)	T-HALF (h) Mean (SD)	Tmax (h) Median (Min, Max)	CLT/F (L/h) Geom. Mean (CV%)	Vz/F (L) Geom. Mean (CV%)	Percentage Protein Bound (%) Mean (SD)
Healthy (n = 6)	136 (31)	465 (34)	438 (34)	12.9 (5.54)	1.00 (0.50, 2.00)	21.5 (35)	370 (36)	92.1 (1.92)

Distribution
Extravascular distribution was demonstrated by higher steady state volume of distribution in the rat (1.6 L/kg), dog (0.8 L/kg) and monkey (0.8 L/kg) compared to the total body water values of 0.7, 0.6 and 0.7 L/kg, in each of these species, respectively.

Dapagliflozin distributes to tissues rapidly and widely following oral exposure in the rat. Concentrations of dapagliflozin were slightly higher in plasma compared to the blood but were qualitatively similar, implying blood clearance will approximate plasma clearance. The tissues with the highest drug concentrations were those involved with absorption and elimination processes such as the gastrointestinal tract, liver and kidneys. Tissues with the lowest concentration were the brain and the bone. The dapagliflozin concentration in all organs declined over 24-48 hours indicating a lack of drug accumulation and was less than 1% at 48 hours. Stably transfected (human embryonic kidney) HEK-293 cells expressing human (h) hOCT2 or hOAT3 transporters or stably transfected MDCK cells expressing the hOAT1 transporter were used to determine the inhibitory capacity of dapagliflozin or the dapagliflozin 3-O-glucuronide in these renal cell lines. Dapagliflozin was a weak inhibitor of hOAT3 with an IC_{50} of 33 μM but did not inhibit hOAT1 or hOCT2. Dapagliflozin 3-O-glucuronide was also a weak inhibitor of hOAT3 with an IC_{50} of 100 μM and hOAT1 (29%) but did not inhibit hOCT2. As the C_{max} of dapagliflozin is less than 1 μM the weak transporter inhibition of hOAT3 is unlikely to be clinically relevant.

Plasma protein binding and blood partitioning
The serum protein binding of dapagliflozin at 10 μM was 97%, 96%, 91% and 96% in the rat, dog, monkey and human. The extent of blood cell partitioning was determined in the rat, dog, monkey and human with dapagliflozin at 10 μM. The ratio of concentration in the blood to plasma was 0.57, 0.58, 0.71 and 0.69 in the rat, dog, monkey and human. The percent distribution to blood cells was 8% and 19% in the dog and monkey and negligible in the rat and human. Equilibrium dialysis showed no differences among the species for plasma protein binding which was high in all species tested (table below):

Table 10. Percent Binding of Dapagliflozin to Protein in the Rat, Dog, Mouse, Rabbit and Human Plasma at 500 and 5000 ng/mL

	Rat	Dog	Mouse	Rabbit	Human
BMS-512148	95.1 ± 0.77	93.1 ± 0.96	92.8 ± 1.20	94.2 ± 0.52	91.0 ± 0.65

Metabolism
In Vitro metabolism
In vitro liver microsomal studies show dapagliflozin undergoes oxidative metabolism in the rat, dog, monkey and human characterized by low to intermediate clearance via this route. Dapagliflozin was metabolized in vitro by numerous human cytochrome P450 (CYP) enzymes and the highest metabolism was seen with CYP2D6, CYP1A2,

CYP3A4, CYP2C9, CYP1A2, CYP3A5 and CYP2E1 (from highest to lowest, respectively). At 1 μM CYP1A1 was the predominant CYP isoform responsible for dapagliflozin metabolism. Also, dapagliflozin was found not to be an in vitro inducer of CYP3A4 and dapagliflozin showed weak activity for CYP inhibition with an IC_{50} > 45μM. In vitro hepatocyte preparations showed multiple glucuronide conjugates as metabolites in hepatocyte preparations from mouse, rat, dog, monkey and human. Dapagliflozin 3-O-glucuronide (m15 or BMS-801576) is the predominant metabolite formed from human kidney, liver or intestinal microsomes. The formation of dapagliflozin 3-O-glucuronide was more rapid in human kidney microsomes compared to human liver and intestinal microsomes, respectively (kidney >> liver >> intestinal microsomes). Inhibitor studies with UDP-glucuronosyltransferase (UGT) inhibitors confirmed the involvement of UGT1A9 in the formation of dapagliflozin 3-O-glucuronide. Of note human UGT1A9 is preferentially expressed in the human kidney (Nishimura & Nato, 2006) and both the kidney and the liver contribute to the formation of dapagliflozin 3-O-glucuronide.

The activity of dapagliflozin 3-O-glucuronide against hSGLT1, hSGLT2, GLUT1 and GLUT4 was determined in vitro. The mean EC_{50} for dapagliflozin 3-O-glucuronide was 2.9 μM compared to the parent EC_{50} of 1.2 nM, thus providing 2400-fold selectivity for the parent dapagliflozin. Dapagliflozin 3-O-glucuronide did not inhibit hSGLT1. Dapagliflozin 3-O-glucurionide was also found to minimally inhibit (0-4%) GLUT1/GLUT4 activity in primary human adipocytes at 20 μM. At the highest therapeutic dose (10 mg), dapagliflozin has a plasma concentration of 0.56 μM, thus overall, the dapagliflozin 3-O-glucuronide is unlikely to contribute to the activity of dapagliflozin in vivo.

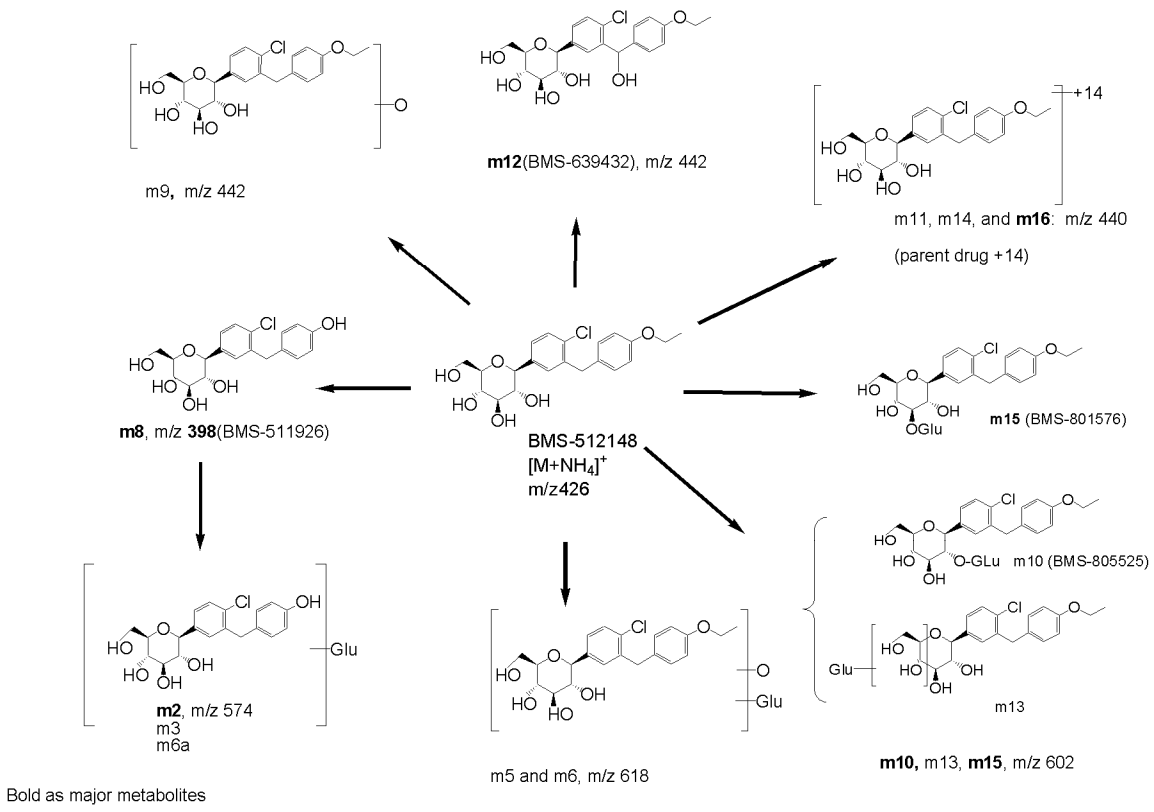
In Vivo Metabolism
While in vivo metabolite profiles were qualitatively similar in all species tested and there were no unique human metabolites, the metabolite profile differed quantitatively following a single radiolabeled dapagliflozin oral treatment. Urinary excretion was the major route of excretion in humans followed by fecal excretion and urinary glucuronides were the major human metabolites. In contrast, urine, biliary and fecal products were the major metabolites in the nonclinical species.

Glucuronides and oxidative metabolites that are further glucuronidated represent 88-98% of the metabolites in animal species. In contrast, only 12% of the human metabolites were oxidative products (including those that were further glucuronidated) and direct glucuronide conjugates of dapagliflozin represent 88% of the metabolites. The primary human metabolite is dapagliflozin 3-O-glucuronide (also known as m15 or BMS-801576) which accounts for 61% of the human dose and is also a major metabolite (25%) in the bile of bile duct cannulated rats. Dapagliflozin represents the major radioactive component (31%) in the urine in bile duct cannulated rats, followed by oxidative metabolites (19%) and dapagliflozin 3-O-glucuronide (10%); but dapagliflozin is also a minor radioactive component in the bile (8%).

Nishimura M and Naito S: Drug Metab. Pharmacokinet.: 21(5): 357-374 (2006).

Dapagliflozin 3-O-glucuronide is the predominant metabolite in human plasma accounting for 42% of total radioactivity AUC, whereas the parent accounts for 39% of total radioactivity AUC. In contrast, dapagliflozin is the major component (65%) of plasma in bile duct cannulated rats.

Figure 11. Proposed Pathway For the In Vivo Biotransformation of [14C]Dapagliflozin in Mouse, Rats, Dogs and Humans (Dapagliflozin 3-O-glucuronide is m15)



Excretion

Excretion of a single oral dose of [^{14}C]dapagliflozin detected as drug-related radioactivity was recovered mostly in the urine for both the rat and the human, but was fecal in the dog. Total recovery of radioactivity was greater than 90% for all species.

Table 11. Recovery of radioactivity in Rats, Dogs, Mice and Humans Following Oral Administration of [^{14}C]Dapagliflozin

Matrix	Recovery of Radioactivity Mean ± SD			
	Rat	Dog	Mice	Human
Urine	39.83± 4.74	21.63 ± 4.58	39.19	75.0 ± 9.0
Feces	48.99 ± 5.07	72.29 ± 1.68	41.01	21.0 ± 8.8
Cage Rinse	3.25± 1.16	5.19 ± 1.13	5.57	NA
Cage Wash	0.93± 0.56	0.25± 0.07	3.83	NA
Cage Wipe	0.54± 0.48	0.06± 0.07	0.36	NA
Carcass	0.38± 0.05	NA	0.25	NA
Total	93.93 ± 0.92	99.43 ± 1.85	90.21	96.0 ± 1.8

The most important elimination pathway for dapagliflozin in humans appears to be metabolism to the glucuronide followed by urinary excretion and then fecal excretion. In rodents elimination appears to be equally distributed by the urinary and fecal pathways. In the dog the fecal elimination pathway predominates.

Dapagliflozin excretion in rat milk: Dapagliflozin was excreted in the milk of lactating rats at a 0.49x ratio to the plasma at each of 1, 15 or 75 mg/kg (Table 12).

Table 12. Milk to Plasma Dapagliflozin Ratios in Dams

Dose (mg/kg/day)	Time (Post Treatment)	Mean Dapagliflozin Concentration (ng/mL)		Mean Milk-to-Plasma Dapagliflozin Concentration Ratio
		Milk	Plasma	
1	Day 10 2h	289.81	581.45	0.49
15	Day 10 2h	2985.54	6195.97	0.49
75	Day 10 2h	10130.30	21178.79	0.49

However, a radioactivity study with a single oral dose [^{14}C]dapagliflozin at 5.19 mg/kg was conducted with rats treated at day 8-9 following parturition. The blood, plasma and milk C_{max} was achieved at 2 hours. Dapagliflozin was also quantifiable at the last collection time point of 24 hours and resulted in half-lives (t ½) of 3.94, 3.87 and 4.84 hours for the blood, plasma and milk, respectively. The $AUC_{(0-\infty)}$ milk to plasma ratio was 0.76 and was greater than the $AUC_{(0-\infty)}$ blood to plasma ratio of 0.63 and by 12 hours the radioactivity of [^{14}C]dapagliflozin approached that of the plasma.

Table 13. Pharmacokinetic Parameters for Radioactivity in Blood, Plasma and Milk Collected from Female Rats Following A Single Oral Administration of [^{14}C]Dapagliflozin at 5 mg/kg

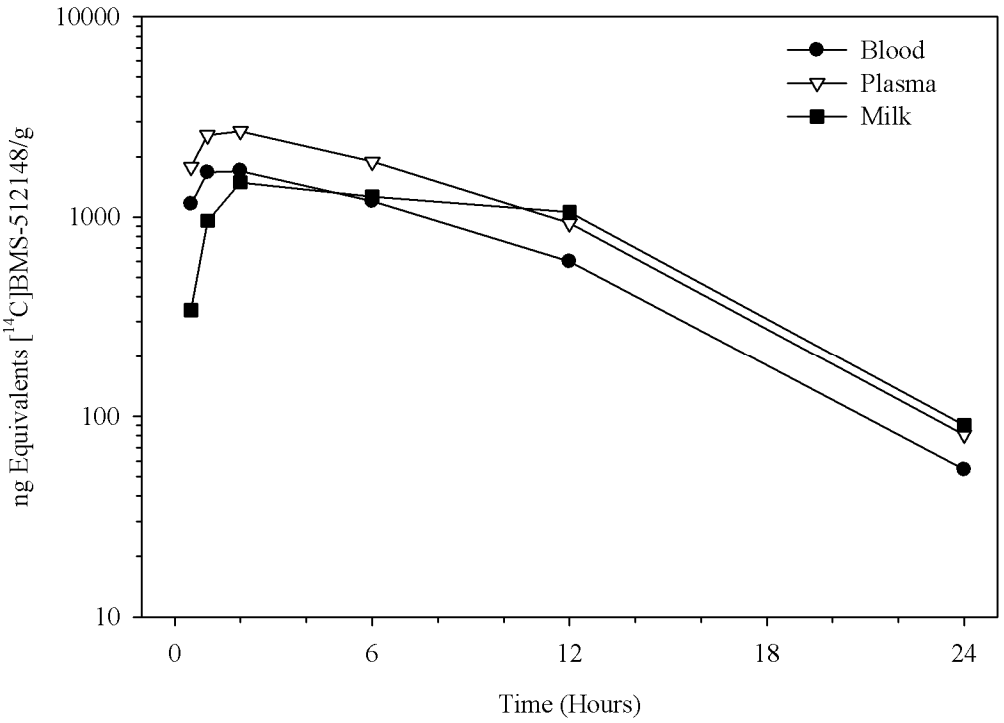
Tissue or matrix	T_{max} (hours)	C_{max} (ng eq/g)	$t_{½}$ (hours)	$AUC_{(0-t)}$ (ng eq·hour/g)	$AUC_{(0-\infty)}$ (ng eq·hour/g)
Blood	2	1700	3.94	17700	18000
Plasma	2	2670	3.87	27700	28200
Milk	2	1480	4.48	20900	21500

eq Equivalents [^{14}C]BMS-512148.

Table 14. C_{max}, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ blood and milk to plasma concentration ratios Following A Single Oral Administration of [^{14}C]Dapagliflozin at 5 mg/kg

	Concentration Ratio		
Tissue or matrix	C_{max}:Plasma C_{max}	$AUC_{(0-t)}$:Plasma $AUC_{(0-t)}$	$AUC_{(0-\infty)}$:Plasma $AUC_{(0-\infty)}$
Blood	0.637	0.639	0.638
Milk	0.554	0.755	0.762

Figure 12. Mean Concentration of Radioactivity in Blood, Plasma and Milk Following a Single Oral Administration of [^{14}C]Dapagliflozin at 5 mg/kg



Pharmacokinetic Drug Interactions
Dapagliflozin was a weak substrate for p-glycoprotein and did not inhibit OAT1 or OCT2. Dapagliflozin and dapagliflozin 3-O-glucuronide were weak inhibitors of hOAT3 with an IC_{50} of 33 and 100 μM, respectively. The C_{max} for dapagliflozin and dapagliflozin 3-O-glucuronide in healthy subjects receiving the maximum clinical dose of 10 mg is 0.49 and 0.56 μM, respectively. These results predict a low probability for pharmacokinetic drug interactions; however dapagliflozin elimination could be affected by inhibitors or inducers of UGT1A9.

5.2 Toxicokinetics

Dedicated toxicokinetic (TK) single dose studies were conducted in the rat and dog. Dedicated repeat dose TK studies were conducted in pregnant rabbits and rats at identical doses used in pivotal embryofetal development studies (Segment 2). All studies were fully reviewed and are summarized below.

Rat (GLP study #DN08040)
Dapagliflozin was administered to SD rats (n=12/sex) at 150 mg/kg and blood collected at 0.5, 1, 2, 4, 8 and 24 hours post-dose for dapagliflozin and dapagliflozin-3-O-glucuronide exposure assessment. No mortality or clinical signs were observed. The TK summary is as follows (sponsor's table):

Table 15. Single Dose Rat TK Summary

Parameter	Dapagliflozin	
	150 mg/kg	
	Male	Female
Cmax (μg/mL)	55.1	68.8
AUC(0-24h) (μg•h/mL)	992	1250
Parameter	**3-O-Glucuronide**	
	Male	**Female**
Cmax (μg/mL)	0.253	0.386
AUC(0-24h) (μg•h/mL)	3.53	4.44

Dapagliflozin-3-O-glucuronide exposure was approximately 0.36% of that of dapagliflozin. Dapagliflozin C_{max} and $AUC_{(0\text{-}24h)}$ were approximately 1.25-fold greater in the females compared to the males. This single dose rat study resulted in exposure to dapagliflozin at 2133x and 2688x MRHD (10 mg, 0.465 ng.h/mL) in males and females, respectively.

Dog (GLP study #DN08039)
Dapagliflozin was administered to Beagle dogs (n=4/sex) at 120 mg/kg and blood collected at 0.5, 1, 2, 4, 8 and 24 hours post-dose for dapagliflozin and dapagliflozin-3-O-glucuronide (BMS-801576) exposure assessment. No mortality or clinical signs were observed, although emesis was noted in 2 males and 2 females at 1 hour and 15 minutes post-dose, respectively. The data from the female dogs was excluded from the analysis. The TK summary is in the sponsor's table below.

Dapagliflozin-3-O-glucuronide exposure was approximately 5% of that of dapagliflozin and approximately 1.9-fold greater in the female than the male. Dapagliflozin C_{max} and $AUC_{(0\text{-}24h)}$ were approximately 1.35-fold greater in the females compared to the males. This single dose dog study resulted in exposure to dapagliflozin at 2752x and 3677x MRHD (10 mg, 0.465 ng.h/mL) in males and females, respectively.

Table 16. Single Dose Dog TK Summary

Parameter	Dapagliflozin	
	120 mg/kg	
	Male	Female[a]
Cmax (μg/mL)	116	159
AUC(0-24 h) (μg•h/mL)	1280	1710
Parameter	**BMS-801576**	
	Male	**Female[a]**
Cmax (μg/mL)	5.85	11.3
AUC(0-24 h) (μg•h/mL)	55.9	106

[a] Mean values were calculated for n = 2.

Pregnant Rat (GLP study #DN04052)
Pregnant dams were treated with dapagliflozin by oral gavage from GD 6 to GD 15 at 37.5, 75, 150 and 300 mg/kg and were evaluated for exposure to dapagliflozin or it's 0-deethylated metabolite BMS-511926. Blood was collected from 4/5 dams per dose group at 0.5, 1, 2, 4, 8 and 24 hours post-dose at GD 15. Plasma TK results are shown for dapagliflozin only in the sponsor's table below:

Table 17. Pregnant Rat Toxicokinetic Summary

Toxicokinetic Parameters of Dapagliflozin in Rat Plasma					
Parameter	**Study Day**	**Dose (mg/kg/day)**			
		37.5	**75**	**150**	**300**
		Female	**Female**	**Female**	**Female**
Cmax (μg/mL)	15	21.7	47.1	67.8	106
AUC (μg•h/mL)	15	327	670	1090	1840

Based on maternal plasma, exposure to dapagliflozin was generally dose proportional in the pregnant rat.

Pregnant Rabbit (GLP study #DN04051)

Pregnant dams were treated with dapagliflozin by oral gavage from GD 7 to GD 19 at 20, 60 and 180 mg/kg/day and were evaluated for exposure to dapagliflozin or it's 0-deethylated metabolite BMS-511926. Blood was collected at 0.5, 1, 2, 4, 8 and 24 hours post-dose at GD 19. Plasma TK results are shown for dapagliflozin only in the sponsor's table below:

Table 18. Pregnant Rabbit Toxicokinetic Summary

Toxicokinetic Parameters of Dapagliflozin in Rabbit Plasma				
Parameter	**Study Day**	**Dose (mg/kg/day)**		
		20	**60**	**180**
		Female	**Female**	**Female**
Cmax (μg/mL)	GD 19	6.36	30.1	85.4
AUC (μg•h/mL)	GD 19	29.7	138	554

Based on maternal plasma, exposure to dapagliflozin increased in a greater than dose proportional manner at 60 mg/kg/day and was also approximately dose proportional at 180 mg/kg day.

6 General Toxicology

6.1 Single-Dose Toxicity

Single dose toxicity studies were conducted in the CD-1 mice, Sprague-Dawley (SD) rats and the Beagle dog. Dapagliflozin was administered by oral gavage in all studies.

Dapagliflozin was lethal in mice at ≥3000 mg/kg and in SD rats at ≥750 mg/kg. A summary of these studies is reported below.

Table 19. Summary of Single Dose Toxicology Studies

SINGLE DOSE TOXICOLOGY STUDIES			
SPECIES/ STUDY	**NOAEL**	**MULTIPLE OF MRHD (10 mg: 0.465 ng.hr/mL)**	**FINDINGS**
Mouse, CD-1 0, 375, 750, 1500 and 3000 mg/kg GLP study #DN02075	750 mg/kg	n.a.	Death 4/5 males and 2/5 females at 3000 mg/kg. Hunched posture and reduced activity at ≥1500 mg/kg No exposure data
Rat, SD 0, 375, 750, 1500 and 3000 mg/kg GLP study #DN02076	375 mg/kg	n.a.	Treatment-related deaths at 750, 1500, 3000mg/kg At ≥750 mg/kg: chromorhinorrhea, reduced BW, unkempt appearance, diarrhea, reduced activity and hunched posture. No exposure data.
Dog, Beagle (Female only) 0, 200, 500 and 1000 mg/kg GLP study #DN06059	1000 mg/kg	n.a.	No adverse effects noted but emesis was observed in all treatment groups. No exposure data.

n.a. – not applicable

6.2 Repeat-Dose Toxicity

General toxicity was assessed in Sprague-Dawley (SD) rats and in Beagle dogs in GLP studies up to 6 month and 12 months in duration, respectively. Exposure to dapagliflozin ranged from 85x to 3097x the 10mg MRHD in the rat and 128x to 3312x the 10mg MRHD in the dog, respectively. In addition, a 3 month toxicity study was conducted in the CD-1 mice. All studies were fully reviewed and are summarized below.

Mice: BMS-512148 Three Month Oral Toxicity Study in Mice
0, 50, 150, 250 and 400 mg/kg

Key Findings

- Dapagliflozin-related deaths were noted at both 250 and 400 mg/kg and necessitated removal of the 400 mg/kg group at day 28.
- Clinical signs in all treatment groups included: rough haircoat, abdominal distension, hunched posture and decreased activity.
- Food consumption was increased (14-49%) in all treatment groups.
- Body weight gain (4-23%) was noted in all groups up to 250 mg/kg.
- No macroscopic or microscopic findings were observed except for an increased prostatic weight (20-23%) in the 150 and 250 mg/kg males.
- The NOAEL is 150 mg/kg which is 653x and 1068x MRHD in males and females respectively.

Rat: BMS-512148: Six –Month Oral Toxicity in Rats (GLP Study #DN05030)
0, 5, 25 and 150 mg/kg

Key Findings

- Mortality was observed at 150 mg/kg in both males and females and cause of death was not determined.
- Body weight was reduced at 25 mg/kg (6%) and 150 mg/kg (8%) in the males at 6 months.
- Increased food consumption and water consumption in all treatment groups.
- Urinary volume, total urine glucose, calcium and phosphorus were increased in all treatment groups.
- Total urinary protein was increased 2-5 fold in all treatment groups with renal microscopic correlates at 150 mg/kg.
- Serum 1, 25 dihydroxy vitamin D (males only) and urinary deoxypyridinolene was reduced in all treatment groups.
- Kidney weight increased (20-81%) in all treatment groups.
- Increased adrenal gland weight (13-36%) that correlated with increased hypertrophy/vacuolation of the zona glomerulosa at 25 and 150 mg/kg.
- Target organ toxicities at 150 mg/kg were observed in the:
    - Kidney (minimal to moderate: chronic progressive nephropathy; minimal to slight: mineral deposits, tubule epithelia hyperplasia and urothelial hyperplasia).
    - Sternum and femur (minimal to marked: increased trabecular bone).

  - Heart and vessels (mineralization).
  - Spleen and liver (extramedullary hematopoiesis in females only).
  - Trachea (mucosal mineralization).
  - Harderian gland (increased pophyrin deposition).
- The NOAEL is 25 mg/kg which is 346x and 675x MRHD in males and females respectively.

Dog: BMS-512148: One Year Oral Toxicity in Dogs (GLP Study #DN05030)
0, 5, 20 and 120 mg/kg

Key Findings

- Mortality was observed in one 5 mg/kg female due to an aspiration pneumonia dosing accident. One 120 mg/kg female was euthanized due to moribundity at study day 172 that was attributed to acute renal toxicity. The sponsor suggests that this was due to tetracycline administered as a bone labeling agent given 4 days prior to the moribund state.
- Mean body weights were 13-17% lower in the treated males and 1-6% lower in the treated females at 12 months.
- Dapagliflozin-dependent dose-related changes included:
  - Increase in urine volume, total urine glucose, calcium and phosphorus.
  - Decreased urine osmolality.
  - Increase in food consumption and water consumption.
  - Increase in absolute and relative adrenal gland weight (males and females), liver (males) and kidney (males).
- No gross microscopy changes were observed.
- Minimal to slight suburothelial inflammation was present in the renal pelvis and/or papilla of:
  - One 120 mg/kg female at 6 month necropsy
  - One 120 mg/kg female at 12 month necropsy
  - One 5 mg/kg female at 6 month necropsy
  - One 20 mg/kg female at 12 month necropsy
- Urothelial hyperplasia of the bladder and/or lining of the renal pelvis or papilla sometimes accompanied the suburothelial inflammation in the kidney.
- The NOAEL was 20 mg/kg which is 561x and 619x MRHD in males and females respectively.

7 Genetic Toxicology

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

BMS-512148: Investigative reverse mutation study in *Salmonella typhimurium* and *Escherichia coli*

Study no.:	DS03100
Study report location:	eCTD
Conducting laboratory and location:	BMS Pharmaceutical Research Institute Dept. of Genetic Toxicology Syracuse, NY USA
Date of study initiation:	May 6th 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BMS-512148, 2J612857 and 82%

Key Study Findings

A previous non-GLP Ames assay with and without S9 was used to determine the concentration to be tested in the present study. Dapagliflozin was not mutagenic in any bacterial strain tested in the non-GLP study. In the present study dapagliflozin was tested using S. Typhimurium and E. Coli strains (with S9 only) up to a concentration of 5000 μg/plate. Dapagliflozin did not induce a dose-dependent increase in histidine and tryptophan revertants.

Results of this study are also consistent with prior GLP study that evaluated up to 2mg dapagliflozin per plate in the Ames assay.

Study Validity

Study was valid. Dose selection for the plate incorporation method was adequate based on the limit dose of 5000 μg/plate. Dapagliflozin was tested in triplicate cultures and the positive control gave the expected results.

Results

When tested to a maximum concentration of 5000 μg/plate, dapagliflozin was not mutagenic in the presence of S9 (sponsor's table below).

Table 20. Mean Revertant Counts

In the Presence of S9 Metabolic Activation[1]

Test Article	(µg/plate)	TA98	TA100	TA1535	TA1537	WP2 *uvrA*
BMS-512148 (in DMSO)	0	31 ± 4	128 ± 13	11 ± 2	5 ± 1	17 ± 5
	62	27 ± 4	128 ± 6	10 ± 2	5 ± 2	-
	125	33 ± 4	137 ± 17	13 ± 1	5 ± 1	18 ± 3
	250	27 ± 3	129 ± 7	12 ± 3	6 ± 3	16 ± 4
	500	28 ± 2	132 ± 6	12 ± 6	6 ± 3	17 ± 4
	1000	22 ± 6	116 ± 21	10 ± 3	7 ± 2	17 ± 6
	2000	19 ± 2	77 ± 7	11 ± 2	7 ± 2	16 ± 4
	5000	-	-	-	-	11 ± 3
2-AA	2.5	2564	2694	510	374	-
2-AA	10	-	-	-	-	551

[1]Mean ± Standard Deviation
2-AA, 2-animoanthracene; 2-NF, 2-nitrofluorene; Na Az, sodium azide;
9-AA, 9-aminoacridine; MMS, methyl methane-sulfonate
- Not tested
* Unable to score due to excessive cytotoxicity

7.2 *In Vitro* Assays in Mammalian Cells

BMS-512148: Cytogenetic study in Chinese Hamster Ovary cells

Study no.: DS03038
Study report location: eCTD
Conducting laboratory and location: (b) (4)
Date of study initiation: February 10th 2003
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BMS-512148, 2J61857 and 82%

Key Study Findings

Dapagliflozin was clastogenic in the presence of metabolic activation S9

Methods

Cell line: CHO
Concentrations in definitive study: 12.5, 25, 50, 75, 100, 125, 150, 175, 200,

	210 and 250 μg/mL
Basis of concentration selection:	From preliminary cytotoxicity test
Negative control:	DMSO
Positive control:	MMC (-S9) and CP (+S9)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	+S9: 4 hr and 16 hr and -S9: 4 hr and 16 hr and 20 hr alone.

Study Validity

Duplicate cultures for each concentration were used in the definitive assay. A minimum of 200 metaphase spreads were examined and scored for chromatid-type and chromosome-type aberrations. The criteria for a valid test were defined as: the frequency of cells with structural chromosome aberrations in the solvent control must be within the range of the historical control. The percentage of cells with chromosome aberration in the positive control must be statistically increased relative to the solvent control. The assay results will be considered positive when the percentage of cells with aberration is increased in a dose-responsive manner with one or more concentrations being statistically significant. Based on these, the study is deemed valid.

Results

In the 4-hr treatment with S9, a statistically significant (ss) and dose-dependent increase in the frequency of chromosome aberrations (structural) was observed. The frequency of chromosome aberrations was 3, 11, and 25% at concentrations of 100, 150, and 250 μg/mL, respectively, compared to 0% in the vehicle control and 27% in the positive control. There was no significant increase in the frequency of chromosome aberrations in the 4-hr or 20-hr treatment in the absence of S9 with 50% cell cytotoxicity (cell growth inhibition) (see sponsor's tables below)

Table 21. Summary of Chromosomal Aberrations in CHO with Dapagliflozin

Treatment (µg/mL)	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored	Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations Numerical (%)	Structural (%)
DMSO	+	4	15.4	200	0.000	±0.000	3.5	0.0
BMS-512148								
50	+	4	12.3	200	0.000	±0.000	4.5	0.0
100	+	4	10.8	200	0.030	±0.171	1.5	3.0*
150	+	4	13.8	200	0.125	±0.374	6.0	11.0**
250	+	4	8.0	100	0.410	±0.818	2.5	25.0**
CP, 10	+	4	11.5	100	0.470	±1.453	2.0	27.0**
DMSO	-	20	15.1	200	0.000	±0.000	3.5	0.0
BMS-512148								
25	-	20	15.2	200	0.000	±0.000	4.0	0.0
50	-	20	15.1	200	0.000	±0.000	2.5	0.0
150	-	20	14.3	200	0.000	±0.000	3.5	0.0
MMC, 0.1	-	20	13.1	100	0.490	±0.937	4.5	28.0**

Treatment: Cells from all treatment conditions were harvested 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: *, p≤0.05; **, p≤0.01; using Fisher's exact test.

Table 22. Summary of Chromosomal Aberrations in CHO with Dapagliflozin with 50% cell cytotoxicity (cell growth inhibition) in the absence of S9

Treatment (µg/mL)	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored	Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations Numerical (%)	Structural (%)
DMSO	-	4	12.4	200	0.000	±0.000	4.0	0.0
BMS-512148								
50	-	4	7.7	200	0.000	±0.000	4.0	0.0
100	-	4	10.1	200	0.000	±0.000	3.5	0.0
210	-	4	5.9	200	0.005	±0.071	3.5	0.5
MMC, 0.2	-	4	9.5	200	0.135	±0.409	3.5	11.5**

Treatment: Cells from all treatment conditions were harvested 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: *, p≤0.05; **, p≤0.01; using Fisher's exact test.

Table 23. Historical Control Values For Chromosomal Aberration Assay (Sponsor's Tables)

IN VITRO MAMMALIAN CYTOGENETIC TEST USING
CHINESE HAMSTER OVARY (CHO) CELLS

HISTORICAL CONTROL VALUES
STRUCTURAL ABERRATIONS
2000-2002

NON-ACTIVATED TEST SYSTEM

Historical Values	Solvent (%)	Positive Control[2] (%)
Mean	1.1	20.7
±SD[1]	1.3	11.6
Range	0.0-5.5	7.5-87.0

S9-ACTIVATED TEST SYSTEM

Historical Values	Solvent (%)	Positive Control[3] (%)
Mean	1.4	29.5
±SD[1]	1.5	16.9
Range	0.0-6.5	8.0-84.0

1 SD = standard deviation.
2 Positive control for non-activated studies, Mitomycin C (MMC, 0.08-0.2 µg/mL).
3 Positive control for S9-activated studies, cyclophosphamide (CP, 10-60 µg/mL).

IN VITRO MAMMALIAN CYTOGENETIC TEST USING
CHINESE HAMSTER OVARY (CHO) CELLS

HISTORICAL CONTROL VALUES
COMBINED NUMERICAL ABERRATIONS
(POLYPLOID AND ENDOREDUPLICATED CELLS)
2000-2002

NON-ACTIVATED TEST SYSTEM

Historical Values	Solvent (%)	Positive Control[2] (%)
Mean	2.1	2.8
±SD[1]	1.4	1.6
Range	0.0-7.5	0.0-8.5

S9-ACTIVATED TEST SYSTEM

Historical Values	Solvent (%)	Positive Control[3] (%)
Mean	2.9	2.7
±SD[1]	1.8	1.4
Range	0.0-11.0	0.0-6.5

1 SD = standard deviation.
2 Positive control for non-activated studies, Mitomycin C (MMC, 0.08-0.2 µg/mL).
3 Positive control for S9-activated studies, cyclophosphamide (CP, 10-60 µg/mL).

BMS-512148: Comparative Cytogenetic study in Chinese Hamster Ovary cells

Study no.:	DS03090
Study report location:	eCTD
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 23 2003
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	BMS-512148-05 (b) (4) batch 2J61857), 82% and BMS-512148-01 ((b) (4) batch 53702-019-26), 92%

Key Study Findings

Dapagliflozin ((b) (4) and also (b) (4) dapagliflozin were clastogenic in the presence of metabolic activation (S9).

Methods

Cell line:	CHO
Concentrations in definitive study:	200, 250, 300, 350 and 400 μg/mL
Basis of concentration selection:	From preliminary cytotoxicity test
Negative control:	DMSO
Positive control:	CP (+S9)
Formulation/Vehicle:	DMSO and DMSO + propylene glycol
Incubation & sampling time:	+S9: 4 hr and 16 hr and -S9: 4 hr and 16 hr and 20 hr alone.

Study Validity

Duplicate cultures for each concentration were used in the definitive assay. A minimum of 200 metaphase spreads were examined and scored for chromatid-type and chromosome-type aberrations. The criteria for a valid test were defined as that: the frequency of cells with structural chromosome aberrations in the solvent control must be within the range of the historical control. The percentage of cells with chromosome aberration in the positive control must be statistically increased relative to the solvent control. The assay results will be considered positive when the percentage of cells with aberration is increased in a dose-responsive manner with one or more concentrations being statistically significant. Based on these, the study is deemed valid.

Results

With BMS-512148-05 ((b) (4) in the presence of S9 activation, the percentage of cells with structural aberrations was ss increased at concentrations of 200, 250, and 300 µg/mL. The corresponding frequency of structural aberrations was 3, 3, and 43.9% compared to 0% in the vehicle control (see sponsor's summary below).

With BMS-512148-01 (b) (4) in the presence of S9 activation, the percentage of cells with structural aberrations was ss increased at concentration s of 200, 250, and 300 µg/mL. The corresponding frequency of aberrations was 2.5, 3, and 46% compared to 0% in the vehicle control (see sponsor's summary below).

Table 24. Summary of Chromosomal Aberrations in CHO with Dapagliflozin

Treatment (µg/mL)	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored	Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations Numerical (%)	Cells With Aberrations Structural (%)
DMSO + Propylene glycol	+	4	8.6	200	0.030	±0.198	4.0	3.0
DMSO	+	4	9.8	200	0.000	±0.000	1.5	0.0
BMS-512148-05 (b) (4)								
200	+	4	7.3	200	0.030	±0.171	6.0*	3.0*
250	+	4	7.5	200	0.040	±0.262	4.5	3.0*
300	+	4	1.2	66‡	0.879	±1.353	0.0	43.9**
CP, 10	+	4	5.7	200	0.350	±1.138	0.5	14.5**
DMSO + Propylene glycol	+	4	8.6	200	0.030	±0.198	4.0	3.0
DMSO	+	4	9.8	200	0.000	±0.000	1.5	0.0
BMS-512148-01 ((b) (4)								
200	+	4	8.1	200	0.025	±0.157	6.0*	2.5*
250	+	4	6.2	200	0.030	±0.171	7.5**	3.0*
300	+	4	3.5	100‡	0.810	±1.361	2.0	46.0**
CP, 10	+	4	5.7	200	0.350	±1.138	0.5	14.5**

‡ Numerical aberrations are out of 200 cells scored.
Treatment: Cells from all treatment conditions were harvested 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: *, p≤0.05; **, p≤0.01; using Fisher's exact test.

Table 25. Historical Control Values For Chromosomal Aberration Assay (Sponsor's Tables)

IN VITRO MAMMALIAN CYTOGENETIC TEST USING
CHINESE HAMSTER OVARY (CHO) CELLS

HISTORICAL CONTROL VALUES
STRUCTURAL ABERRATIONS
2000-2002

NON-ACTIVATED TEST SYSTEM

Historical Values	Solvent (%)	Positive Control[2] (%)
Mean	1.1	20.7
±SD[1]	1.3	11.6
Range	0.0-5.5	7.5-87.0

S9-ACTIVATED TEST SYSTEM

Historical Values	Solvent (%)	Positive Control[3] (%)
Mean	1.4	29.5
±SD[1]	1.5	16.9
Range	0.0-6.5	8.0-84.0

1 SD = standard deviation.
2 Positive control for non-activated studies, Mitomycin C (MMC, 0.08-0.2 µg/mL).
3 Positive control for S9-activated studies, cyclophosphamide (CP, 10-60 µg/mL).

IN VITRO MAMMALIAN CYTOGENETIC TEST USING
CHINESE HAMSTER OVARY (CHO) CELLS

HISTORICAL CONTROL VALUES
COMBINED NUMERICAL ABERRATIONS
(POLYPLOID AND ENDOREDUPLICATED CELLS)
2000-2002

NON-ACTIVATED TEST SYSTEM

Historical Values	Solvent (%)	Positive Control[2] (%)
Mean	2.1	2.8
±SD[1]	1.4	1.6
Range	0.0-7.5	0.0-8.5

S9-ACTIVATED TEST SYSTEM

Historical Values	Solvent (%)	Positive Control[3] (%)
Mean	2.9	2.7
±SD[1]	1.8	1.4
Range	0.0-11.0	0.0-6.5

1 SD = standard deviation.
2 Positive control for non-activated studies, Mitomycin C (MMC, 0.08-0.2 µg/mL).
3 Positive control for S9-activated studies, cyclophosphamide (CP, 10-60 µg/mL).

BMS-512148: Investigative Cytogenetics and Cytotoxicity Study in Chinese Hamster Ovary cells

Study no.:	DS04143
Study report location:	eCTD
Conducting laboratory and location:	BMS Pharmaceutical Research Institute Dept. of Genetic Toxicology Syracuse, NY USA
Date of study initiation:	June 8th 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BMS-512148-05, 2J61857 and 81.8%

Key Study Findings

Dapagliflozin was clastogenic in the presence of metabolic activation (S9) at concentrations that were not excessively cytotoxic.

Methods

Cell line:	CHO
Concentrations in definitive study:	170-270 μg/mL
Basis of concentration selection:	From previous cytogenetic studies
Negative control:	DMSO
Positive control:	CP (+S9)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	+S9: 4 hr

Study Validity

Duplicate cultures for each concentration were used in the definitive assay. A minimum of 200 metaphase spreads were examined and scored for chromatid-type and chromosome-type aberrations. The criteria for a valid test were defined as that: the frequency of cells with structural chromosome aberrations in the solvent control must be within the range of the historical control. The percentage of cells with chromosome aberration in the positive control must be statistically increased relative to the solvent control. The assay results will be considered positive when the percentage of cells with aberration is increased in a dose-responsive manner with one or more concentrations being statistically significant. Based on these, the study is deemed valid.

Results

In the definitive trial, 4 hour exposure to dapagliflozin, in the presence of S9 activation, resulted in a concentration dependent ss increase in structural aberrations. Numerical aberrations were not increased. The corresponding frequency of structural aberrations was 9 and 20% compared to 1% in the vehicle control (see sponsor's summary table below). Cell growth inhibition ranged from 34-54% in cells with structural aberrations.

Table 26. Summary of Chromosomal Aberrations at 4 hr in CHO with Dapagliflozin and Metabolic Activation

	CYTOTOXICITY					PERCENT CELLS WITH ABERRATIONS	
TREATMENT	CELL GROWTH INHIBITION	MITOTIC INDEX	CELLS SCORED	TOTAL STRUCTURAL ABERRATIONS[1]	STRUCTURAL ABERRATIONS PER CELL[2]	NUMERICAL[3]	STRUCTURAL[4]
DMSO	0%	4.2%	200	2	0.010	4.0%	1.0%
BMS-512148 (µg/ml)							
170	31%	-[5]	-	-	-	-	-
180	30%	-	-	-	-	-	-
190	6%	-	-	-	-	-	-
200	34%	2.5%	200	5	0.025	6.0%	2.5%
210	14%	-	-	-	-	-	-
220	40%	2.7%	200	22	0.110	4.0%	9.0%**
230	58%	3.1%	-	-	-	4.0%	-
240	54%	1.3%	200	55	0.275	6.0%	20.0%**
250	66%	-	-	-	-	-	-
260	56%	-	-	-	-	-	-
270	69%	-	-	-	-	-	-
Cyclophosphamide (µg/ml)							
10	40%	1.3%	100	83	0.830	3.0%	56.0%**

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

Two-week Oral Investigative Study in Rats with Micronucleus Evaluation

Study no: DN03086
Study report location: eCTD
Conducting laboratory and location: Bristol-Myer Squibb Pharmaceutical Research Institute, One Squibb Drive, New Brunswick, NJ 08903 and 6000 Thompson Road, East Syracuse, NY 13057
Date of study initiation: August 26th 2003
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BMS-512148, 2J61857 and 82%

Key Study Findings

Dapagliflozin was negative in the in vivo rat bone marrow micronucleus assay.

Methods

Doses in definitive study:	0, 75, 150, 200 and 250 mg/kg
Frequency of dosing:	Daily
Route of administration:	PO gavage
Dose volume:	075 – 2.5 mL/kg
Formulation/Vehicle:	90% PEG400 in water
Species/Strain:	Rat/Sprague-Dawley
Number/Sex/Group:	7 males per group
Satellite groups:	500 and 700 mg.kg for TK evaluation
Basis of dose selection:	DRF study
Negative control:	90% PEG400 in water
Positive control:	None. CP used in DRF and HC data provided

Study Validity

TK data confirm exposure. Positive result induced by cyclophosphamide (CP) in DRF study.

Results

Treatment with dapagliflozin for 14 days in the rat did not result in an increase in bone marrow micronucleated polychromatic erythorcytes (MN-PCE).

Table 27. Summary of Rat Micronucleus Bone Marrow Assessment (sponsor's table)

Summary of Bone-Marrow Analysis

Article	Dose (mg/kg)	Sex	No. Rats Evaluated	Mean % PCE (±SD)	Mean % MN-PCE (±SD)
vehicle (90% PEG 400)	0	M	5	35 ± 3.7	0.11 ± 0.03
BMS-512148	75	M	5	35 ± 3.0	0.19 ± 0.09
BMS-512148	150	M	5	37 ± 2.8	0.21 ± 0.11
BMS-512148	200	M	5	36 ± 2.9	0.18 ± 0.13
BMS-512148	250	M	5	37 ± 2.4	0.16 ± 0.07

Table 28. Summary of Historical Control (HC) Data in Two week Rat Bone Marrow Studies (sponsor's table)

Summary of Historical Negative-Control Data in Rat Bone Marrow from Two-Week Oral Studies

Study Number	Control Article	Sex	Number of Animals Evaluated	Mean % PCE (±SD)	Mean % MN-PCE (±SD)
96037	85% Capmul in Water	M	4	46 ± 6.0	0.36 ± 0.26
		F	4	44 ± 3.6	0.23 ± 0.08
97206	PEG-400	M	4	59 ± 3.1	0.12 ± 0.05
		F	4	55 ± 2.0	0.19 ± 0.15
98341	D5W/Tween-80/ETOH/PEG-300	M	5	59 ± 10.2	0.19 ± 0.06
		F	5	49 ± 5.7	0.23 ± 0.12
98023	M-Q H_2O	M	4	52 ± 5.2	0.29 ± 0.10
		F	4	55 ± 4.9	0.30 ± 0.10
98651	PEG-400/Tween-80	M	5	59 ± 3.7	0.30 ± 0.10
		F	5	60 ± 4.7	0.25 ± 0.11
DM00018	0.5% Methocel	M	5	44 ± 2.4	0.07 ± 0.07
		F	5	52 ± 5.1	0.14 ± 0.07
DN02055	PEG-400	M	5	39 ± 4.7	0.20 ± 0.07

7.4 Other Genetic Toxicity Studies

BMS-512148: One Month Oral In Vivo/In Vitro Cytogenetics Study in Rat Peripheral Blood Lymphocytes

Study no.: DS05023
Study report location: eCTD
Conducting laboratory and location: (b) (4)
Date of study initiation: March 11 2005
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BMS-512148, 4B73220 and 81.1%

Key Study Findings

Dapagliflozin was not clastogenic in peripheral blood lymphocytes (PBL) from rats treated with dapagliflozin for one month at up to 200 mg/kg.

Methods

Doses in definitive study:	0, 25, 100, 150 and 200 mg/kg
Frequency of dosing:	Daily
Route of administration:	PO gavage
Dose volume:	4 mL/kg
Formulation/Vehicle:	90% PEG 400 in water
Species/Strain:	Rat/Sprague-Dawley (SD)
Number/Sex/Group:	10/sex/group
Satellite groups:	None
Basis of dose selection:	Prior cytogenetic studies
Negative control:	90% PEG 400 in water
Positive control:	Cyclophosphamide (CP) 60 mg/kg

Study Validity

The criteria for a valid test were defined as: the frequency of cells with structural chromosome aberrations in the solvent control must be within the range of the historical control. The percentage of cells with chromosome aberration in the positive control must be statistically increased relative to the solvent control. The assay results will be considered positive when the percentage of cells with aberration is increased in a dose-responsive manner with one or more concentrations being statistically significant. Based on the positive control data where chromosomal aberration was detected, the study is deemed valid.

Results

There was no statistically significant (ss) increase in the number of cells with structural chromosomal aberrations in either the male and female rats when treated with dapagliflozin at 100, 150 or 200 mg/kg. CP induced a ss increase in the in the number of cells with structural chromosomal aberrations in both males and females (sponsor's table below):

Table 29. Male Summary of Chromosomal Aberrations in PBL with Dapagliflozin Treatment for One Month.

Assay No.: 26947-0-444 Lab No.: CY050605
Test Article: BMS-512148 Initiation of Dosing: 04/06/05

Treatment	Dose Level	Harvest Time (~ hr after culture initiation)	Number of Animals	Total Number of Cells Analyzed for Aberrations	% –g Group Mean ± S.E.	% +g Group Mean ± S.E.	Judge-ment (+/-)[a]	% Polyploidy Group Mean ± S.E.	% Endoreduplication Group Mean ± S.E.	Judge-ment (+/-)[b]	% Mitotic Index Group Mean ± S.E.
Controls											
90% PEG 400	0.0 mg/kg	46	5	500	0.4 ± 0.24	2.8 ± 0.97	-	0.2 ± 0.20	0.0 ± 0.00	-	3.8 ± 0.22
Cyclophosphamide	60 mg/kg	46	5	250	76.0 ± 2.61	80.4 ± 1.60	+	0.0 ± 0.00	0.0 ± 0.00	-	1.3 ± 0.46
Test Article											
	100 mg/kg	46	5	500	1.2 ± 0.58	4.8 ± 1.16	-	0.2 ± 0.20	0.0 ± 0.00	-	4.5 ± 0.79
	150 mg/kg	46	5	500	1.2 ± 0.58	3.8 ± 0.86	-	0.0 ± 0.00	0.0 ± 0.00	-	4.5 ± 0.88
	200 mg/kg	46	5	500	2.2 ± 0.92	6.4 ± 1.96	-	0.4 ± 0.24	0.0 ± 0.00	-	3.1 ± 0.58

% -g = % of cells with chromosome aberrations.
% +g = % of cells with chromosome aberrations + % of cells with gaps.
90% PEG 400 = 90% polyethylene glycol 400 v/v in water
[a] Significantly greater in -g than the corresponding vehicle control, $p \leq 0.05$.
[b] Significantly greater in polyploidy and endoreduplication than the corresponding vehicle control, $p \leq 0.05$.

Table 30. Female Summary of Chromosomal Aberrations in PBL with Dapagliflozin Treatment for One Month.

Assay No.: 26947-0-444 Lab No.: CY050605
Test Article: BMS-512148 Initiation of Dosing: 04/06/05

Treatment	Dose Level	Harvest Time (~ hr after culture initiation)	Number of Animals	Total Number of Cells Analyzed for Aberrations	% –g Group Mean ± S.E.	% +g Group Mean ± S.E.	Judge-ment (+/-)[a]	% Polyploidy Group Mean ± S.E.	% Endoreduplication Group Mean ± S.E.	Judge-ment (+/-)[b]	% Mitotic Index Group Mean ± S.E.
Controls											
90% PEG 400	0.0 mg/kg	46	5	500	0.4 ± 0.24	4.2 ± 0.66	-	0.0 ± 0.00	0.0 ± 0.00	-	3.8 ± 1.10
Cyclophosphamide	60 mg/kg	46	5	350	60.4 ± 9.13	67.7 ± 7.60	+	0.0 ± 0.00	0.0 ± 0.00	-	1.4 ± 0.52
Test Article											
	100 mg/kg	46	5	500	0.8 ± 0.37	4.4 ± 1.29	-	0.0 ± 0.00	0.0 ± 0.00	-	4.2 ± 0.79
	150 mg/kg	46	5	500	0.2 ± 0.20	3.6 ± 0.98	-	0.0 ± 0.00	0.0 ± 0.00	-	1.8 ± 0.43
	200 mg/kg	46	5	500	0.8 ± 0.37	3.2 ± 0.86	-	0.0 ± 0.00	0.0 ± 0.00	-	2.8 ± 0.34

% -g = % of cells with chromosome aberrations.
% +g = % of cells with chromosome aberrations + % of cells with gaps.
90% PEG 400 = 90% polyethylene glycol 400 v/v in water
[a] Significantly greater in -g than the corresponding vehicle control, $p \leq 0.05$.
[b] Significantly greater in polyploidy and endoreduplication than the corresponding vehicle control, $p \leq 0.05$.

Unscheduled DNA Synthesis (UDS) Assay: The test system was male rat hepatocytes. Dapagliflozin was administered as a single oral dose to male rats at 175, 350 and 700 mg/kg (n=10/group), respectively. Dimethylnitrosamine (DMN) at 35 mg/kg and 90% PEG 400 in water (w/v) served as the positive and negative control, respectively. Animals were euthanized at 2-4 hours or 12-16 hours post-dose and primary hepatocyte cultures established at both time points. Hepatocytes were incubated with ^{3}H-thymidine for 4 hours and the nuclear net grains counted for each group using autoradiography. The mean net nuclear grain (NG) counts were not increased for the dapagliflozin-treated animals. The positive control (DMN) mean NG counts were 24.8 at 2-4 hours

and 30.3 at 12-16 hours, respectively, suggesting ≥ 98% cells in repair. Sponsor's tables below:

Table 31. Two to Four hour Exposure for the UDS Assay for Dapagliflozin

Group	Ear Tag Number	Slide Code	No. of Cells Scored	per Animal: Mean Grain Counts ± S.D.[1] Nuclear	Cytoplasmic	Net per Nucleus	Cells in Repair		per Treatment Group: Mean Net ± S.D.#[2]	Cells in Repair
90% Polyethylene 400 (PEG 400) in water (w/v)										
	151	32	150	5.7 ± 2.6	7.0 ± 3.0	-1.3 ± 2.7	1%			
Vehicle	152	27	150	6.1 ± 2.6	7.2 ± 3.3	-1.1 ± 3.1	3%		-1.3 ± 0.2	2%
	153	28	150	4.4 ± 2.3	6.0 ± 3.0	-1.5 ± 2.5	1%			
BMS-512148 (mg/kg)										
	194	19	150	6.0 ± 3.6	7.3 ± 3.4	-1.3 ± 3.3	3%			
175	195	20	150	6.1 ± 3.2	7.1 ± 3.2	-1.0 ± 3.1	3%		-1.0 ± 0.3	3%
	196	34	150	5.0 ± 2.3	5.8 ± 2.3	-0.7 ± 2.3	1%			
	161	11	150	5.1 ± 2.4	5.9 ± 2.9	-0.8 ± 2.9	3%			
350	162	33	150	6.2 ± 2.7	7.7 ± 3.1	-1.4 ± 2.8	1%		-1.1 ± 0.3	2%
	163	35	150	5.5 ± 2.8	6.4 ± 3.6	-0.9 ± 2.5	1%			
	166	12	150	6.0 ± 3.2	6.8 ± 3.6	-0.9 ± 3.0	3%			
700	167	23	150	5.7 ± 2.7	7.0 ± 2.6	-1.3 ± 2.8	1%		-1.2 ± 0.3	3%
	168	36	150	6.0 ± 3.4	7.5 ± 3.1	-1.5 ± 3.3	5%			
Positive Control: Dimethylnitrosamine (mg/kg)										
	171	22	150	30.1 ± 10.4	3.2 ± 2.2	26.9 ± 10.6	99%			
35	172	39	+					*	24.8 ± 2.9	98%
	173	37	150	26.4 ± 11.2	3.7 ± 2.2	22.7 ± 11.2	97%			

[1] Standard deviation reflecting slide to slide variation
[2] S.D.#: Standard deviation reflecting variation between animals
* Significant (see protocol Section 9.0, Evaluation of Test Results)
+ Not scored due to a lack of scorable cells on all slides.

Table 32. Twelve to Sixteen hour Exposure for the UDS Assay for Dapagliflozin

Group	Ear Tag Number	Slide Code	No. of Cells Scored	per Animal: Mean Grain Counts ± S.D.[1] Nuclear	Cytoplasmic	Net per Nucleus	Cells in Repair		per Treatment Group: Mean Net ± S.D.#[2]	Cells in Repair
90% Polyethylene 400 (PEG 400) in water (w/v)										
	1	21	150	7.8 ± 3.9	11.4 ± 4.4	-3.6 ± 4.4	3%			
Vehicle	2	26	150	6.0 ± 2.9	8.4 ± 3.1	-2.4 ± 2.9	1%		-2.1 ± 1.6	3%
	3	25	150	4.8 ± 2.8	5.1 ± 3.4	-0.4 ± 3.1	5%			
BMS-512148 (mg/kg)										
	6	29	150	7.0 ± 3.1	7.8 ± 3.2	-0.9 ± 3.2	4%			
175	7	30	150	8.1 ± 3.8	10.2 ± 4.1	-2.1 ± 3.5	4%		-1.4 ± 0.7	5%
	8	18	150	8.0 ± 3.7	9.2 ± 3.6	-1.1 ± 3.5	7%			
	14	40	150	8.0 ± 3.7	10.1 ± 4.3	-2.1 ± 3.8	5%			
350	15	38	150	6.1 ± 3.0	6.9 ± 3.1	-0.9 ± 3.0	4%		-1.6 ± 0.6	4%
	13	17	150	8.0 ± 3.6	9.8 ± 4.5	-1.8 ± 3.5	3%			
	16	31	150	8.3 ± 4.2	10.8 ± 4.5	-2.5 ± 4.0	3%			
700	17	14	150	7.7 ± 3.1	8.1 ± 4.6	-0.4 ± 3.6	7%		-1.6 ± 1.1	5%
	19	15	150	8.2 ± 3.7	10.1 ± 4.0	-1.9 ± 3.8	4%			
Positive Control: Dimethylnitrosamine (mg/kg)										
	21	16	150	36.7 ± 13.8	4.3 ± 2.8	32.4 ± 13.8	100%			
35	22	13	150	35.3 ± 11.7	5.4 ± 4.8	30.0 ± 11.8	99%	*	30.3 ± 2.0	100%
	23	24	150	32.7 ± 11.3	4.2 ± 2.6	28.5 ± 11.1	100%			

[1] Standard deviation reflecting slide to slide variation

[2] S.D.#: Standard deviation reflecting variation between animals

* Significant (see protocol Section 9.0, Evaluation of Test Results)

8 Carcinogenicity

BMS-512148 Oral Carcinogenicity Study in Rats

Study no.:	DN06073
Study report location:	eCTD
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	January 23 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BMS-512148, Dapagliflozin, Lot # 5J04457-02, purity 80.8-81.6%.
CAC concurrence:	Yes

Key Study Findings

Statistically Significant Neoplastic Findings

- Skin tumors (benign keratoacanthoma) were independently identified by FDA biostatistics staff using a pair-wise analysis and showed statistical significance ($p<0.04$) and increased incidence in the mid dose males when compared to the water control males.

Non-Neoplastic Findings:

- The sponsor eliminated the HD dose (25 mg/kg) at week 25. ECAC concurred.
- Kidney: Increased incidence and severity of chronic progressive nephropathy in the high dose males. This resulted in increased incidence of decedents in the high dose males.
- Adrenal gland: Hypertrophy/vacuolation was noted dose-independently in the dapagliflozin treated animals.
- Bone: The incidence of trabecular bone was increased in the high dose males and females. The severity of increased trabecular bone was slightly increased in the high dose females.

Maximum Clinical Exposure: 10mg/day, 465 ng.h/ml. The high dose tested in this study achieved exposures of 131-fold and 186-fold the clinical exposure in males and females, respectively.

Adequacy of Carcinogenicity Study

The final study report of a GLP-compliant standard two year oral gavage carcinogenicity study in the Sprague Dawley rat was reviewed and the results were discussed at a meeting of the Executive Carcinogenicity Assessment Committee (ECAC). The ECAC and the division considers the rat study an adequate assessment of carcinogenic potential because dapagliflozin reached a ≥25x exposure margin in both males and

females and showed the high dose to be at the MTD due to reduced body weight compared to the controls.

Appropriateness of Test Models

The sponsor chose doses of dapagliflozin (BMS-512148) at 0.5, 2, 10 and 25 mg/kg/day based on the recommendations of the ECAC. The 25 mg/kg dose was removed (b) (4) Overall treatment was well tolerated and the results showed no dose-limiting toxicity up to the highest dose tested. Exposure at the high dose (10 mg/kg) provided approximately 131x and 186x MRHD in males and females, respectively, based on total exposure (AUC_{0-24}).

Evaluation of Tumor Findings

There were no tumor increases in any dapagliflozin treatment group that were considered treatment-related or biologically significant.

Methods

Doses:	0, 0, 0.5, 2, 10 and 25mg/kg
Frequency of dosing:	Once daily
Dose volume:	4mL/Kg
Route of administration:	P.O. (Oral gavage)
Formulation/Vehicle:	C1: 90% (v/v) PEG 400 C2:Distilled water
Basis of dose selection:	Dose selection was done with ECAC concurrence.
Species/Strain:	Hsd: Sprague-Dawley: SD® Rats (b) (4)
Number/Sex/Group:	70/sex/group
Age:	6 weeks
Animal housing:	The animals were individually housed in suspended, stainless steel, wire-mesh type cages
Paradigm for dietary restriction:	Certified Global Rodent Diet No. 2018C or 2016C (Harlan Teklad) and water *ad libitum*
Dual control employed:	Yes
Interim sacrifice:	No
Satellite groups:	TK; 10/sex/group dosed for 6 months
Deviation from study protocol:	None that affected study outcome

Observations and Results

Mortality

Due to a reduction in survival, males were terminated between weeks 89 to 91. Females were terminated as scheduled. At termination the vehicle (90% PEG 400 in water) treated males had a slightly lower survivorship than the 10 mg/kg males (29% vs

31%). The summary of survivorship is shown in the sponsor's tables below (high dose 25 mg/kg animals were excluded). Sponsor's table below:

Table 33. Summary of Survivorship in the Rat

Text Table 3.3A – Summary of Survivorship										
	Males					Females				
Dose(mg/kg/day)	0[a]	0[b]	0.5	2	10	0[a]	0[b]	0.5	2	10
No. of Rats	70	70	70	70	70	70	70	70	70	70
No. of Deaths Prior to Termination	50	48	39	40	48	42	37	39	45	43
No. of Survivors at Termination[c]	20	22	31	30	22	28	33	31	25	27
Percent Survivorship	29%	31%	44%	43%	31%	40%	47%	44%	36%	39%

[a]Vehicle-control group

[b]Water control group

[c]Termination occurred in Weeks 89 to 91 for males and Weeks 105 to 106 for females.

In the males the most common non-neoplastic cause of death was chronic progressive nephropathy (CPN) that resulted in a higher incidence of death in the 10 mg/kg males due to increased microscopic severity of CPN. In the females the most common cause of death was benign and malignant mammary and pituitary tumors (sponsor's tables and figures below).

Table 34. Major Causes of Death in Rats Treated with Dapagliflozin

Dose (mg/kg/day):	0[a]	0[b]	0.5	2	10
No. of Rats (M/F):	70/70	70/70	70/70	70/70	70/70
Sex:	M/F	M/F	M/F	M/F	M/F
Number of Unscheduled Decedents	50/42	48/37	39/39	40/45	48/43
Chronic Progressive Nephropathy	24/2	22/1	24/4	20/5	36/5
Mammary Tumor	0/8	0/15	0/14	0/12	0/12
Pituitary Tumor	2/14	1/11	1/4	0/8	1/9

[a]vehicle control group
[b]water control group

Figure 13. Proportion of Male Rats Surviving as a Function of Time

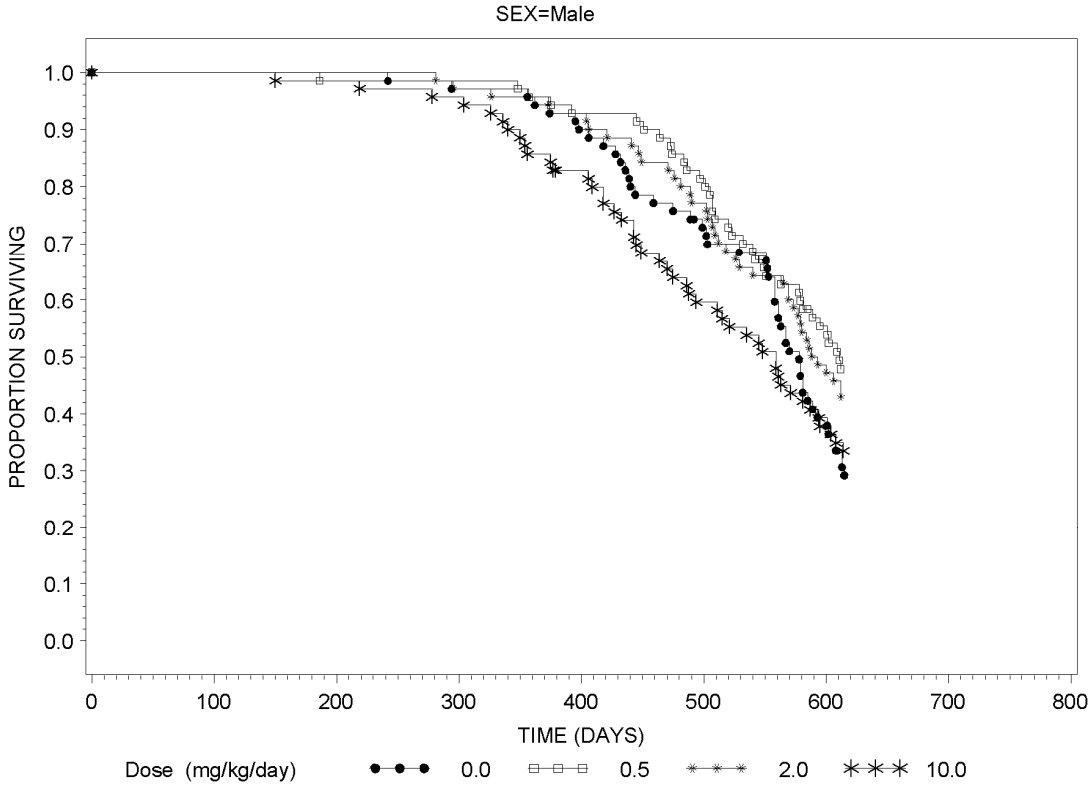


Table 35. KM Survival Estimate in the Male Rat

Male Rats: Mortality due to all causes, followed by Kaplan-Meier (KM) survival estimates at terminal sacrifice and life-table trend tests for dose-related trends in mortality

Group	1[b]	3	4	5
Dose (mg/kg/day)	**0**	**0.5**	**2**	**10**
Natural Death / Moribund Sacrifice	49	36	40	46
Terminal Sacrifice	20	31	30	22
Accidental Death	1	3	0	2
TOTAL	70	70	70	70
KM Survival Estimate at Terminal Sacrifice	0.29	0.48	0.43	0.33
Two-Sided Trend Test P-Values[a]		P=0.0406	P=0.3465	P=0.1300

[a] Trend test P-values are reported in the column of the highest dose-group included in the trend test.

[b] Vehicle-control group

Figure 14. Proportion of Female Rats Surviving as a Function of Time

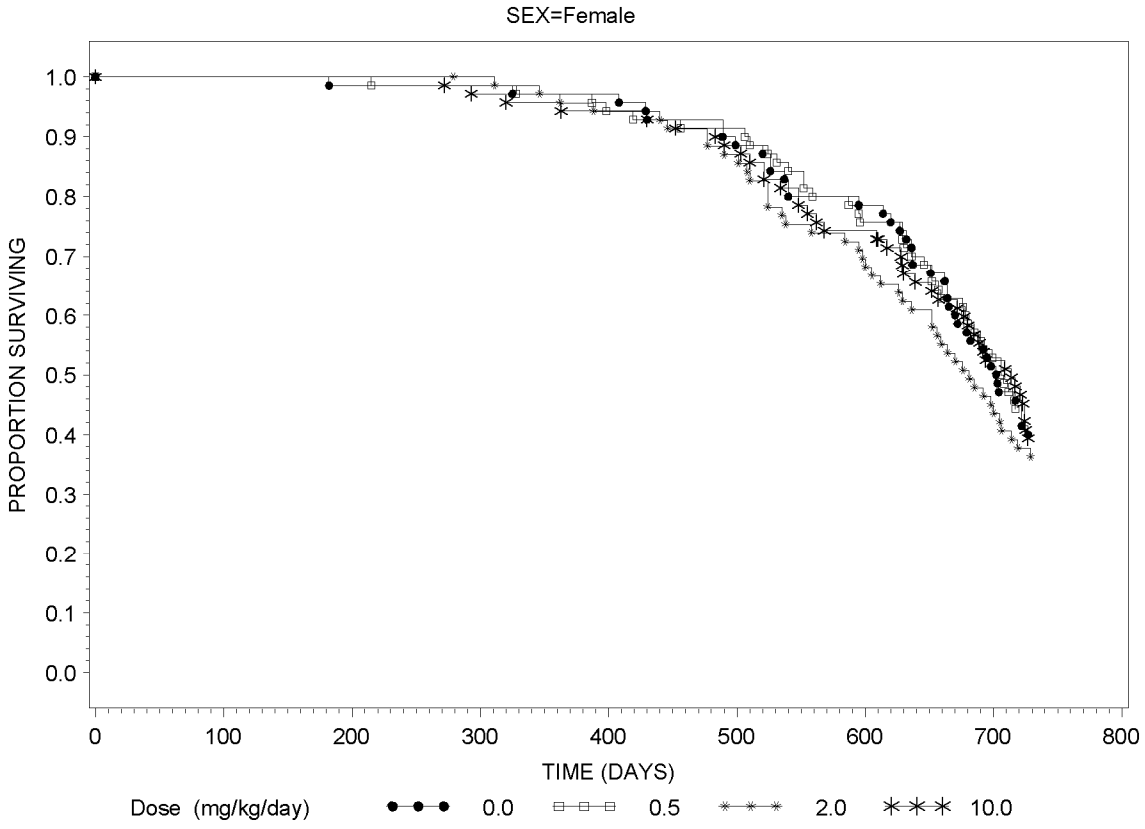


Table 36. KM Survival Estimate in the Female Rat

Female Rats: Mortality due to all causes, followed by Kaplan-Meier (KM) survival estimates at terminal sacrifice and life-table trend tests for dose-related trends in mortality

Group	1[b]	3	4	5
Dose (mg/kg/day)	**0**	**0.5**	**2**	**10**
Natural Death / Moribund Sacrifice	42	39	44	42
Terminal Sacrifice	28	31	25	27
Accidental Death	0	0	1	1
TOTAL	70	70	70	70
KM Survival Estimate at Terminal Sacrifice	0.40	0.44	0.36	0.39
Two-sided Trend Test P-Value [a]		P=0.7084	P=0.3301	P=0.8817

[a] Trend test P-values are reported in the column of the highest dose-group included in the trend test.

[b] Vehicle-control group

Clinical Signs

Clinical signs were generally unremarkable. Beginning in week 50 until week 89, the incidence of dermal scabs was increased dose-independently in all dapagliflozin-treated males, with the highest incidence occurring in the low dose males. Beginning week 58 until week 104, the incidence of dermal scabs was increased dose-independently in all dapagliflozin treated females. The increased incidence of dermal scabs did not correlate with skin hyperplasia/neoplasia. Skin tumors (benign keratoacanthoma) were independently identified by FDA biostatistics staff using a pair-wise analysis and showed statistical significance ($p<0.04$) and increased incidence in the mid dose males when compared to the water control males (4, 0, 3, 5, 3 tumors in vehicle, water vehicle, LD, MD and HD, respectively); but were without a dose response.

Table 37. Incidence of Dermal Scabs in the Rat

Dapagliflozin treatment (mg/kg/day)	Total Dermal Scabs Incidence	
	Male (Weeks 50-89)	Female (Week 58-104)
0 (vehicle control)	319	15
0 (water control)	303	31
0.5	839	378
2	690	428
10	594	424

Body Weights

Mean body weights were statistically significantly (ss) reduced in males in all treatment groups. In males the ss reduction of mean body weight showed a dose proportional trend for the onset of weight reduction and the duration of treatment. For example, for males at 10 mg/kg ss reduced mean body weight began at week 6 until the end of the study (week 89) and ranged from 3 to 12%. For males at 2 mg/kg ss reduced mean body weight began at week 17 until week 85 and ranged from 3 to 7%. For males at 0.5 mg/kg ss reduced mean body weight began at week 25 until week 85 and ranged from 2 to 6%. In the males reduced mean body weight was dose dependent beginning at week 25 until week 89 (except for weeks 69 and 77). Reduced body weight was also reflected in a dose dependent reduced body weight gain in both males and females; and which was ss reduced in the high dose males (see table below). Reduced mean body weight did not correlate with an increase in food consumption. See sponsor's figure below.

Mean body weights were statistically significantly (ss) reduced in the females at 10 mg/kg beginning at week 57 until week 85 and again at study termination (week 101) and ranged from 3 to 8%. The reduction in body weight did not correlate with increased

food consumption. From week 11 to week 37, mean body weight was ss increased in the water control group and ranged from 4 to 9%. See sponsor's figures below.

Figure 15. Male Rat Body Weights

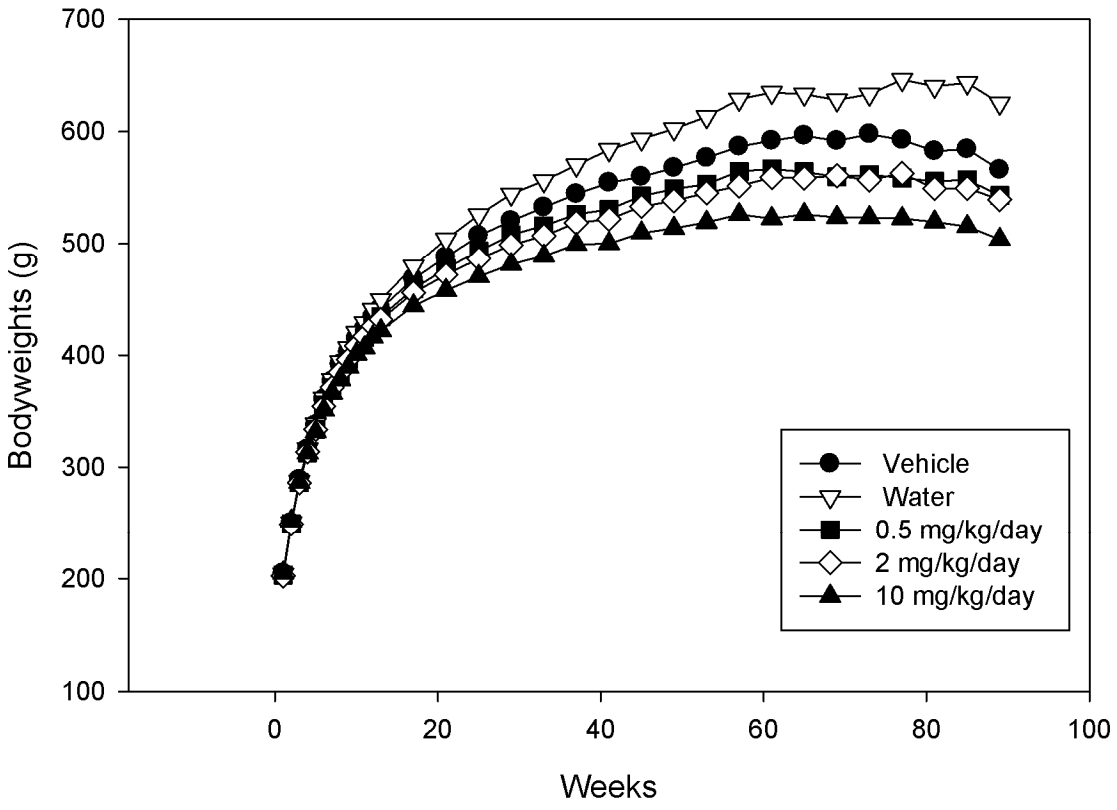


Figure 16. Female Rat Body Weights

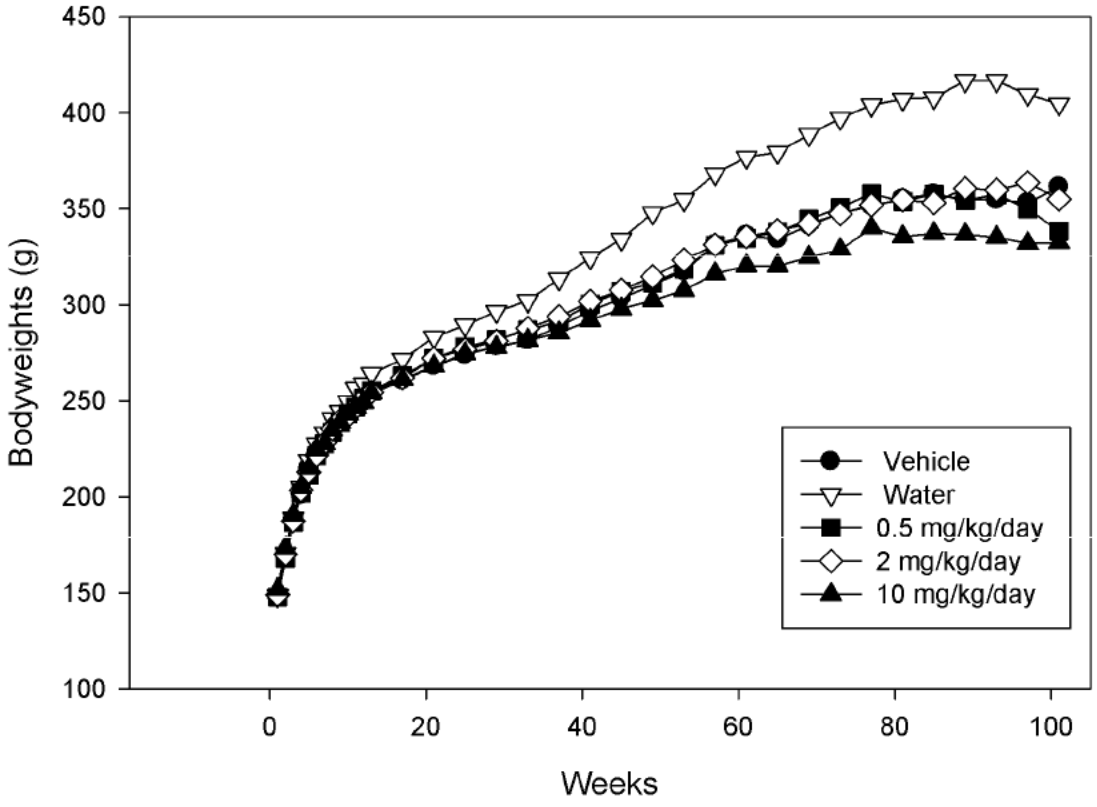


Table 38. Body Weight Summary

Body Weight Summary				
Treatment Mg/kg/day	Male		Female	
	BW (g)	BW Gain (g)	BW (g)	BW Gain (g)
Vehicle	566.1	406.1	361.4	233.4
Water	625.1	472.5	404.6	275
0.5	543.1	390.4	338.4	210.4
2	539.4	381.5	355	227.7
10	503.9*	346.1*	332.4	205.9

BW – Body weight, *p<0.05 (sponsor's assessment)

Feed Consumption

Beginning at week 1 mean food consumption was statistically significantly increased in all dapagliflozin treated animals until the study termination. The increases ranged from 4 to 22%, 7 to 29% and 15 to 33% in the 0.5, 2 and 10 mg/kg/day treated animals, respectively. The increase in food consumption did not correlate with reduced mean body weight.

Clinical Pathology-Urinalysis

Urinalysis was conducted at study day 182 from the vehicle control toxicokinetic subset of animals to determine urinary glucose and provide evidence of a lack of cross-contamination and dapagliflozin-induced gluosuria. Glucosuria was absent in the vehicle control animals.

Gross Pathology

There was a slight dose-dependent increase in pale discoloration of the kidneys seen in the male rats and a dose-independent increase in pale kidneys seen in the female rats. There was an increased incidence of kidney cysts and kidney irregular surfaces particularly for the male rats. The renal gross pathology observations correlated microscopically with a dose-related increase in severity of chronic progressive nephropathy, particularly in the male rat. In the adrenals there was an increased incidence of pale discoloration in all treated males and in the high dose (10 mg/kg) females, respectively. The pale discoloration correlated with hypertrophy/vacuolation of the zona glomerulosa observed microscopically. Sponsor's table below:

Table 39. Incidence of Dapagliflozin-Related Gross Pathologic Findings

Dose (mg/kg/day):	0[a]	0[b]	0.5	2	10
No. of Rats (M/F):	70/70	70/70	70/70	70/70	70/70
Sex:	M/F	M/F	M/F	M/F	M/F
KIDNEYS: Number Examined	70/70	70/70	70/70	70/70	70/70
Discolored, Pale	16/13	14/17	23/22	26/28	35/20
Cyst(s)	9/0	9/2	20/1	16/5	20/3
Irregular Surface	22/12	12/11	22/11	25/13	33/13
ADRENAL GLAND:Number Examine	70/70	70/70	70/70	70/70	70/70
Discolored, Pale	8/16	9/19	14/15	16/16	21/23

[a]vehicle control group
[b]water control group

There were no other apparent gross pathology treatment-related findings in early decedents or at scheduled necropsy. There were no apparent drug-related increases in palpable masses.

Histopathology

Peer Review: Yes

Neoplastic
There was no statistically significant increase found by trend test comparison in the incidence of any tumor type among males or females in any BMS-512148 treated group, when compared to the vehicle control group in the sponsor's analysis or in the independent FDA statistical analysis. However the skin tumors (keratoacanthoma) were independently identified by FDA biostatistics staff using a pair-wise analysis and showed statistical significance ($p<0.04$) and increased incidence in the mid dose males when compared to the water control males (4, 0, 3, 5, 3 tumors in vehicle, water vehicle, LD, MD and HD, respectively); but were without a dose response. This is considered to be an incidental finding. Historical control data were not submitted.

Reviewer's Note on Statistical Analysis
Due to removal of the 25 mg/kg/day at week 25 the high dose was excluded from the sponsor's and the FDA's statistical analysis for the purpose of identifying significant trends in tumor incidence. Biometrics conducted pair wise tests against both of the vehicle and water control groups either combined or separately. Sexes were analyzed separately.

Non Neoplastic
Kidney
In the males treatment-related microscopic changes occurred in the kidney at all doses. Chronic progressive nephropathy (CPN) was present in all male rats including the vehicle and water treated groups. However, a treatment-related increase in severity (up to severe) of CPN was observed in the dapagliflozin-treated rats, particularly at 10 mg/kg and in the males. Other microscopic findings included: an increased incidence and severity of minimal to severe vacuolation of the cortical tubule epithelium and an increased incidence and severity of minimal to marked cortical tubule atypical hyperplasia, particularly in the males. Vacuolation of the cortical tubule epithelium was not observed in the distilled water-treated rats and could be attributable to the 90% PEG 400 in water vehicle. CPN was also observed in female rats but there were minimal or no difference between dapagliflozin or vehicle/water treated female rats. Moderate to marked atypical hyperplasia was only present in the dapagliflozin-treated males. There was also increased incidence of mineralization of the cortex in the high dose males when compared to the vehicle control males (4, 2, 4, 6 and 9 in vehicle, water vehicle, LD, MD and HD, respectively). Renal kidney mineralization of the collecting ducts was previously been observed at high doses (>2000x MRHD) in the 6 month rat study. Sponsor's table below:

Table 40. Incidence of Dapagliflozin-Related Non-Neoplastic Microscopic Findings in the Kidney

Dose (mg/kg/day):		0[a]	0[b]	0.5	2	10
No. of Rats (M/F):		70/70	70/70	70/70	70/70	70/70
Sex:		M/F	M/F	M/F	M/F	M/F
KIDNEYS:	Number examined	70/70	70/70	70/70	70/70	70/70
Chronic Progressive Nephropathy:	Total	70/59	69/59	70/55	70/51	70/60
	Minimal	6/23	2/18	1/21	1/24	2/16
	Slight	7/11	4/21	5/10	8/8	5/18
	Moderate	20/15	17/14	14/13	10/7	11/11
	Marked	16/7	27/4	17/8	23/3	12/10
	Severe	21/3	19/2	33/3	28/9	40/5
Vacuolation, Cortical Tubule Epithelium:	Total	12/2	0/0	17/1	17/4	26/1
	Minimal	0/1	0/0	1/0	0/1	2/0
	Slight	0/1	0/0	1/1	2/1	1/1
	Moderate	8/0	0/0	3/0	6/1	6/0
	Marked	4/0	0/0	8/0	7/0	17/0
	Severe	0/0	0/0	4/0	2/1	0/0
Atypical Tubule Hyperplasia:	Total	27/0	18/0	44/0	42/0	45/0
	Minimal	19/0	16/0	25/0	15/0	27/0
	Slight	8/0	2/0	16/0	17/0	11/0
	Moderate	0/0	0/0	3/0	10/0	6/0
	Marked	0/0	0/0	0/0	0/0	1/0

[a]vehicle control group
[b]water control group

Adrenal Gland

In the adrenal gland there was a treatment related but dose-independent increase in the incidence and severity of minimal to moderate hypertrophy/vacuolation of the zona glomerulosa in both males and females. The incidence and severity of hypertrophy/vacuolation of the zona glomerulosa was also slightly increased in the dapagliflozin-treated rats particularly in the males. Sponsor's table below:

Table 41. Incidence of Dapagliflozin-Related Non-Neoplastic Microscopic Findings in the Adrenal Gland

Dose (mg/kg/day):		0[a]	0[b]	0.5	2	10
No. of Rats (M/F):		70/70	70/70	70/70	70/70	70/70
Sex:		M/F	M/F	M/F	M/F	M/F
ADRENAL GLAND:	Number examined	70/70	70/70	70/70	70/70	70/70
Hypertrophy/Vacuolation, Zona Glomerulosa:	Total	32/11	18/7	60/25	55/32	64/34
	Minimal	23/6	17/4	28/12	23/12	36/17
	Slight	8/4	1/3	26/12	25/17	22/14
	Moderate	1/1	0/0	6/1	7/3	6/3

[a]vehicle control group
[b]water control group

Bone (Sternum and Femur)

There was a minimal to slight increase in the trabecular bone of the 10 mg/kg males and females. The severity of the increased trabecular bone was slightly increased in the 10 mg/kg females (see tables below). Increased trabecular bone in the sternum and femur were also observed at high doses (>2000x MRHD) in the 6 month rat study. Sponsor's table below:

Table 42. Trabecular Bone Microscopic Findings With Severity-Femur

Increased Trabecular Bone Microscopic Findings – All Animals										
Femur with Joint /Severity	Vehicle		Water Control		0.5 mg/kg/day		2 mg/kg/day		10 mg/kg/day	
	M	F	M	F	M	F	M	F	M	F
# examined	70	70	69	70	70	70	70	69	70	70
Minimal	0	2	0	1	0	0	0	1	8	3
Slight	0	0	0	0	0	0	0	0	0	1
Total Incidence	**0**	**2**	**0**	**1**	**0**	**0**	**0**	**1**	**8**	**4**

Table 43. Trabecular Bone Microscopic Findings With Severity-Sternum

Increased Trabecular Bone Microscopic Findings – All Animals										
Sternum /Severity	Vehicle		Water Control		0.5 mg/kg/day		2 mg/kg/day		10 mg/kg/day	
	M	F	M	F	M	F	M	F	M	F
# examined	69	68	70	70	70	68	70	66	63	64
Minimal	0	2	0	0	0	1	0	1	5	3
Slight	0	0	0	0	0	0	0	1	1	2
Total Incidence	**0**	**2**	**0**	**0**	**0**	**1**	**0**	**2**	**6**	**5**

Table 44. Incidence of Dapagliflozin-Related Non-Neoplastic Microscopic Findings in the Bone

Dose (mg/kg/day):		0[a]	0[b]	0.5	2	10
No. of Rats (M/F):		70/70	70/70	70/70	70/70	70/70
Sex:		M/F	M/F	M/F	M/F	M/F
BONES (STERNUM AND FEMUR):						
	Number examined	70/70	70/70	70/70	70/69	70/70
Increased Trabecular Bone:	Total	0/3	0/1	0/1	0/2	9/6
	Minimal	0/3	0/1	0/1	0/1	8/4
	Slight	0/0	0/0	0/0	0/1	1/2

[a]vehicle control group
[b]water control group

Additional non-neoplastic findings considered noteworthy and that are particular to the dapagliflozin-treated males include: increased mineralization of the heart vasculature and stomach, increased dilatation of the urinary bladder and arteritis of the pancreas (see table below). Stomach and vasculature mineralization have previously been observed at high doses (>2000x MRHD) in the 6 month rat study. Urinary bladder dilatation and pancreatic arteritis are new findings.

Table 45. Heart Microscopic Findings With Severity

Heart Microscopic Findings – All Animals										
Mineralization, vasculature /Severity	Vehicle		Water Control		0.5 mg/kg/day		2 mg/kg/day		10 mg/kg/day	
	M	F	M	F	M	F	M	F	M	F
# examined	69	70	67	69	64	69	64	68	56	68
Minimal	0	0	1	0	2	0	3	1	4	0
Slight	0	0	1	1	2	1	2	0	10	0
Moderate	1	0	1	0	2	0	1	0	0	1
Marked	0	0	0	0	0	0	0	1	0	1
Total Incidence	**1**	**0**	**3**	**1**	**6**	**1**	**6**	**2**	**14**	**2**

Table 46. Stomach Microscopic Findings With Severity

Stomach Microscopic Findings – All Animals										
Mineralization /Severity	Vehicle		Water Control		0.5 mg/kg/day		2 mg/kg/day		10 mg/kg/day	
	M	F	M	F	M	F	M	F	M	F
# examined	64	70	63	70	65	68	60	68	49	68
Minimal	1	0	0	0	0	0	0	0	1	0
Slight	3	0	5	0	3	0	5	1	12	0
Moderate	1	0	2	0	2	2	4	0	7	2
Marked	1	0	0	0	0	0	1	1	1	0
Total Incidence	**6**	**0**	**7**	**0**	**5**	**2**	**10**	**2**	**21**	**2**

Table 47. Urinary Bladder Microscopic Findings With Severity

Urinary Bladder Microscopic Findings – All Animals										
Dilatation /Severity	Vehicle		Water Control		0.5 mg/kg/day		2 mg/kg/day		10 mg/kg/day	
	M	F	M	F	M	F	M	F	M	F
# examined	70	70	70	70	70	70	70	70	70	70
Minimal	1	0	0	0	0	0	1	0	1	0
Slight	3	0	2	0	2	0	2	0	3	0
Moderate	2	0	1	0	4	1	3	0	7	1
Marked	3	1	1	0	0	0	1	1	1	0
Total Incidence	**9**	**1**	**4**	**0**	**6**	**1**	**7**	**1**	**12**	**1**

Table 48. Pancreas Microscopic Findings With Severity

Pancreas Microscopic Findings – All Animals										
Arteritis /Severity	Vehicle		Water Control		0.5 mg/kg/day		2 mg/kg/day		10 mg/kg/day	
	M	F	M	F	M	F	M	F	M	F
# examined	56	60	60	68	55	62	61	67	46	62
Minimal	4	1	3	0	5	0	2	0	7	3
Slight	5	6	6	0	7	5	5	3	10	2
Moderate	2	2	0	1	3	2	2	0	5	3
Marked	3	1	1	1	0	1	0	0	1	0
Severe	0	0	0	0	0	0	0	0	1	0
Total Incidence	**14**	**10**	**10**	**2**	**15**	**8**	**9**	**3**	**24**	**8**

Toxicokinetics

In this 2-year rat carcinogenicity study the sponsor administered dapagliflozin at 0.5, 2, 10 mg/kg. On SD 184 blood was obtained from satellite TK animals. T_{max} ranged from 0.5 to 2 hr and C_{max} and $AUC_{0\text{-}24h}$ was approximately dose proportional. Systemic exposure in the males was 0.7 to 0.8x lower compared to the female animals. Sponsor's table below:

Table 49. Toxicokinetic Summary

Parameter	Day	BMS-512148					
		0.5 mg/kg		2 mg/kg		10 mg/kg	
		Male	Female	Male	Female	Male	Female
Cmax (µg/mL)	184	0.377	0.502	1.29	1.84	8.05	8.84
AUC(0-24) (µg•h/mL)	184	3.12	4.04	11.8	16.0	60.7	86.6

Dosing Solution Analysis

The formulations were stable from study day 0 of preparation through to study day 8 and were prepared weekly. The concentration verification throughout the study were within 10% of the nominal concentration.

BMS-512148 Oral Carcinogenicity Study in Mice

Study no.:	DN06072
Study report location:	eCTD
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 9 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BMS-512148, Dapagliflozin, Lot # 5J04457-02, purity 80.8-81.2%.
CAC concurrence:	Yes

Key Study Findings

Statistically Significant Neoplastic Findings

- No statistically significant tumors were found by trend analysis.
- Pair-wise analysis showed an increase in benign bronchiolo alveolar adenoma in the low dose males when compared to the vehicle control males (p = 0.0423) but this was without a dose response. In addition, malignant lymphoma of the lymph/reticulocyte system was noted in the mid dose females when compared to vehicle controls (p=0.0069), but this was without a dose response.

Non-Neoplastic Findings:

- The sponsor eliminated the HD dose (120 mg/kg in males and 60 mg/kg in females) at week 22. ECAC concurred, noting that the AUC endpoint would still be met by exposure at the next lowest dose.
- Urogenital system lesion: Increased incidence and severity of urogenital system lesions in the mid and high dose males was exacerbated by dapagliflozin. This resulted in increased incidence of decedents in the mid and high dose males.
- Dilated renal pelvis (hydronephrosis) and distended renal bladder of increasing incidence and severity was observed in the mid and high dose males.

Maximum Clinical Exposure: 10mg/day, 465 ng.h/ml. The high dose tested in this study achieved exposures of 72-fold and 105-fold the clinical exposure in males and females, respectively.

Adequacy of Carcinogenicity Study

The final study report of a GLP-compliant standard two year oral gavage carcinogenicity study in the CD-1 mice was reviewed and the results were discussed at a meeting of the executive carcinogenicity Assessment Committee (ECAC). The ECAC and the division considers the negative carcinogenic finding in the mouse study as an adequate tumor assessment because dapagliflozin reached a ≥25x exposure margin in both males and females.

Appropriateness of Test Models

The sponsor chose doses of dapagliflozin (BMS-512148) at 5, 15, 40 and 120 mg/kg/day in the males and 2, 10, 20 and 60 mg/kg/day in the females, respectively, based on the recommendations of the ECAC. The 120 and 60 mg/kg doses were removed (b) (4) Overall treatment was well tolerated and the results showed no dose-limiting toxicity up to the highest dose tested. Exposure at the high dose (40 mg/kg for males and 20 mg/kg for the females) provided approximately 72x and 105x MRHD in males and females, respectively, based on total exposure (AUC_{0-24}).

Evaluation of Tumor Findings

There were no tumor increases in any dapagliflozin treatment group that were considered treatment-related or biologically significant.

Methods

Doses:	M: 0, 0, 5, 15, 40 and 120 mg/kg; F: 0, 0, 2, 10, 20 and 60 mg/kg
Frequency of dosing:	Once daily
Dose volume:	4mL/Kg
Route of administration:	P.O. (Oral gavage)
Formulation/Vehicle:	C1: 90% (v/v) PEG 400 C2:Distilled water
Basis of dose selection:	Dose selection was done with ECAC concurrence.
Species/Strain:	CD-1® Rats (b) (4) (b) (4)
Number/Sex/Group:	50/sex/group
Age:	5 weeks
Animal housing:	The animals were individually housed in suspended, stainless steel, wire-mesh type cages
Paradigm for dietary restriction:	Certified Global Rodent Diet No. 2018C (Harlan Teklad) and water *ad libitum*
Dual control employed:	Yes
Interim sacrifice:	No
Satellite groups:	TK; 18/sex/group dosed for 6 months
Deviation from study protocol:	None that affected study outcome

Observations and Results

Mortality

Males and females were terminated as scheduled. At termination the survival rates were slightly lower in the 15 and 40 mg/kg males compared to the vehicle (90% PEG

400 in water) and water treated males (see table below). Dapagliflozin slightly reduced survivorship in the 2 mg/kg females relative to the vehicle control (32% vs 47%) but was not dose dependent. The summary of survivorship is shown in the sponsor's tables below and shows no differences between the vehicle and treated groups (high dose 120 mg/kg males and 60 mg/kg female animals were excluded).

Table 50. Summary of Survivorship in the Mouse

Mortality Summary										
	Males					Females				
Dose(mg/kg/day)	**0[a]**	**0[b]**	**5**	**15**	**40**	**0[a]**	**0[b]**	**2**	**10**	**20**
No. of Mice	60	60	60	60	60	60	60	60	60	60
No. of Deaths Prior to Termination	34	36	34	42	40	32	39	41	38	35
No. of Survivors at Termination	26	24	26	18	20	28	21	19	22	25
Percent Survivorship	43%	40%	43%	30%	33%	47%	35%	32%	37%	42%
[a]Vehicle-control group [b]Water control group										

In the males the most common non-neoplastic cause of death was attributed to urogenital system lesions (mouse urologic syndrome) that show a treatment related increase in the mid and high dose males. These lesions are associated with dilated renal pelves and urinary bladder distension, both of which show a treatment-related increase in the mid and high dose males both in the macroscopic and microscopic findings (see macroscopic and microscopic subsections below). These renal and bladder lesions are an occasional background lesion and dapagliflozin appears to increase the incidence of this lesion in the mid and high dose males. All other causes of death were not dose related. Sponsor's table below:

Table 51. Major Causes of Death in Mice Treated with Dapagliflozin

Major Causes of Preterminationa Deaths										
	Males					Females				
Dose (mg/kg/day)	**0[b]**	**0[c]**	**5**	**15**	**40**	**0[b]**	**0[c]**	**2**	**10**	**20**
Urogenital System – non-neoplastic lesions[d]	9%	11%	15%	19%	30%	0	0	0	0	0
Lymphoreticular System - neoplasms	3%	8%	12%	2%	8%	25%	31%	12%	32%	9%
Kidney – chronic progressive nephropathy	6%	3%	3%	0	3%	6%	15%	20%	11%	6%
Amyloidosis	12%	11%	12%	10%	8%	6%	3%	12%	3%	9%
Uterus/Cervix – neoplasms	-	-	-	-	-	22%	8%	2%	13%	3%
Lung – neoplasms	12%	8%	0	5%	8%	6%	5%	2%	3%	6%
Liver – neoplasms	12%	3%	3%	5%	0	0	0	2%	0	3%
Heart – non-neoplastic lesion	0	11%	15%	2%	5%	0	3%	2%	0	0
Other causes	17%	25%	25%	12%	20%	19%	14%	28%	25%	35%
Undetermined	32%	17%	15%	45%	18%	16%	21%	20%	13%	29%

[a]Percentage of pretermination deaths attributed to a specific cause of death. .

[b]Vehicle-control group

[c]Water control group

[d]Non-neoplastic urogenital system lesions were comprised of combinations of the following: 1) Kidney lesions (chronic progressive nephropathy, pyelitis, pyelonephritis, dilated pelves); 2) Urinary bladder lesions (distended, erosions/ulcers/subacute/chronic inflammation; 3) Prostate Gland and Seminal Vesicles (subacute/chronic inflammation, purulent inflammation/abscesses, fibrosis).

Figure 17. Proportion of Male Mice Surviving as a Function of Time

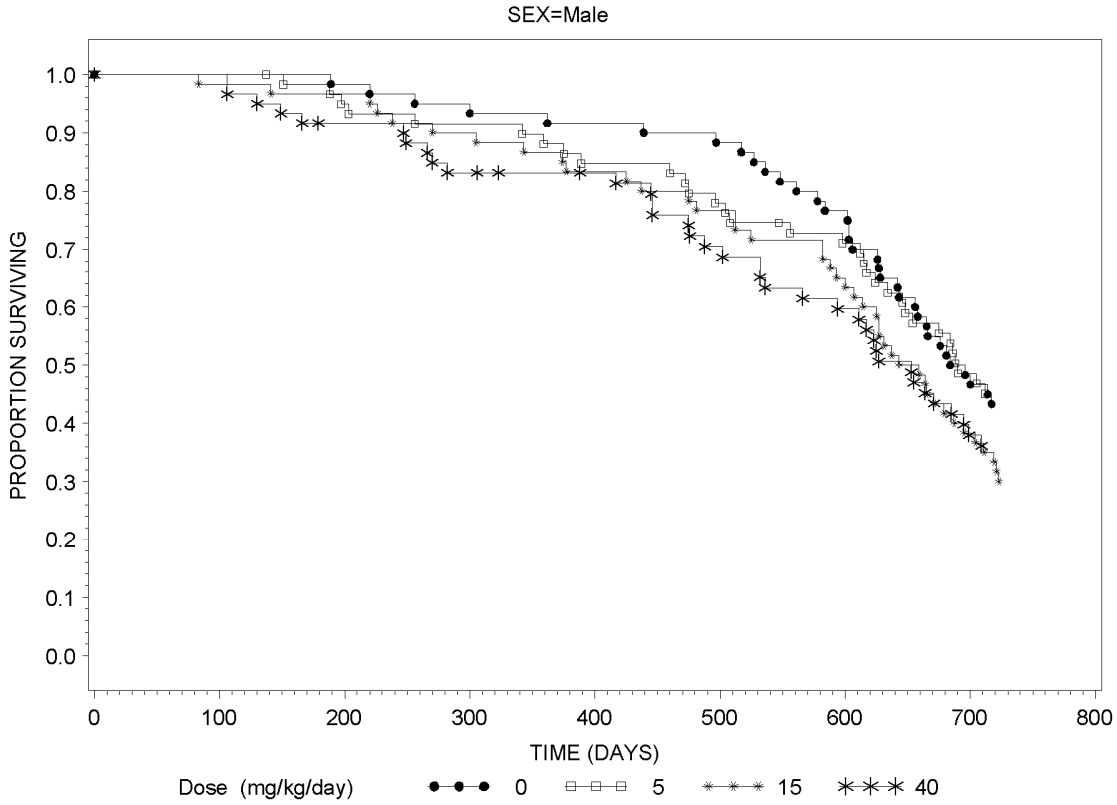


Table 52. KM Survival Estimate in the Male Rat

Male Mice: Mortality due to all causes, followed by Kaplan-Meier (KM) survival estimates at terminal sacrifice and life-table trend tests for dose-related trends in mortality

Group	1[b]	3	4	5
Dose (mg/kg/day)	**0**	**5**	**15**	**40**
Natural Death / Moribund Sacrifice	34	32	42	36
Terminal Sacrifice	26	26	18	20
Accidental Death	0	2	0	4
TOTAL	60	60	60	60
KM Survival Estimate at Terminal Sacrifice	0.43	0.45	0.30	0.36
Two-Sided Trend Test P-Values[a]		P=0.9634	P=0.0861	P=0.1383

[a] Trend test P-values are reported in the column of the highest dose-group included in the trend test.

[b] Vehicle-control group

Figure 18. Proportion of Female Mice Surviving as a Function of Time

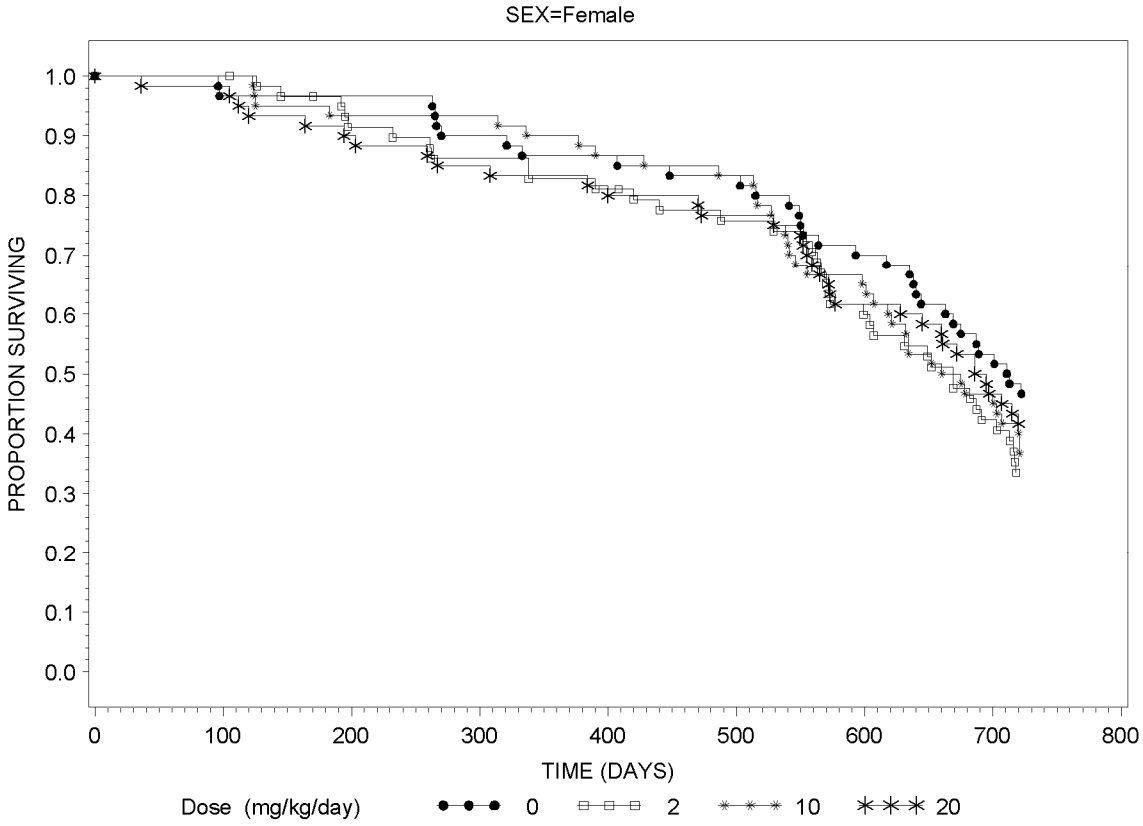


Table 53. KM Survival Estimate in the Female Rat

Female Mice: Mortality due to all causes, followed by Kaplan-Meier (KM) survival estimates at terminal sacrifice and a life-table trend test for dose-related trends in mortality

Group	1[b]	3	4	5
Dose (mg/kg/day)	**0**	**2**	**10**	**20**
Natural Death / Moribund Sacrifice	32	38	38	35
Terminal Sacrifice	28	19	22	25
Accidental Death	0	3	0	0
TOTAL	60	60	60	60
KM Survival Estimate at Terminal Sacrifice	0.47	0.33	0.37	0.42
Two-sided Trend Test P-Value [a]		P=0.1592	P=0.4868	P=0.8570

[a] Trend test P-values are reported in the column of the highest dose-group included in the trend test.

[b] Vehicle-control group

Clinical Signs

Clinical signs were generally unremarkable with the exception of a slightly increased incidence of abdominal distension in the mid and high dose males. Abdominal distension correlated with enlarged kidneys and urinary bladder distension observed both macro- and microscopically.

Body Weights

Treatment with dapagliflozin had no effect on the mean body weight in both males and females at the end of treatment (see table below). Mean body weights and mean body weight gain were statistically significantly (ss) reduced in males for weeks 21 to 41, and did not correlate with decreased food consumption. The reduced body weight ranged from 4-8% and was not dose-dependent. The reduced mean body weight gain were inversely related to dose with statistically significant reduction ranging from 18-25% at 5 mg/kg, 13-20% at 15 mg/kg and 10-15% at 40 mg/kg, respectively. However, mean body weight gain was also ss increased 28% in the high dose males at week 101.

In contrast, there was a ss increase in body weight at weeks 4, 6, 11-12, 25 and 29 in the high dose females. The increase in body weight ranged from 3-4% and was associated with increased food consumption. Body weight gain was however, unchanged at the end of the treatment (see table below). In addition, there were sporadic instances of body weight gain and reduction that were ss but were of low incidence and not dose-dependent in the dapagliflozin-treated females up to week 29.

Figure 19. Male Mice Body Weights

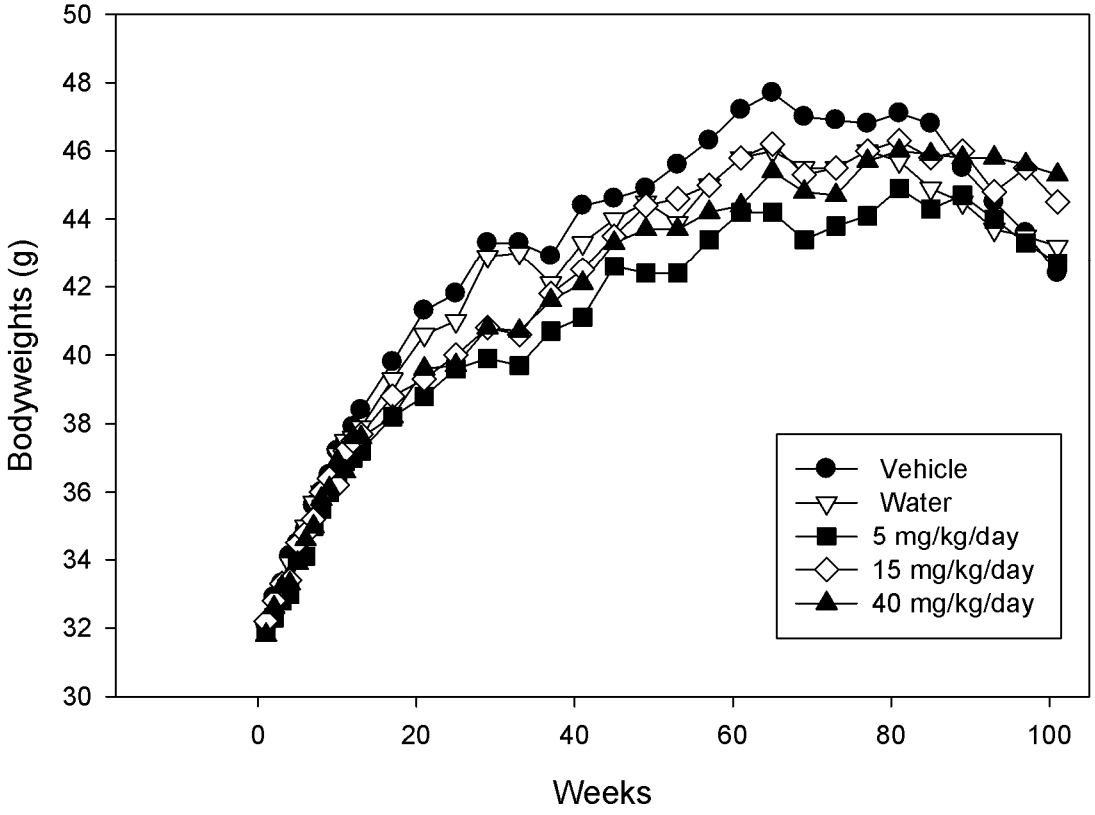


Figure 20. Female Mice Body Weights

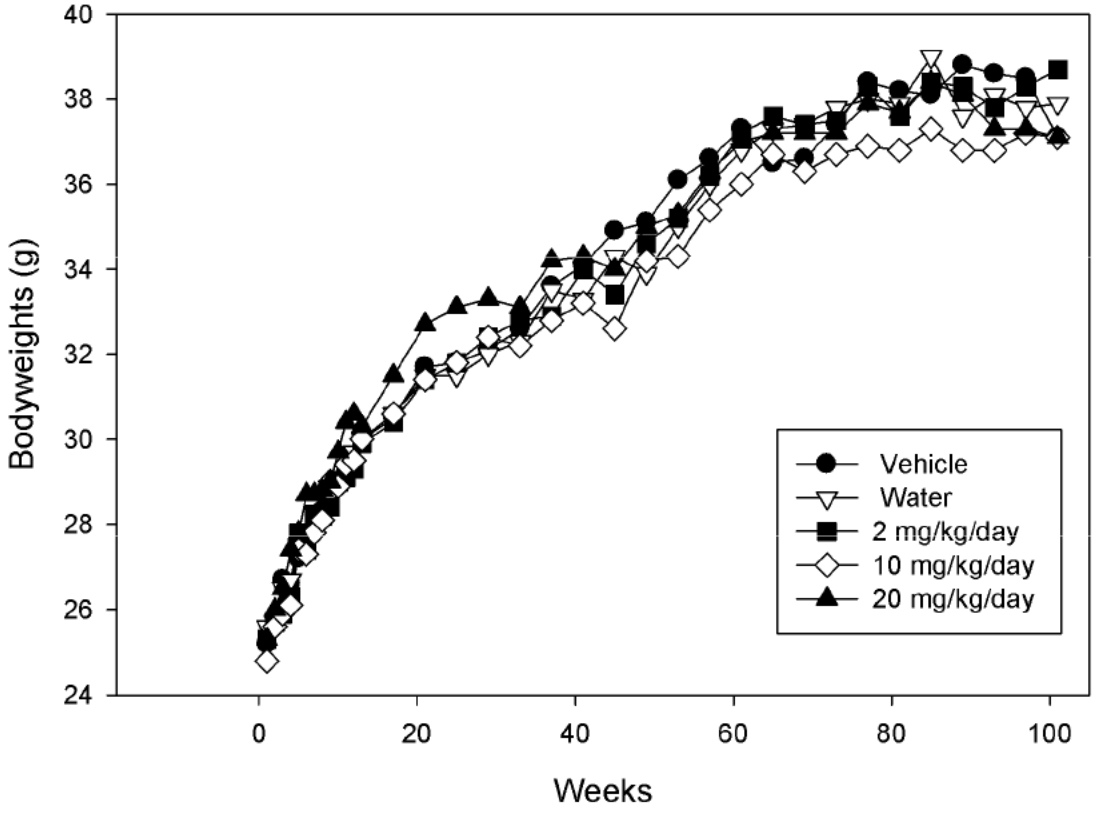


Table 54. Body Weight Summary

BODY WEIGHT SUMMARY					
MALE			FEMALE		
TREATMENT MG/KG/DAY	BW (G)	BW GAIN (G)	TREATMENT MG/KG/DAY	BW (G)	BW GAIN (G)
VEHICLE	**42.4**	**12.7**	**VEHICLE**	**37.1**	**13.2**
WATER	**43.2**	**13.1**	**WATER**	**37.9**	**13.5**
5	**42.7**	**12.3**	**2**	**38.7**	**15.7**
15	**44.5**	**14.2**	**10**	**37.1**	**14.1**
40	**45.3**	**16.2***	**20**	**37.1**	**12.3**

BW – Body weight, BW gain relative to baseline, *p<0.05 (sponsor's assessment)

Feed Consumption

In general mean food consumption was statistically significantly increased in all dapagliflozin-treated animals, particularly high dose males and females. The incidence and magnitude of food consumption was dose-related. The increases ranged from 8 to 20%, 6 to 30% and 9 to 41% in the 5, 15 and 40 mg/kg/day treated males, respectively and 9 to 34%, 9-45% and 11-66% in the 2, 10 and 20 mg/kg/day treated females, respectively. The magnitude and dose dependence of increased in food consumption did not correlate with a dose-dependent increased mean body weight.

Gross Pathology

Macroscopically in the kidney there was an increase in the incidence of enlarged kidneys, cysts and renal pelvic dilatation in the dapagliflozin-treated males. The renal kidney dilatation was dose-dependent. The enlarged kidneys correlated microscopically with dilated pelvis which showed a dose and severity-related increase. The enlarged kidneys did not correlate to chronic progressive nephropathy. In addition, an increased incidence of urinary bladder distension and ureter distension was observed particularly in the mid and high dose males. Relative to the vehicle control there was a slight increase in urinary bladder calculus in the high dose males (0 vs 4) (see sponsor's tables below). Macroscopic kidney and urinary bladder findings were unremarkable in the females, with the possible exception of slightly increased pelvic dilatation in the high dose females (n= 6 vehicle control vs n=9 high dose female). Sponsor's tables below:

Table 55. Incidence of Dapagliflozin-Related Gross Pathologic Findings in Mice (1)

Dose (mg/kg/day) (M/F):		0[a]	0[b]	5/2	15/10	40/20
No. of Mice (M/F):		60/60	60/60	60/60	60/60	60/60
Sex:		M/F	M/F	M/F	M/F	M/F
KIDNEYS:	Number Examined	60/60	60/60	60/60	60/60	60/60
Enlarged		8/3	9/4	14/2	13/6	17/3
Cyst(s)		25/4	24/5	30/4	29/5	31/6
Pelvis dilated		11/6	7/5	14/4	27/7	28/9
URINARY BLADDER:	Number Examined	60/60	60/60	60/60	60/60	60/60
Distended		16/-	15/2	14/1	28/1	31/-

- Indicates absence of finding in group
[a]vehicle control
[b]water control

Table 56. Incidence of Dapagliflozin-Related Gross Pathologic Findings in Mice (2)

	-- Males --					-- Females --				
Group:	1	2	3	4	5	1	2	3	4	5
Number in subgroup(s) 1:	60	60	60	60	60	60	60	60	60	60
Ureters										
Distended	4	4	4	9	7	0	2	1	3	1
Calculus	0	0	2	0	0	0	0	0	0	0
Cyst	1	0	0	0	1	0	0	0	0	0
Urinary Bladder										
Discolored	3	0	0	0	1	0	0	1	1	0
Distended	16	15	14	28	31	0	2	1	1	0
Calculus	0	1	3	1	4	0	0	0	0	0
Urinary Bladder										
Abnormal Contents	2	3	3	3	4	0	0	0	0	0
Thickened	1	1	3	1	2	0	1	0	0	0
Cyst	0	0	0	1	0	0	0	0	0	0
Calculi	1	0	0	1	0	0	0	0	0	0

There were no other apparent gross pathology treatment-related findings in early decedents or at scheduled necropsy. There were no apparent drug-related increases in palpable masses.

Histopathology

Peer Review: Yes

Neoplastic

There was no statistically significant increase found by trend test comparison in the incidence of any tumor type among males or females in any BMS-512148 treated group, when compared to the vehicle control group in the sponsor's analysis or in the independent FDA statistical analysis. However, pair-wise analysis showed hepatocellular adenomas in male mice. Adenomas were found in 4 vehicle control males and 14 water control males (p= 0.0087). In addition, pair-wise assessment of lymphatic system lymphomas in female mice showed 4 in the vehicle treated female mice and 10 in the water control female mice (p=0.0263).

Independent analysis by FDA biostatistics staff using a pair-wise method showed statistical significance and increased incidence for benign bronchiolo alveolar adenoma in the low dose males when compared to the vehicle control males (p = 0.0423); but these were without a dose response (see table below). In addition, malignant lymphoma of the lymph/reticulocyte system was noted in the mid dose females (p=0.0069), but this was without a dose response. Historical control data were not submitted.

Table 57. Non-dose dependent Tumor Incidence with Dapagliflozin Treatment

			0 mg	5 mg	15 mg	40 mg	P_Value			
			Vehicle	Low	Med	High	Dos	P_Value	P_Value	P_Value
	Organ Name	Tumor Name	N=60	N=60	N=60	N=60	Resp	C vs. L	C vs. M	C vs. H
Male Mice	LUNGS	BRONCHIOLO/ALVEOLAR	6	13	10	7	0.4564	0.0423	0.1387	0.2931

			0 mg	2 mg	10 mg	20 mg	P_Value			
Female Mice			**Vehicle**	Low	Med	High	Dos	P_Value	P_Value	P_Value
	Organ Name	Tumor Name	N=60	N=60	N=60	N=60	Resp	C vs. L	C vs. M	C vs. H
	LYMPH/RETIC SYS	MALIGNANT LYMPHOMA	4	7	14	7	0.1468	0.1833	**0.0069***	0.1959
			Water							
	ADRENAL GLANDS	SUBCAPSULAR CELL ADE	0	1	1	3	0.0474	0.4800	0.5063	0.1249

Reviewer's Note on Statistical Analysis

Due to removal of the high dose male and female groups at week 22/23, the high dose was excluded from the sponsor's and the FDA's statistical analysis for the purpose of identifying significant trends in tumor incidence. Biometrics conducted pair wise tests against both of the vehicle and water control groups either combined or separately. Sexes were analyzed separately.

Non-Neoplastic

Kidney

Increased incidence and severity of dilated renal pelves (hydronephrosis) and distended renal bladder were observed microscopically in the mid and high dose males and correlated with gross necropsy findings for these tissues (sponsor's table below). Note that the PEG400 vehicle appears to have increased the incidence of hydronephrosis compared to the water control in males, but a treatment effect beyond the vehicle is still apparent in mid- and high-dose groups.

Table 58. Incidence of Dapagliflozin-Related Non-Neoplastic Microscopic Findings in the Kidney

Dose (mg/kg/day) (M/F):		0[a]	0[b]	5/2	15/10	40/20
No. of Mice (M/F):		60/60	60/60	60/60	60/60	60/60
Sex:		M/F	M/F	M/F	M/F	M/F
KIDNEYS:	Number examined	60/60	60/60	60/60	60/60	60/60
Pelvis dilated (hydronephrosis)		17/7	10/5	20/8	34/14	37/11
Minimal		2/-	-/-	2/-	5/-	3/-
Slight		9/5	2/3	6/6	7/13	5/8
Moderate		5/2	8/1	12/2	19/1	23/3
Marked		1/-	-/1	-/-	3/-	6/-
URINARY BLADDER:	Number examined	60/59	60/59	60/58	60/60	60/60
Distended		18/-	19/2	19/2	30/1	29/1
Minimal		-/-	-/-	-/-	-/-	1/-
Slight		4/-	6/-	4/1	9/-	6/-
Moderate		14/-	12/1	15/1	21/1	20/1
Marked		-/-	1/1	-/-	-/-	2/-

- Indicates absence of finding in group
[a]vehicle control
[b]water control

Toxicokinetics

In this 2-year mouse carcinogenicity study the sponsor administered dapagliflozin at 5, 15 and 40 mg/kg in males and 2, 10 and 20 mg/kg in females, respectively. On SD 182 blood was obtained from satellite TK animals. C_{max} and AUC_{0-T} were dose-proportional at 5 and 15 mg/kg in the males but not dose proportional at 40 mg/kg. C_{max} and AUC_{0-T} were approximately dose proportional in the 2, 10 and 20 mg/kg females. Systemic exposure in the males was lower compared to the female animals, despite a lower dose regimen in the females. Sponsor's table below:

Table 59. Toxicokinetic Summary

Parameter	Day	BMS-512148					
		5 mg/kg	2 mg/kg	15 mg/kg	10 mg/kg	40 mg/kg	20 mg/kg
		Male	Female	Male	Female	Male	Female
Cmax (µg/mL)	182	1.04	1.09	3.20	6.25	14.1	16.0
AUC(0-T) (µg•h/mL)	182	2.00	5.09	6.41	24.0	33.5	48.6

For AUC(0-T), T = 8 (males) or 24 (females) hours post dose.

Dosing Solution Analysis

The formulations were stable from study day 0 of preparation through to study day 8 and were prepared weekly. The concentration verification throughout the study were within 10% of the nominal concentration.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

A GLP-compliant fertility study was conducted in the rat. The study report was fully reviewed and is summarized here.

BMS-512148: Oral Study of Fertility and Early Embryonic Development in Rats

Study no.:	DN4014
Study report location:	eCTD
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 27 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BMS-512148, lot # 2J61857, and 81.8%

Methods

Doses:	F: 0, 3, 15 or 75 mg/kg; M: 0, 15, 75 or 300/210 mg/kg
Frequency of dosing:	Daily
Dose volume:	1 mL/kg
Route of administration:	P.O. (Oral gavage)
Formulation/Vehicle:	90% PEG 400 in water
Species/Strain:	Rat/Sprague-Dawley
Number/Sex/Group:	25/sex/group
Satellite groups:	10/sex/group at 3, 15 or 75 mg/kg only
Study design:	Standard 2 week pre-treatment prior to co-habitation with treatment naïve animals. Males were treated with dapagliflozin at 310 mg/kg for the first 4 days but the dose was lowered to 210 mg/kg due to mortality. Males continued to be treated with dapagliflozin for 2 weeks during mating and until study day 43. Pre-treated females continued to be dosed with dapagliflozin until GD 7.
Deviation from study protocol:	None that affected study outcome

Key Study Findings

- Significant mortality (n=11) and moribundity was observed in the 300/210 mg/kg high dose male group between study day 4 and 22.

- No effects were observed on mating or fertility indices in the males except for altered spermatogenesis (reduced sperm motility, sperm counts and morphologic abnormalities) in the 300/210 mg/kg high dose males.
- The NOAEL for male fertility is 15 mg/kg (160x the MRHD (10 mg)) due to mortality, altered spermatogenesis in the high dose males and also significantly reduced body weight gain in the high and mid dose males (≥20%).
- No effects were observed on mating or fertility indices in the females. The NOAEL for female fertility was 15 mg/kg (188x MRHD (10 mg)) due to significantly reduced body weight gain in the high dose females.
- The NOAEL for embryonic development was 3 mg/kg (39x the MRHD (10 mg)) due to increased embryo-lethality (resorptions) at 15 and 75 mg/kg.
- Exposure in the females was 39x, 188x and 998x MRHD (10mg). Exposure in the males was 160x, 675x and 1707x MRHD (10 mg).

9.2 Embryonic Fetal Development

An exploratory non-GLP embryo-fetal development study was conducted in the rat. GLP-compliant embryo-fetal development studies were also investigated in rat and rabbit. All study reports were fully reviewed and are summarized here.

Rat (non-GLP study #DN05081).
Pregnant dams were dosed with dapagliflozin by oral gavage (15/group) once daily from GD 6 to GD 12 at 0, 150, 225 or 300 mg/kg. On GD day 10 dams were evaluated for exposure to dapagliflozin or it's 0-deethylated metabolite BMS-511926 and also for serum glucose, calcium, phosphorus. Blood was collected at 0.5, 1, 2, 4, 8 and 24 hours post-dose at GD 10. All dams were euthanized at GD 12 at 4 hours post-dose. Litters (5/group) were evaluated for viability and for exposure to dapagliflozin or it's 0-deethylated metabolite BMS-511926 or litters (5/group) were evaluated for glucose, lactate, pyruvate, total protein and phosphorus content.

Dapagliflozin caused maternal toxicity in all treatment groups including mortality and moribundity at 300 mg/kg. Clinical signs included mucoid/liquid feces, brown perioral substance, brown stained fur and urine stained coat. In addition, reduced body weight gain (16g and 0g at 150 and 225 mg/kg, respectively) was noted in the low and mid dose groups and body weight loss (-15g) occurred at the high dose, relative to 33g in the control group. Embryo-lethality was observed at ≥225 mg/kg and consisted of resporbed conceptuses (10% and 56% at 225 and 300 mg/kg, respectively, relative to 6% in the control). At 225 and 300 mg/kg there was reduced: total embryonic protein (17% and 32%, less than control, respectively), pyruvate (9% and 36% less than control, respectively) and glucose (43% and 67% less than control, respectively).

Maternal serum glucose was reduced from 2 to 8 hours at 150 (91-97% of the control) and 300 mg/kg (82-91% of control) and from 1 to 8 hours at 225 mg/kg (87-97% of control). A minimal increase in maternal plasma calcium was observed at all time points (3-14%).

Based on AUC and C_{max} maternal systemic exposure to dapagliflozin were similar at 150 and 225 mg/kg and increased dose-proportionately from 225 to 300 mg/kg. Embryonic dapagliflozin exposure was similar in all treatment groups (sponsor's tables below). Due to the 51% reduced body weight gain at 150 mg/kg a NOAEL could not be established.

Table 60. Maternal Dapagliflozin Exposure

Toxicokinetic Parameters of Dapagliflozin in Rat Plasma				
Parameter	**Study Day**	**Dose (mg/kg/day)**		
		150	**225**	**300**
		Female	**Female**	**Female**
Cmax (µg/mL)	GD 10	109	85.7	129
AUC (µg•h/mL)	GD 10	1590	1380	1900

Table 61. Embryonic Dapagliflozin Exposure

Dose (mg/kg/day)	**Dapagliflozin Mean Concentration**	**BMS-511926 Mean Concentration**
	µg/mL	**µg/mL**
150	8.618	0.018
225	8.847	0.016
300	6.955	0.014

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BMS-512148: Oral Study of Embryo-Fetal Development in the Rat

Study no.: DN031202
Study report location: eCTD
Conducting laboratory and location: (b) (4)
Date of study initiation: October 13 2003
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BMS-512148, lot # 2J61857, and 82%

Methods

Doses: 0, 37.5, 75, 150, and 300 mg/kg
Frequency of dosing: Daily

Dose volume:	3 mL/kg
Route of administration:	PO (Oral gavage)
Formulation/Vehicle:	90% PEG 400 in water
Species/Strain:	Rat/Sprague-Dawley
Number/Sex/Group:	25/group
Satellite groups:	None
Study design:	GD6 – GD 15
Deviation from study protocol:	None that affected study outcome

Key Study Findings

- The NOAEL for maternal toxicity was not established (< 37.5 mg/kg/d; <703x the MRHD 10 mg) due to reduced food consumption (FC) and reduced body weight gain.
- The NOAEL for embryo-fetal development was 75 mg/kg (1441x the MRHD 10 mg).
- Exposure in the dams was 703x, 1441x and 2344x MRHD (10mg) from a pregnant rat TK (bridging) study with identical dapagliflozin exposures.
- 3/25 HD dams were found dead and 1/25 was moribund sacrificed; critical clinical signs including dehydration, ↓motor activity, pale extremities, cold to touch, bradypnea, labored breathing, gasping occurred in these animals.
- Dose-dependent decreases in body weight gain were observed in all dapagliflozin-treated animals during the treatment period, and remained decrease in the high dose (HD) group in the postdose recovery period (GD 16 to 20). Decreased body weight gain was associated with decreased FC at all dose levels.
- At 150 and 300 mg/kg, there were total of 21 and 22 late gestation fetal deaths, respectively; and a total of 84 early resorptions at 300 mg/kg were also noted. Reduction of live litter size at 300 mg/kg and ↓fetal body weights at 150 and 300 mg/kg were observed.
- Dose-dependent significant findings in the offspring were malformations: in the blood vessels, fused/wavy ribs and verterbral centra, duplicated manubira and skeletal variations: reduced ossifications (pelvis, vertebrae and sternal centra) were observed at 150 and 300 mg/kg.

BMS-512148: Oral Study of Embryo-Fetal Development in the Rabbit

Study no.:	DN03111
Study report location:	eCTD
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 30 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BMS-512148, lot # 2J61857, and 82%

Methods

Doses:	0, 20, 60 and 180 mg/kg
Frequency of dosing:	Daily
Dose volume:	1 mL/kg
Route of administration:	P.O. (Oral gavage)
Formulation/Vehicle:	90% PEG 400 (v/v) in water
Species/Strain:	Rabbit/New Zeland White
Number/Sex/Group:	22F/group
Satellite groups:	None
Study design:	GD7 – GD 19
Deviation from study protocol:	None that affected study outcome

Key Study Findings

- The NOAEL for maternal toxicity was not established (< 20 mg/kg/d; <64x the MRHD 10 mg) due to reduced body weight gain (≥36%) in all dapagliflozin-treated groups.
- The NOAEL for embryo-fetal development was 180 mg/kg (1191x the MRHD 10 mg) due to no treatment-related findings.
- Exposure in the dams was 64x, 297x and 1191x MRHD (10mg) from a pregnant rabbit TK (bridging) study with identical dapagliflozin exposures.
- Litter endpoints were unaffected by dapagliflozin treatment.
- Malformations and variations were not observed in the offspring.

9.3 Prenatal and Postnatal Development

The pre- and postnatal study and the juvenile study in rats both showed irreversible renal pathology (minimal to marked pelvic dilatation and minimal to moderate tubular dilation) in post-weaning pups. Renal injury was associated with increased kidney weight and increased blood urea nitrogen. The juvenile animals also appear to be more susceptible to adrenal vacuolation (minimal to slight) that was irreversible in recovery

animals. Increased trabecular bone (minimal to slight) was also observed in the high dose juvenile animals. The pre- and postnatal study showed no effects on developmental behavior or reproductive performance other than a slight delay in preputial separation.

Dapagliflozin (BMS-512148): Oral Study of Pre- and Post-Natal Development in Rats

Study no.:	DN09008
Study report location:	eCTD SN: 0333
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 17th 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Dapagliflozin (BMS-512148), 8D42425 and 81.4%

Key Study Findings

F_0 Generation: Body weight gain was slightly suppressed (7%) during gestation (GD 6 - 21) in the high dose (75 mg/kg) females. However, body weight gain rebounded above control females in all treatment groups during lactation. The 75 mg/kg dose had a slight reductive effect on body weight gain during lactation and 75 mg/kg is considered the LOAEL for maternal toxicity

Treatment with dapagliflozin at 1, 15 or 75 mg/kg provide exposure multiples of approx. 19x, 249x and 1415x MRHD.

F_1 Generation: High Dose: Pup weight was reduced at birth, during lactation and in the post-natal period in the high dose animals. Consequently, mature high dose pregnant F_1 females also had lower body weights during gestation. However, this had no effect on the mating or fertility indices. Increased incidence and severity of renal pelvic dilatation was observed in the high dose F_1 animals exposed to dapagliflozin in utero and from their mother's milk. The 15 mg/kg dose is considered the NOAEL.

Low and Mid-Dose: Pups in the low and mid-dose groups had normal body weight at birth, but gained less weight than the control pups during the lactation period when exposure to drug would occur via milk intake. In the post-weaning period, body weight in the low dose pups became comparable to the control pups, whereas the mid-dose pups approached control.

F_2 Generation: Unremarkable regarding body weight and gross physical condition. The internal organs including the kidneys were not evaluated in F_2 animals.

Reviewer Comments: F_1 renal pelvic dilation in the high dose animals occurred in the presence of minimal parental toxicology. As dapagliflozin (BMS-512148) exposure

occurred during a period of renal maturation pre- and post-natally, this suggests that dapagliflozin (BMS-512148) is a renal developmental toxicant at high doses. The NOAEL of 15mg/kg provides a 249x multiple of clinical exposure, so the clinical risk of the renal finding in rats is predicted to be marginal.

Reduced weight gain in all dosed groups during the lactation period suggests that exposure to drug via milk can negatively impact neonatal growth, though the effect appears reversible once exposure to drug stops (e.g., low and mid-doses). Because this effect on weight gain in lactating pups occurred at all doses of dapagliflozin, the label should recommend against using dapagliflozin in nursing women.

Once weaned, control F_1 animals that were not used for mating were used in the juvenile rat study and were treated with dapagliflozin at either 0, 1, 15 or 75 mg/kg from PND 21 to PND 90 (study DN09009).

SD Rats, Segment 3 0, 1, 15 and 75 mg/kg Adverse Effect	**NOAEL $AUC_{(0\text{-}24h)}$ ng.h/mL**	**Multiple of MRHD (506 ng.h/mL)**
Dams High dose: Slight body weight loss during gestation that recovers during lactation. Weight loss during gestation is considered adverse.	15 mg/kg AUC: 116	249x
F_1 Generation: High dose: incidence and severity of renal pelvic dilation increased. Incidence and severity of pelvic dilatation in control males is within the historical range. Significantly reduced weight and weight gain and stunted growth.	15 mg/kg	249x
F_1 Generation: Development Possible developmental toxicant. Reduced weight gain during lactation at all doses tested	< 1 mg/kg	< 19x
F_2 Generation No adverse effects	75 mg/kg	1415x

Methods

Doses:	0, 1, 15 and 75 mg/kg
Frequency of dosing:	Daily
Dose volume:	1 mL/kg
Route of administration:	PO (gavage)
Formulation/Vehicle:	90% PEG 400 in DI water (v/v)
Species/Strain:	Rat/Crl: CD(SD)
Number/Sex/Group:	30 F control, 25 F/group dapagliflozin
Satellite groups:	None
Study design:	Tx GD 6 to PND 20-22
Deviation from study protocol:	None that affected study outcome.

F_0 Dams

Survival:	Twice daily
Clinical signs:	Detailed examinations daily, pre-dose and 2 hr post-dose, 3x/day from GD 20 to parturition.
Body weight:	GD 0, 3, daily GD 6 to 21 and at termination.
Feed consumption:	Daily: GD 6 to 21 and LD 0 to 14.
Uterine content:	Implantation site scars were counted when appropriate. Non-pregnant animals were evaluated for evidence of implantation sites.
Necropsy observation:	
Toxicokinetics:	Dams: PND 4 at 0.5, 1, 2, 4, 8 and 24 hours.
Dosing Solution Analysis	Yes
Other:	

F_1 Generation

Survival:	Twice daily
Clinical signs:	Examined on LD 0 for malformations, sex, weight, and live status and daily for general condition until weaning. Detailed observations daily as adults
Body weight:	At birth, LD 4, 7, 14, and 21. Adults 2x/week. Mated females weighed GD 0, 6, 9, 12, 15, 18, 21 and at termination. At development landmarks.
Feed consumption:	Weekly after weaning and GD 0-6, 6-9, 9-12, 12-15, 15-18, 18-21 for mated F_1 mated females.
Physical development:	Yes
Neurological assessment:	Motor activity in a Figure 8 maze, auditory startle habituation and “E” water maze.
Reproduction:	No
Other:	

F_2 Generation

Survival:	No. of live/dead fetuses counted
Body weight:	At GD 21
External evaluation:	Yes
Male/Female ratio:	Yes
Other:	

Observations and Results

F_0 Generation

Mortality: F_0: One 1 mg/kg female was euthanized on GD 22 for dystocia.

Clinical Signs: Salivation (wet fur) and /or red or brown staining of the mouth/jaw and muzzle were observed from GD 9 to the end of lactation in the 75 mg/kg females.

Body Weight:
Gestation: Mean female body weight was statistically significantly (ss) lower (↓3.6%) at GD7 following the first dose at GD 6 in the 75 mg/kg group. Although not statistically significant, mean body weight was 3-4% lower in the 75 mg/kg group for the remainder of the gestation period. Overall, body weight gain in the 75 mg/kg group was comparable to the control during gestation from GD 6 to 21.
Lactation: At LD 12 mean body weight was ss increased 4.2% in the 75 mg/kg group. During the remainder of lactation F_0 mean body weight was ss increased 4 to 8% in the 75 mg/kg group. Overall, there was a dose dependent increase of body weight gain from LD 0-20 that was ss at 15 mg/kg (↑71%) and 75 mg/kg (↑147%); thus showing recovery and exceeding the body weight loss observed during gestation (see table below).

Table 62. Body Weight Summary in the Dams

F_0 Female Body Weight				
Study Time	Dose, mg/kg	BW gain (g)	% increment/ Decrement	BW % control
Gestation GD 6-21	0	143.4	-	100%
	1	144.2	↑1%	100%
	15	142.3	↓1%	100%
	75	133.4	↓7%	98%
Lactation LD 0-20	0	25.3	-	100%
	1	34.2	↑35%	101%
	15	43.3	↑71%**	103%
	75	62.6	↑147%***	108%***

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ from sponsor's assessment

Sponsor's figures F_0:

Figure 21. Summary Data: Female Body Weights during Gestation

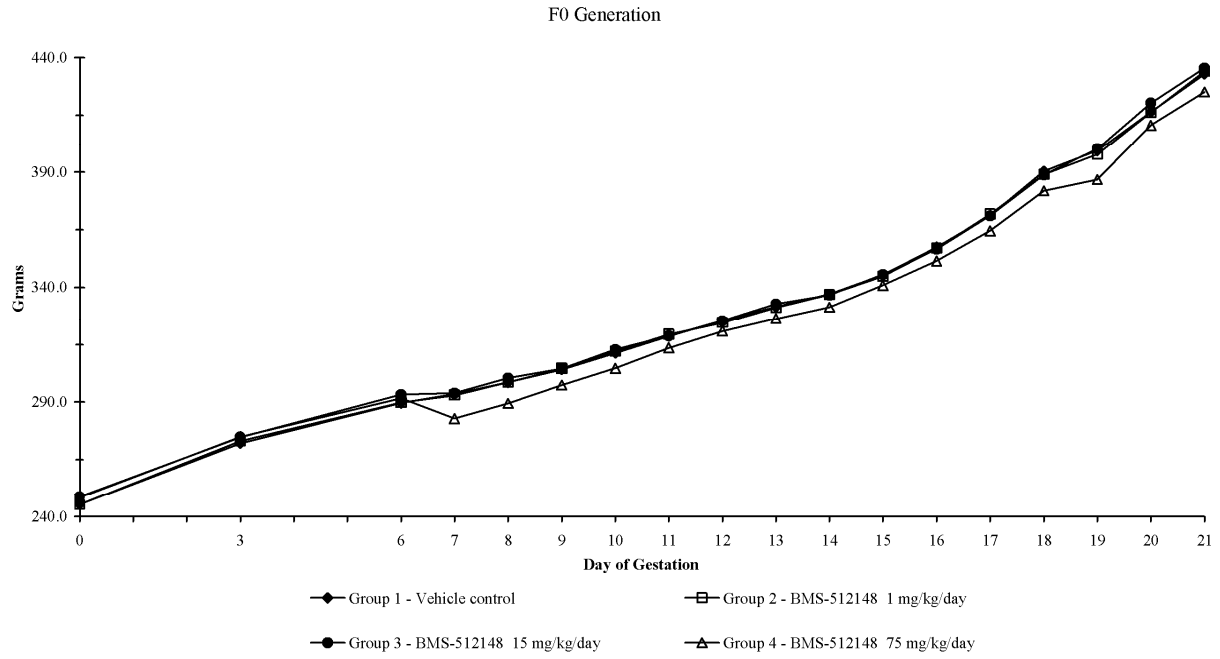
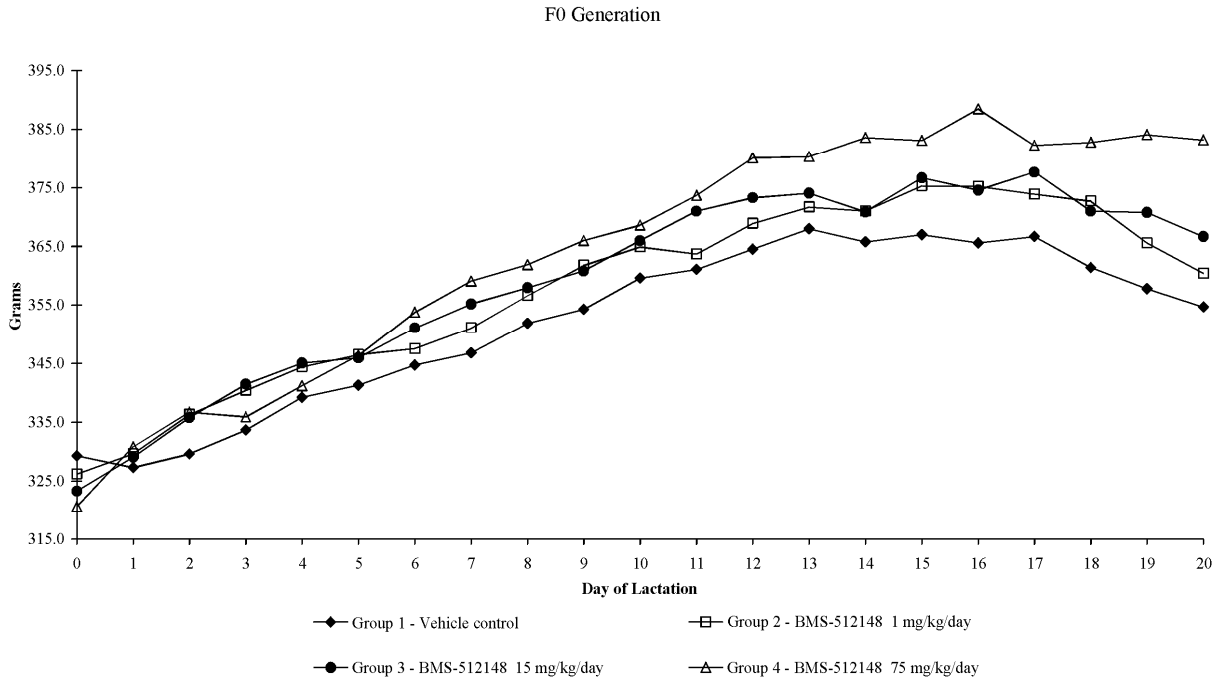


Figure 22. Summary Data: Female Body Weights during Lactation



Food Consumption: Food consumption (FC) was statistically significantly (ss) reduced 32% from GD 6-7 (1st dose) and resulted in significant body weight loss for this interval. From GD 9-21 the low dose FC was ss increased 9-17%. From GD 7-21 the mid dose FC was ss increased 12-25%. From GD 9-18 the high dose FC was ss increased 11-32%. From GD 6-21, overall FC was ss increased 13%, 18% and 13% for low, mid and high dose groups, respectively. Increased FC during gestation did not correlate with increased body weight for the mid and high dose dapagliflozin-treated groups.

From LD 0-2 and LD 5-9 FC was ss increased 10-48% in the high dose lactating females (F_0). From LD 0-3, 5-7, 8-9 and 11-12 FC was ss increased 12-43% in the mid dose lactating females. From LD 3-4, 8-9 and 11-12, FC was ss increased 13-17% in the low dose lactating females. Overall from LD 0-14 FC was ss increased 8%, 12% and 9%, in the low, mid and high dose lactating females, respectively. Increased FC correlated with increased body weight and body weight gain during lactation.

F_0 necropsy: Unremarkable. Sporadic findings appear unrelated to treatment.

F_0 microscopy: The F_0 dam euthanized for dystocia was found with dark fluid in the uterine horns and an enlarged placenta.

F_0 Reproductive Performance: There were no treatment-related effects on reproductive performance. Sponsor's tables:

Table 63. Reproductive Performance Indices

Incidence Data
F0 Generation

Group 1 - Vehicle control
Group 2 - BMS-512148 1 mg/kg/day
Group 3 - BMS-512148 15 mg/kg/day
Group 4 - BMS-512148 75 mg/kg/day

Group	No. of Mated Females	No. of Pregnant Females	Pregnancy Rate (%)	Gestation Index (%)	Dead Pups		Malformed Pups	
					Litters Affected	Pups Affected	Litters Affected	Pups Affected
1	30	30	100.0	100.0	1	8	0	0
2	25	24	96.0	95.8	3	3	1 &&	1
3	25	24	96.0	100.0	1	1	0	0
4	25	24	96.0	100.0	2	2	0	0

&& For litter 273 there is 1 pup included in both dead and malformed
Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 *** - P ≤ 0.001 (Fisher's)

Group Mean Data
F0 Generation

Group 1 - Vehicle control
Group 2 - BMS-512148 1 mg/kg/day
Group 3 - BMS-512148 15 mg/kg/day
Group 4 - BMS-512148 75 mg/kg/day

Group	Summary Information	Length of Gestation (Days)	Duration of Parturition (h)	Sex Ratio (% Males)	Number of Pups at Birth/Litters			No. of Implant Scars	Live Birth Index (%)
					Live	Dead	Malformed		
1	Mean	21.6	3.050	50.33	12.7	0.3	0.00	13.6	93.03
	SD	0.5	1.195	14.06	2.7	1.5	0.00	2.2	14.09
	N	30	8	30	30	30	30	30	30
2	Mean	21.6	3.780	52.72	13.0	0.1	0.00	13.9	93.71
	SD	0.5	-	16.00	1.4	0.3	0.00	1.5	7.66
	N	23	1	23	23	23	23	23	23
3	Mean	21.5	3.536	50.84	13.0	0.04	0.04	13.9	93.62
	SD	0.6	1.946	15.50	2.3	0.20	0.20	2.1	7.93
	N	24	5	24	24	24	24	24	24
4	Mean	21.6	3.318	50.29	12.8	0.1	0.00	13.9	92.25
	SD	0.5	0.535	9.90	2.3	0.3	0.00	2.2	8.40
	N	24	4	24	24	24	24	24	24

Significantly different from control group (group 1) value: a - P ≤ 0.05 b - P ≤ 0.01 c - P ≤ 0.001 (Wilcoxon)

F_0 Toxicokinetics in Dams

At PND 4 $AUC_{0\text{-}24h}$ increased in a slightly less than dose proportional manner. Mean T_{max} was 2 hours at 1 and 15 mg/kg and 8 hours at 75 mg/kg. Sponsor's table:

Table 64. F_0 Toxicokinetic Summary

Parameter	Dapagliflozin		
	1 mg/kg/day	15 mg/kg/day	75 mg/kg/day
Cmax (µg/mL)	0.765	9.15	42.1
AUC(0-24 h) (µg•h/mL)	8.73	116	658
Tmax (h)	2.0	2.0	8.0

F_1 Weaning period

F_1 Mortality: One vehicle control female pup was euthanized at PND 8 due to a suspected broken muzzle. At necropsy clots were present in the nasal cavity and the lungs were dark.

F_1 Viability: There were no treatment related differences on the viability or survival of the F_1 pups though to LD 21. Sponsor's table below:

Table 65. F_1 Generation Viability Data

Summary Data: Viability (%)

F1 Generation Pups
Day Post Partum

Group 1 - Vehicle control
Group 2 - BMS-512148 1 mg/kg/day
Group 3 - BMS-512148 15 mg/kg/day
Group 4 - BMS-512148 75 mg/kg/day

Group	Summary Information	Day 4 Viability Index	Day 7 Survival Index	Day 14 Survival Index	Day 21 Lactation Index
1	Mean	96.19	99.57	99.14	99.14
	SD	18.35	2.32	3.22	3.22
	N	30	29	29	29
2	Mean	98.62	100.00	100.00	100.00
	SD	3.77	0.00	0.00	0.00
	N	23	23	23	23
3	Mean	99.41	99.48	99.48	99.48
	SD	2.04	2.55	2.55	2.55
	N	24	24	24	24
4	Mean	98.36	98.33	97.81	97.81
	SD	4.10	8.16	8.45	8.45
	N	24	24	24	24

Significantly different from control group (group 1) value: § - P ≤ 0.05 §§ - P ≤ 0.01 §§§ - P ≤ 0.001 (Wilcoxon)

F_1 Group mean litter size: There were no significant differences in average litter size. Sponsor's tables below:

Table 66. F_1 Generation Litter Data

Summary Data: Litter Size

F1 Generation Pups
Day Post Partum

Group 1 - Vehicle control
Group 2 - BMS-512148 1 mg/kg/day
Group 3 - BMS-512148 15 mg/kg/day
Group 4 - BMS-512148 75 mg/kg/day

Group	Summary Information	Day 0 Males	Day 0 Females	Day 0 Total	Day 4 (Pre-Cull) Males	Day 4 (Pre-Cull) Females	Day 4 (Pre-Cull) Total
1	Mean	6.3	6.4	12.7	6.3	6.6	12.9
	SD	2.0	2.0	2.7	2.0	1.6	2.2
	N	30	30	30	29	29	29
2	Mean	6.8	6.2	13.0	6.7	6.1	12.8
	SD	2.0	2.3	1.4	1.9	2.3	1.5
	N	23	23	23	23	23	23
3	Mean	6.6	6.4	13.0	6.6	6.3	13.0
	SD	2.3	2.3	2.3	2.3	2.2	2.3
	N	24	24	24	24	24	24
4	Mean	6.5	6.4	12.8	6.4	6.3	12.7
	SD	1.8	1.7	2.3	1.8	1.8	2.4
	N	24	24	24	24	24	24

Summary Data: Litter Size

F1 Generation Pups
Day Post Partum

Group 1 - Vehicle control
Group 2 - BMS-512148 1 mg/kg/day
Group 3 - BMS-512148 15 mg/kg/day
Group 4 - BMS-512148 75 mg/kg/day

Group	Summary Information	Day 4 (Post Cull) Males	Day 4 (Post Cull) Females	Day 4 (Post Cull) Total	Day 7 Males	Day 7 Females	Day 7 Total
1	Mean	3.9	4.0	7.9	3.9	4.0	7.9
	SD	0.3	0.3	0.4	0.3	0.3	0.4
	N	29	29	29	29	29	29
2	Mean	4.2	3.8	8.0	4.2	3.8	8.0
	SD	0.7	0.7	0.0	0.7	0.7	0.0
	N	23	23	23	23	23	23
3	Mean	4.1	3.9	8.0	4.1	3.9	8.0
	SD	0.7	0.7	0.0	0.7	0.7	0.2
	N	24	24	24	24	24	24
4	Mean	4.0	3.9	7.9	3.9	3.9	7.8
	SD	0.2	0.4	0.6	0.4	0.6	1.0
	N	24	24	24	24	24	24

Summary Data: Litter Size

F1 Generation Pups
Day Post Partum

Group 1 - Vehicle control
Group 2 - BMS-512148 1 mg/kg/day
Group 3 - BMS-512148 15 mg/kg/day
Group 4 - BMS-512148 75 mg/kg/day

Group	Summary Information	Day 14 Males	Day 14 Females	Day 14 Total	Day 21 Males	Day 21 Females	Day 21 Total
1	Mean	3.9	4.0	7.9	3.9	4.0	7.9
	SD	0.3	0.3	0.4	0.3	0.3	0.4
	N	29	29	29	29	29	29
2	Mean	4.2	3.8	8.0	4.2	3.8	8.0
	SD	0.7	0.7	0.0	0.7	0.7	0.0
	N	23	23	23	23	23	23
3	Mean	4.1	3.9	8.0	4.1	3.9	8.0
	SD	0.7	0.7	0.2	0.7	0.7	0.2
	N	24	24	24	24	24	24
4	Mean	3.9	3.8	7.8	3.9	3.8	7.8
	SD	0.4	0.6	1.0	0.4	0.6	1.0
	N	24	24	24	24	24	24

F_1 Body Weight:

Pup weight was statistically significantly (ss) reduced at birth (↓7% in males, and ↓6% in females) and at post natal day (PND) 4 pre-cull (↓12% in males and ↓11% in females) in the 75 mg/kg animals. Male pup weight was further ss reduced 13-23% at PND 4 (post-cull), 7, 14, 17 and 21 of the weaning period in the 75 mg/kg group. Female pup weight was ss reduced 12-22% at PND 4 (post-cull), 7, 14, 17 and 21 in the 75 mg/kg (high dose) females. Decreased body weight gain at 75 mg/kg in both males and females is considered the LOAEL.

Male pup weight was statistically significantly (ss) reduced 6-7% at PND 4 (post-cull) and during the weaning period in the 15 mg/kg (mid dose) males. In the 15 mg/kg females, pup weight was ss reduced 5-6% at each of PND 14, 17 and 21.

Table 67. Body Weight Summaries

Body Weight of Live F_1 Newborns				
	Control n=30	1 mg/kg n=25	15 mg/kg n=25	75 mg/kg n=25
Males (g)	7.23	7.11	7.14	6.73**
Females (g)	6.77	6.71	6.8	6.36**

* p < 0.05; ** p < 0.01; *** p < 0.001 from sponsor's assessment

F_1 MALES: Body Weight to LD 21				
Study Time	Dose, mg/kg	BW gain (g)	% Decrement	BW % control
LD 0 to 21	0	59.48	-	100%
	1	57.12	↓4%	97%
	15	54.32	↓9%	93%
	75	46.81	↓21%	81%

F_1 FEMALES: Body Weight to LD 21				
Study Time	Dose, mg/kg	BW gain (g)	% Decrement	BW % control
LD 0 to 21	0	55.8	-	100%
	1	54.73	↓2%	98%
	15	52.42	↓6%	95%
	75	44.46	↓20%	81%

F_1 Visceral, Skeletal Examination: Very few pups (≤ 5/group) were evaluated for external/internal findings. No major abnormalities were reported in those few pups examined.

F_1 Post-weaning Period

Post-weaning Viability: One adult 75 mg/kg male was found dead at PND 74. There were no clinical signs prior to the death and there were no macroscopic or microscopic findings for this animal.

Post-weaning physical exam: Unremarkable.

Post-weaning body weight: Mean body weight was statistically significantly reduced 5-6% from PND 25 to 32 in the 15 mg/kg males. In the first 2 weeks post-weaning, male and female body weights were ss reduced 9-16% in the 75 mg/kg group and were ss

reduced 7-9% for subsequent weeks up to PND 84. The reduced body weight did not correlate with reduced food consumption in the F_1 animals. Sponsor's figures:

Figure 23. F_1 Post-weaning Male Body Weight

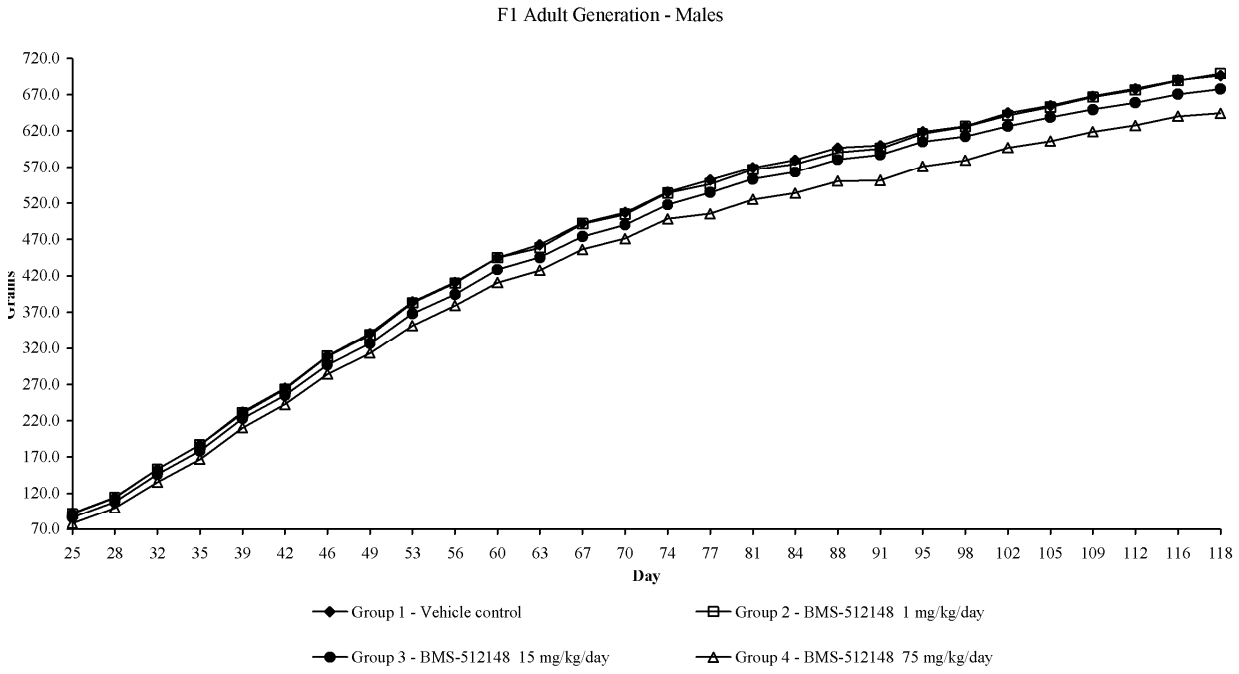


Figure 24. F_1 Post-weaning Female Body Weight

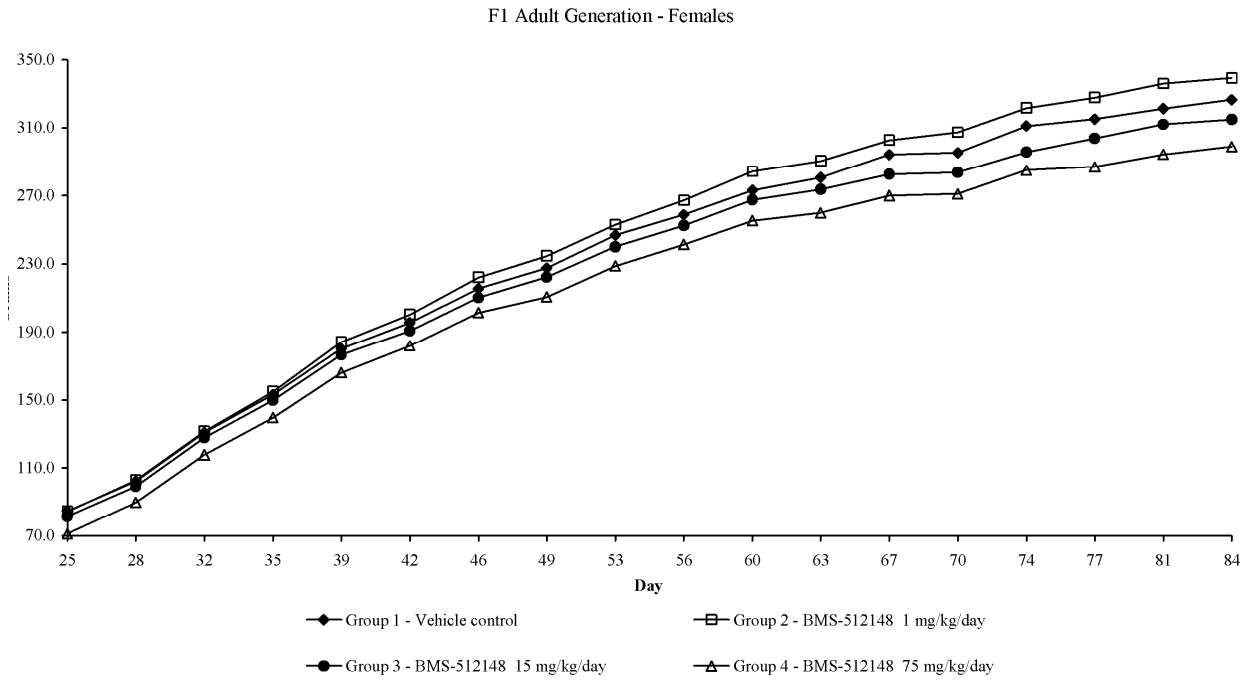


Table 68. Post-Weaning Body Weight Summary

Post-Weaning Body Weight				
Post weaning week	Control	1 mg/kg	15 mg/kg	75 mg/kg
Females Week 1	102.3	102.9	99.3	**89.8*** **
Week 12	326.5	339.3	314.9	**299*** **
Males Week 1	114.8	114.1	**108.3***	**99.8*** **
Week 12	580.3	574.7	563.3	**534.1****

* p < 0.05; ** p < 0.01; *** p < 0.001 from sponsor's assessment

F_1 Physical exam: Dapagliflozin had no effect on the number of days to reach sexual maturity (vaginal opening) in the females. However, there was a slight delay in the age of preputial separation at 15 mg/kg (1 day) and 75 mg/kg (2 days). The delay in preputial separation may have been due to the lower body weight observed for these groups. Sponsor's tables:

Table 69. F_1 Vaginal Opening

F1 Adult Generation
Females

Group 1 - Vehicle control
Group 2 - BMS-512148 1 mg/kg/day
Group 3 - BMS-512148 15 mg/kg/day
Group 4 - BMS-512148 75 mg/kg/day

Group	Summary Information	Day of Development	Body Weights on Day of Development
1	Mean	31.7	126.2
	SD	1.4	12.6
	N	29	29
2	Mean	31.7	128.9
	SD	1.0	8.0
	N	23	23
3	Mean	31.8	126.1
	SD	0.8	11.8
	N	24	24
4	Mean	32.3	120.1
	SD	1.7	13.8
	N	24	24

Significantly different from control group (group 1) value: § - P ≤ 0.05 §§ - P ≤ 0.01 §§§ - P ≤ 0.001 (Wilcoxon) (Day of Development)
Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 *** - P ≤ 0.001 (Dunnett) (Body Weight Day of Development)
† - P ≤ 0.05 †† - P ≤ 0.01 ††† - P ≤ 0.001 (Dunn)

Table 70. F_1 Preputial Separation

F1 Adult Generation
Males

Group 1 - Vehicle control
Group 2 - BMS-512148 1 mg/kg/day
Group 3 - BMS-512148 15 mg/kg/day
Group 4 - BMS-512148 75 mg/kg/day

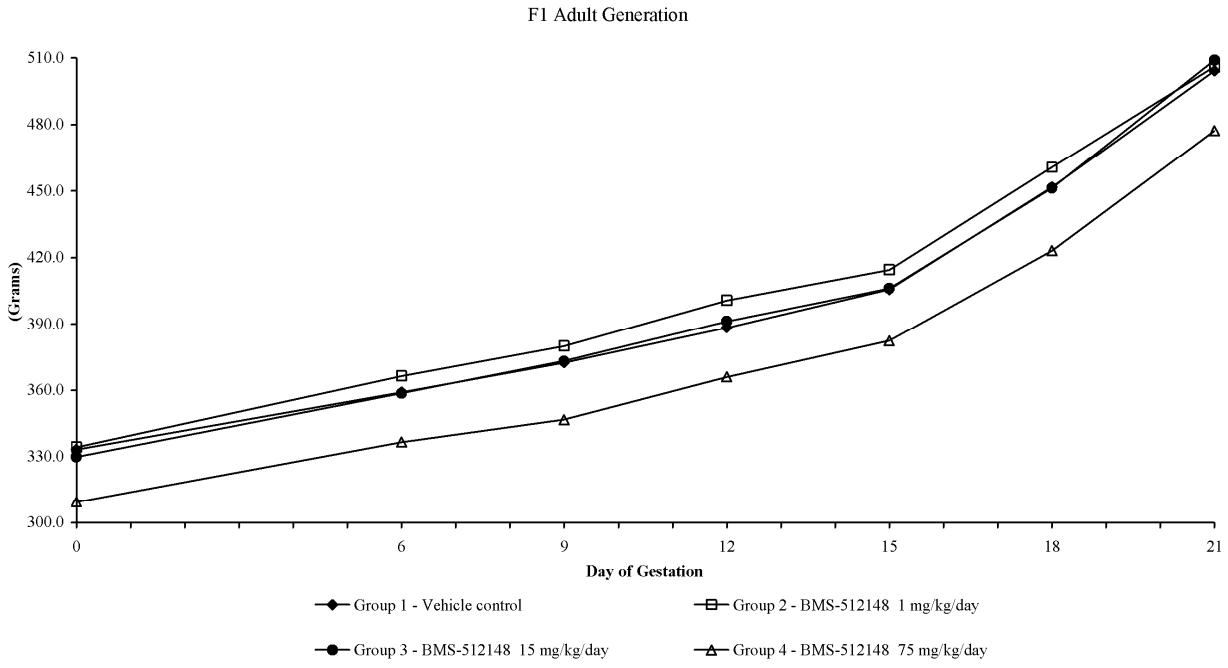
Group	Summary Information	Day of Development	Body Weights on Day of Development
1	Mean	42.2	267.2
	SD	1.5	21.7
	N	29	29
2	Mean	43.0	273.9
	SD	1.7	25.7
	N	23	23
3	Mean	43.3 §	267.7
	SD	2.0	22.9
	N	24	24
4	Mean	44.6 §§§	270.2
	SD	1.1	22.2
	N	24	24

Significantly different from control group (group 1) value: § - P ≤ 0.05 §§ - P ≤ 0.01 §§§ - P ≤ 0.001 (Wilcoxon) (Day of Development)
Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 *** - P ≤ 0.001 (Dunnett) (Body Weight Day of Development)
† - P ≤ 0.05 †† - P ≤ 0.01 ††† - P ≤ 0.001 (Dunn)

Behavioral Development post-weaning (n= 23-29 per group)
Motor activity: Unremarkable.
Startle habituation: Unremarkable.
E water maze: There were no differences in maze learning or memory.

F_1 Mating to Sacrifice Period
Mortality: None.
Physical Exam: Unremarkable.
Body weight: Mean body weight was statistically significantly reduced 4-7% at GD 0, 9 and 18 in the F_1 pregnant females in the 75 mg/kg group. However, the overall food consumption during gestation did not differ from controls in the 75 mg/kg F_1 females.
Sponsor's figure:

Figure 25. F1 Female Body Weight During Gestation



Reproductive Performance: The mating and fertility indices of the F_1 males and females were comparable to the control animals. There were no treatment related effects on the number of copora lutea, implantation sites, live fetuses, dead fetuses, resorptions, sex ratio, placenta's or pre- and post-implantation losses. Sponsor's tables:

Table 71. F_1 Generation Parental Performance

F1 Generation

Group 1 - Vehicle control
Group 2 - BMS-512148 1 mg/kg/day
Group 3 - BMS-512148 15 mg/kg/day
Group 4 - BMS-512148 75 mg/kg/day

Group	Number Placed for Mating		Number Mating	Mean (SD) Day to Mating	Number Females Pregnant	Mating Index (%)	Fertility Index (%)	Conception Rate (%)
	Males	Females						
1	29	29	27	3.6 (1.9) 25	25	93.1	86.2	92.6
2	23	23	20	3.6 (3.1) 20	17	87.0	73.9	85.0
3	24	24	22	3.8 (2.7) 21	21	91.7	87.5	95.5
4	24	24	24	3.4 (2.3) 24	24	100.0	100.0	100.0

Significantly different from control group (group 1) value: a - $P \leq 0.05$ b - $P \leq 0.01$ c - $P \leq 0.001$ (Wilcoxon - day to mating only)
Significantly different from control group (group 1) value: * - $P \leq 0.05$ ** - $P \leq 0.01$ *** - $P \leq 0.001$ (Fisher's)

Table 72. F_1 Generation Uterine Findings

Group 1 - Vehicle control
Group 2 - BMS-512148 1 mg/kg/day
Group 3 - BMS-512148 15 mg/kg/day
Group 4 - BMS-512148 75 mg/kg/day

Group	Summary Information		Total Number of Corpora Lutea	Total Implantation Sites	Male Fetuses	Female Fetuses	Sex Ratio (% Males)
1	Mean		17.3	15.8	7.2	7.8	50.11
	SD		3.1	4.0	2.4	2.6	14.52
	N		24	24	23	23	23
2	Mean	A	16.5	13.8	6.2	6.4	-
	SD		5.0	5.3	2.7	3.5	-
	N		17	17	17	17	-
	Mean	B	17.5	14.6	6.6	6.8	50.98
	SD		3.0	4.3	2.2	3.2	12.26
	N		16	16	16	16	16
3	Mean		18.0	16.3	7.4	7.9	47.43
	SD		2.4	2.5	2.7	2.0	14.35
	N		20	20	19	19	19
4	Mean		17.0	15.3	6.8	7.7	49.80
	SD		2.2	4.5	2.5	3.1	16.48
	N		24	24	24	24	24

A - Including animal(s) with total resorption / ammonium sulfide
B - Excluding animal(s) with total resorption / ammonium sulfide
Significantly different from control group (group 1) value: § - $P \leq 0.05$ §§ - $P \leq 0.01$ §§§ - $P \leq 0.001$ (Wilcoxon)

Group 1 - Vehicle control
Group 2 - BMS-512148 1 mg/kg/day
Group 3 - BMS-512148 15 mg/kg/day
Group 4 - BMS-512148 75 mg/kg/day

Group	Summary Information		Live Fetuses	Dead Fetuses	Early Resorptions	Middle Resorptions	Late Resorptions
1	Mean		15.2	0.0	0.7	0.0	0.0
	SD		3.8	0.0	0.7	0.0	0.0
	N		24	24	24	24	24
2	Mean	A	12.6	0.0	1.1	0.0	0.0
	SD		5.4	0.0	1.3	0.0	0.0
	N		17	17	17	17	17
	Mean	B	13.4	0.0	1.2	0.0	0.0
	SD		4.5	0.0	1.3	0.0	0.0
	N		16	16	16	16	16
3	Mean		15.4	0.0	0.9	0.0	0.0
	SD		2.7	0.0	0.9	0.0	0.0
	N		20	20	20	20	20
4	Mean		14.5	0.0	0.8	0.1	0.0
	SD		4.3	0.0	0.9	0.4	0.0
	N		24	24	24	24	24

A - Including animal(s) with total resorption / ammonium sulfide
B - Excluding animal(s) with total resorption / ammonium sulfide
Significantly different from control group (group 1) value: § - $P \leq 0.05$ §§ - $P \leq 0.01$ §§§ - $P \leq 0.001$ (Wilcoxon)

Group 1 - Vehicle control
Group 2 - BMS-512148 1 mg/kg/day
Group 3 - BMS-512148 15 mg/kg/day
Group 4 - BMS-512148 75 mg/kg/day

Group	Summary Information		Sum of Resorptions	Preimplantation Loss (%)	Post Implantation Loss (%)	Gravid Uterus Weight (g)
1	Mean		0.7	9.57	3.99	114.1
	SD		0.7	18.50	4.13	27.8
	N		24	24	24	24
2	Mean	A	1.1	15.94	14.80	-
	SD		1.3	20.91	24.53	-
	N		17	17	17	16
	Mean	B	1.2	16.93	9.48	103.3
	SD		1.3	21.18	11.29	31.8
	N		16	16	16	16
3	Mean		0.9	8.98	5.68	117.0
	SD		0.9	11.02	5.61	19.7
	N		20	20	20	20
4	Mean		0.9	11.01	6.98	107.1
	SD		0.9	22.64	10.51	29.6
	N		24	24	24	24

A - Including animal(s) with total resorption / ammonium sulfide
B - Excluding animal(s) with total resorption / ammonium sulfide
Significantly different from control group (group 1) value: § - P ≤ 0.05 §§ - P ≤ 0.01 §§§ - P ≤ 0.001 (Wilcoxon)

Gross Pathology: Approximately equal numbers of rats/group were evaluated for gross findings. An increased incidence of pelvic dilatation was observed in the kidney of F_1 adult males in the 75 mg/kg group. The gross lesion was confirmed microscopically as unilateral or bilateral pelvic dilatation. Reviewer note: The incidence of pelvic dilatation in the high dose males is slightly above (54% vs 0-40%) the historical control kidney pelvic dilatation incidence (see appendix). Sponsor's table for F_1 gross pathology:

Table 73. Gross Pathology

Incidence of Dapagliflozin-Related Gross Finding

	Dose (mg/kg/day):	**0**	**1**	**15**	**75**
	No. of rats (M/F):	**29/25**	**23/20**	**24/21**	**24/24**
	Sex:	**M/F**	**M/F**	**M/F**	**M/F**
Tissue: Kidney					
Dilatation: pelvis		8/4	7/4	9/3	13/6

Microscopic pathology: Low and unequal numbers of rats/group were evaluated histologically. Essentially all males and females examined showed a dilated renal pelvis, both unilateral and bilateral. The severity is apparently increased in females at 75mg/kg. Other histological findings were of low incidence and, given the low numbers of animals examined, difficult to ascribe to drug treatment.

Table 74. F_1 Generation Microscopic Findings in the Kidney

Incidence of Microscopic Finding of Pelvic Dilatation in the Kidneys

Dose (mg/kg/day):	0	1	15	75
No. of rats (M/F):	9/5	7/4	11/3	14/6
Sex:	M/F	M/F	M/F	M/F
Kidney:				
Dilatation: pelvis	9/4	7/4	10/3	13/6
Minimal	2/1	0/2	3/0	3/2
Slight	1/3	3/1	5/0	4/0
Mild	1/0	4/0	1/2	3/1
Moderate	5/0	0/1	1/1	2/3
Marked	-	-	-	1/0

A dash (-) indicates absence of finding in group

F_2 Generation

Body weight: Male and female F_2 fetal litter weights were unremarkable.
Gross Pathology: Major malformations were unremarkable.

10 Special Toxicology Studies

Dapagliflozin (BMS-512148): Oral Study of Toxicokinetics in Lactating Rat Dams and Their Nursing Pups	
Study #	DN10025
Study report location	eCTD SN:0346
CRO/Laboratory name	(b) (4)
CRO/Laboratory address	(b) (4)
Date of study initiation	23rd March 2010
GLP compliance statement	Yes
GLP deviations identified	Yes
QA statement	Yes
Drug, lot #, and % purity	Dapagliflozin (BMS-512148), 8D42425 and 81.3%

Key Study Findings

F_0 Generation: Dapagliflozin exposure ($AUC_{0\text{-}24h}$) in the dams was approximately dose proportional. For 1, 15 and 75 mg/mL, the milk to plasma ratio was 0.49x

F_1 Generation: Systemic exposure to dapagliflozin in the pups was 9 to 15% of that of the dams.

Reviewer Comments: TK evaluation only. No other data was presented by the sponsor. However, drug exposure in the lactating pups was 2x, 29x, and 142x the MRHD, indicating that pups were exposed to pharmacologically and toxicologically relevant levels of drug.

METHODS	
Doses	0, 1, 15 and 75 mg/kg
Frequency of dosing	Daily
Dose volume	1 mL/kg
Route of administration	PO (gavage)
Formulation/Vehicle	90% PEG 400 in DI water (v/v)
Species/Strain	Rat/Crl: CD(SD)
Number/Sex/Group	9 time-mated rats/group
Satellite groups	None
Study design	Tx GD 6 to LD 10
Deviation from study protocol	None that affected study outcome.

IN-LIFE OBSERVATIONS	**FREQUENCY**
Mortality & cageside observations	Twice daily (adults)
Clinical examination	F_0: Detailed examinations weekly and at termination. F_1: Daily
Body weight	F_0: GD 0, 3, daily GD 6 to LD 10. F_1: At birth, LD 4 and 7.
Toxicokinetics	F_0: Blood: PND 10 at 0.5, 1, 2, 4, 8 and 24 hours. F_0: Milk: PND 10 at 2 hours post-dose. F_1: Blood: PND 10 at 0.5, 1, 2, 4, 8 and 24 hours.
Culling	On LD 4, F_1 litters were reduced to 8 pups per litter (4/sex where possible).
POST-MORTEM EVALUATIONS	
Termination	F_0 dams were euthanized on LD 10 or 11; F_1 animals were euthanized at LD 10.

Observations and Results

F_0 Generation

Mortality: F_0: None.

Clinical Signs: Data not submitted in the sponsor's report

Body Weight: Data not submitted in the sponsor's report.

F_0 necropsy: N/A.

F_0 Toxicokinetics

At LD 10 the mean dapagliflozin exposure (AUC_{0-24h}) in the dams were approximately dose proportional between 1 and 15 mg/kg and between 15 and 75 mg/kg. The mean T_{max} ranged from 1 to 8 hours and C_{max} ranged from 0.693 to 26.1 μg/mL and was dose proportional from 1 to 15 mg/kg. At LD 10 the mean milk dapagliflozin concentration was less than dose proportional at 2 hours post-dose. The milk to plasma ratios were 0.49x for each dapagliflozin treatment (1, 15 or 75 mg/kg). Sponsor's tables:

Table 75. Toxicokinetic Summary in Lactating Dams

Parameter	Dapagliflozin		
	1 mg/kg/day	15 mg/kg/day	75 mg/kg/day
C_{max} (μg/mL)	0.693	9.95	26.1
AUC(0-24 h) (μg•h/mL)	7.34	142	427
T_{max} (h)	1.0	4.0	8.0

Table 76. Summary of Milk-to Plasma Dapagliflozin Ratios in Dams

Dose (mg/kg/day)	Time (Post Treatment)	Mean Dapagliflozin Concentration (ng/mL)		Mean Milk-to-Plasma Dapagliflozin Concentration Ratio
		Milk	Plasma	
1	Day 10 2h	289.81	581.45	0.49
15	Day 10 2h	2985.54	6195.97	0.49
75	Day 10 2h	10130.30	21178.79	0.49

F_1 Lactation period

F_1 Mortality: None.

F_1 Viability: Not examined.

F_1 Body Weight: Data not submitted in the report.

F_1 Toxicokinetics

At LD 10 the mean dapagliflozin exposure (AUC_{0-24h}) and C_{max} in the pups were dose proportional and there were no gender differences. The mean T_{max} was 6 hours at each of 1, 15 and 75 mg/kg. Systemic exposures in the pups were 9 to 15% of the dams. Sponsor's table:

Table 77. Toxicokinetic Summary in Pups

Parameter	Dapagliflozin Dose in Dams					
	1 mg/kg/day		15 mg/kg/day		75 mg/kg/day	
	Male	Female	Male	Female	Male	Female
C_{max} (µg/mL)	0.0469	0.0574	0.866	0.769	3.41	3.54
AUC(0-24 h) (µg•h/mL)	0.797	0.775	13.4	13.4	64.0	65.9
T_{max} (h)	6.0	6.0	6.0	6.0	6.0	4.0

Dapagliflozin (BMS-512148): Two Month Oral Developmental Study in Juvenile Rats with a 1-Month Recovery	
Study #	DN09009
Study report location	eCTD SN: 0310
CRO/Laboratory name	(b) (4)
CRO/Laboratory address	(b) (4)
Date of study initiation	29th April 2009
GLP compliance statement	Yes
GLP deviations identified	Yes
QA statement	Yes
Drug, lot #, and % purity	Dapagliflozin (BMS-512148), 8D42425 and 81.4%

Key Study Findings

F_0 Generation: During dosing, weight gain was less at all doses of drug in males and females. By the end of dosing, final body weight was lower in males at all doses but significant in females only at 75mg/kg. Final crown/rump length was shorter in males and females only at 75mg/kg.

There was a dose-dependent increase in blood urea nitrogen which was marked in the mid and high dose animals. Histopatholigically, there was an increased incidence and severity of cortical tubular dilatation and pelvic dilatation in the kidney, particularly in the mid and high dose animals.

Dapagliflozin reduced serum glucose in all mid and high dose animals. Serum calcium was also reduced in the mid dose males and high dose males and females. Dapagliflozin also produced high glucosuria and calciuria in all treated animals and increased polyuria in the mid and high dose animals. Natriuria and phosphuria was also noted in the mid and high dose animals and may be due to diuriesis.

Kidney weight and kidney body/brain weight ratios were increased and correlated with kidney enlargement and microscopically with an increased incidence and severity of cortical tubular dilatation and pelvic dilatation (from minimal to marked). Kidney tubular dilatation and pelvic dilatation of similar incidence and severity was present in recovery animals.

Hemorrhage and ulceration/erosion of the stomach (minimal to slight) was observed in the mid and high dose animals. Reduced zymogen granules in the pancreas (minimal to slight severity) was noted in the high dose animals. Adrenal gland cortical vacuolation with enlargement in some animals and correlating with increased adrenal weight/adrenal to body weight ratios (mid and high dose), was noted in all dapagliflozin-treated animals. Cortical vacuolation was irreversible. Increased trabecular bone (minimal to slight) was noted in the high dose animals.

F_0 mating, fertility and pregnancy were generally successful. The age of preputial separation was delayed 1-2 days in the dapagliflozin-treated males and may have been due to the reduced body weight.

Dapagliflozin exposure in mature male animals at PND 83 was 28-35% less than that of mature females. Systemic exposure was also greater in immature post-weaning animals, suggesting increased metabolic activity of the mature animals.

Treatment with dapagliflozin at 1, 15 or 75 mg/kg provide exposure multiples of approx. 15x, 208x and 1086x MRHD, respectively.

F_1 Generation: Unremarkable.

Reviewer Comments: Urinary glucose increased in all dapagliflozin-treated animals and is likely due to the pharmacological action of dapagliflozin.

Final body weight was reduced at all doses and was markedly reduced at the high dose. There was pathological evidence of renal damage (cortical tubule dilatation and pelvic dilatation) that was irreversible. Similar renal damage was observed in the F_1 generation exposed in utero and during lactation in a pre- and postnatal study conducted at identical doses in the rat. For all treatment group males, the incidence of renal pelvic dilatation is above the historical control incidence range of renal pelvic dilatation (82-100% vs 0-40%).

Adrenal cortical vacuolation was unusual and is likely associated with an aldosterone production in response to diuresis. However it is concerning that adrenal cortical vacuolation was also irreversible.

As dapagliflozin (BMS-512148) exposure occurred during a period of renal maturation post-natally, this suggests that dapagliflozin (BMS-512148) is a renal developmental toxicant.

SD Rats, Juvenile Study 0, 1, 15 and 75 mg/kg Adverse Effect	NOAEL $AUC_{0\text{-}24\ h}$ (ng.h/mL)	Multiple of MRHD (506 ng.h/mL)
F_0: Irreversible kidney pelvic dilatation and tubular dilatation in all dapagliflozin groups. Adrenal cortical vacuolation observed in all groups and recovery animals.	Not established	Not established
F1 Generation	N/A	N/A

METHODS	
Doses	0, 1, 15 and 75 mg/kg
Frequency of dosing	Daily
Dose volume	1 mL/kg
Route of administration	PO (gavage)
Formulation/Vehicle	90% PEG 400 in DI water (v/v)
Species/Strain	Rat/Crl: CD(SD)
Number/Sex/Group	21/sex/group
Satellite groups	11 naïve F/group used to as mating partners
Study design	Tx PND 21 to PND 90
Deviation from study protocol	None that affected study outcome.

IN-LIFE OBSERVATIONS	FREQUENCY
Mortality & cageside observations	Twice daily (adults)
Clinical examination	F_0: Detailed examinations daily, pre-dose, 2 hr post-dose and daily in recovery animals. F_1: PND 0 and daily during lactation. Naïve females: at body weight assessment.
Body weight	F_0: 2x/week and at termination. Mated females at GD 0, 3, 6, 9, 12, 15, 18, 21 and PND 0, 3 and at termination. BW also at physical development landmarks (vaginal opening and preputial separation). F_1: PND 0 and 3. Mated naïve females: GD 0, 6 and 13.
Toxicokinetics	F_0: PND 21 and 83 at 0.5, 1, 2, 4, 8 and 24 hours.
Food consumption	F_0: Weekly until cohabitation; Mated females GD 0-3, 3-6, 6-9, 9-12, 12-15 and 15-18. Mated naïve females GD 0-6 and 6-13.
Breeding procedure	Treated females mated with naïve males from PND 94-97. Treated males mated with a naïve female from PND 93-96 for 14 days.

Growth	Crown to rump length measured at the body weight assessment and at PND 90.
F_0 Maturation	Physical development in the females (vaginal opening) from PND 26 until development. Physical development in the males (preputial separation) from PND 35 until development.
Opthalmoscopy	At the end of treatment and recovery periods: funduscopic (indirect opthalmoscopy) and biomicroscopic (slit lamp) examination.
Estrous Cycles	F_0: Treated recovery females 14 days prior to mating until mating confirmation by vaginal lavage.
POST-MORTEM EVALUATIONS	
Termination	F_0 animals were euthanized at the end of treatment at SD 90. F_0 recovery males were euthanized 3 weeks after the end of the mating period. F_0 dams that failed to mate were euthanized at 28d following mating. F_0 dams that failed to litter were euthanized at 27d following mating. F_0 pregnant dams were fasted and euthanized on PND 4, 5 or 6. Naïve mated females were euthanized at GD 13. Naïve female dams that failed to mate were euthanized at 7d following mating. **Reviewer note**: the disposition of naïve mated males is unknown. Except for found dead or moribund pups, F_1 pups were fasted and euthanized PND 4, 5 or 6.
Macroscopic	Gross pathology performed for all F_0 animals and F_1 pups found dead or dying. F_1 animals undergoing scheduled euthanasia were discarded without examination if externally normal.
Microscopic	F_0 terminal: Control and HD: all tissues; LD and MD: only tissues with gross lesions. F_0 recovery: only tissues with treatment-related findings.
Organ weights	F_0: adrenal glands, brain, heart, kidneys, liver, lungs, ovaries/testes, pituitary, prostate, seminal vesicles, spleen, thymus, thyroid and parathyroid glands and uterus.
Uterine & Ovarian exams	Implantation site scars were counted when appropriate. Non-pregnant animals were evaluated for evidence of implantation sites.
Blood collection	At termination: hematology, CBC, coagulation and urinalysis.

Observations and Results

F_0 Generation

Mortality: F_0: One 1 mg/kg male was found dead at PND 86. There were no macroscopic or microscopic observations and the cause of death is unknown.

Clinical Signs: Salivation, wet fur and /or red staining of the mouth/jaw and muzzle were observed with high incidence during treatment in the F_0 75 mg/kg males and non-pregnant females but not in the recovery animals, pregnant/lactating dams (non-treatment phase) (Sponsor's tables):

Table 78. F_0 Generation Clinical Observations in Males

F0 Generation
Treatment Period
Males

Group 1 - Vehicle control
Group 2 - BMS-512148 1 mg/kg/day
Group 3 - BMS-512148 15 mg/kg/day
Group 4 - BMS-512148 75 mg/kg/day

	Group			
Clinical Sign\Site	1	2	3	4
Number of animals per group	21	21	21	21
Fur Staining Red\Muzzle	3	6	5	14
Fur Wet\Lower Jaw	1	.	.	20
Salivation	.	.	.	21

Table 79. F_0 Generation Clinical Observations in Females

F0 Generation
Treatment Period
Females

Group 1 - Vehicle control
Group 2 - BMS-512148 1 mg/kg/day
Group 3 - BMS-512148 15 mg/kg/day
Group 4 - BMS-512148 75 mg/kg/day

	Group			
Clinical Sign\Site	1	2	3	4
Number of animals per group	21	21	21	21
Fur Wet\Lower Jaw	.	.	.	21
Salivation	.	.	.	21

Body Weight: Mean body weight was statistically significantly (ss) lower (↓10%) at PND 25 in both males and females in the 75 mg/kg group. Thereafter, mean body weight was ss lower in the males (↓6-9%) and females (↓6-8%) during the treatment period. However, the frequency of ss lower mean body weight was more pronounced in the 75 mg/kg males (n=16) than the females (n=4). Terminal body weights were ss and 13%, 12% and 16% lower in the low, mid and high dose males, respectively. Terminal body weights were 7%, 3% and 14% (ss) lower in the low, mid and high dose females, respectively. Mean body weights were comparable to the control animals during the recovery period. Mean body weights and body weight gains were unchanged in the dams during gestation and lactation. Mean body weight gain was ss reduced 36% and 38% at PND 21-25 and PND 84-88, respectively, in the 75 mg/kg males; and 13% and 42% at PND 21-25 in the 15 and 75 mg/kg females, respectively. Increased food consumption did not correlate with increased body weight or body weight gain.

Table 80. Body Weight Summaries.

F_0 Terminal Body Weight (End of Dosing Period)				
	Control n=21	1 mg/kg n=20	15 mg/kg n=21	75 mg/kg n=21
Males (g)	586.05	508.48**	517.47**	493.55***
Females (g)	304.95	283.78	296.27	262.62**

* p < 0.05; ** p < 0.01; *** p < 0.001 (Dunnett)

F_0 Male Body Weight				
Study Time	Dose, mg/kg	BW gain (g)	% decrement	BW % control
PND 21-25	0	25	-	100%
	1	23.4	↓6%	97%
	15	22.8	↓9%	97%
	75	16	↓36%***	90%***
PND 84-88	0	17.8	-	100%
	1	8.8	↓51%**	99%
	15	13.9	↓22%	98%
	75	11	↓38%***	89%***

* p < 0.05; ** p < 0.01; *** p < 0.001 from sponsor's assessment

F_0 Female Body Weight				
Study Time	Dose, mg/kg	BW gain (g)	% increment/ decrement	BW % control
PND 21-25	0	20.7	-	100%
	1	19.4	↓6%	99%
	15	18.1	↓13%**	98%
	75	12.1	↓42%***	89%***

* p < 0.05; ** p < 0.01; *** p < 0.001 from sponsor's assessment

Sponsor's body weight figures:

Figure 26. F_0 Body weight in Males during Dapagliflozin Treatment

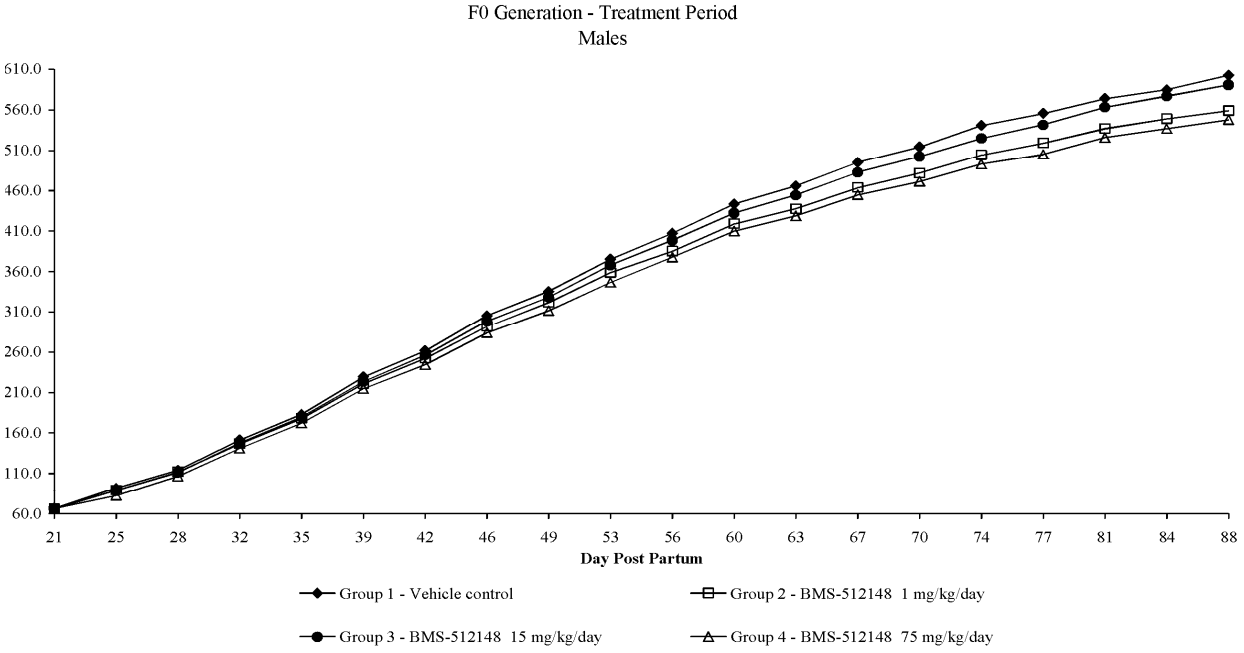


Figure 27. F_0 Body weight in Females during Dapagliflozin Treatment

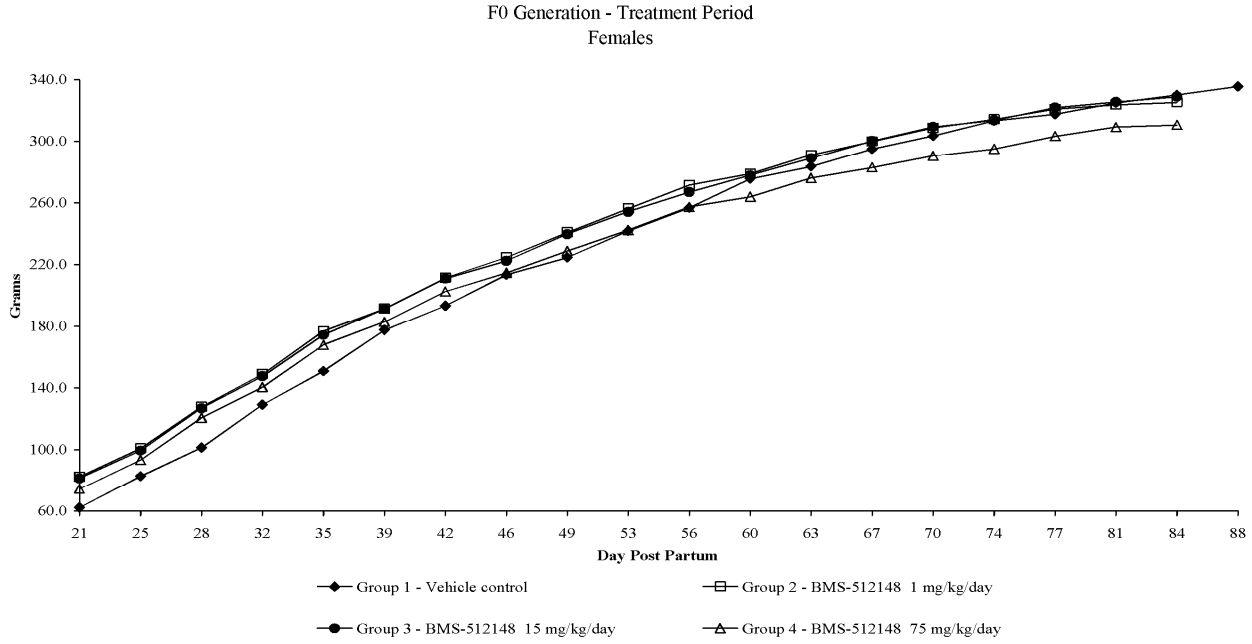


Figure 28. F_0 Body weight in Recovery Males

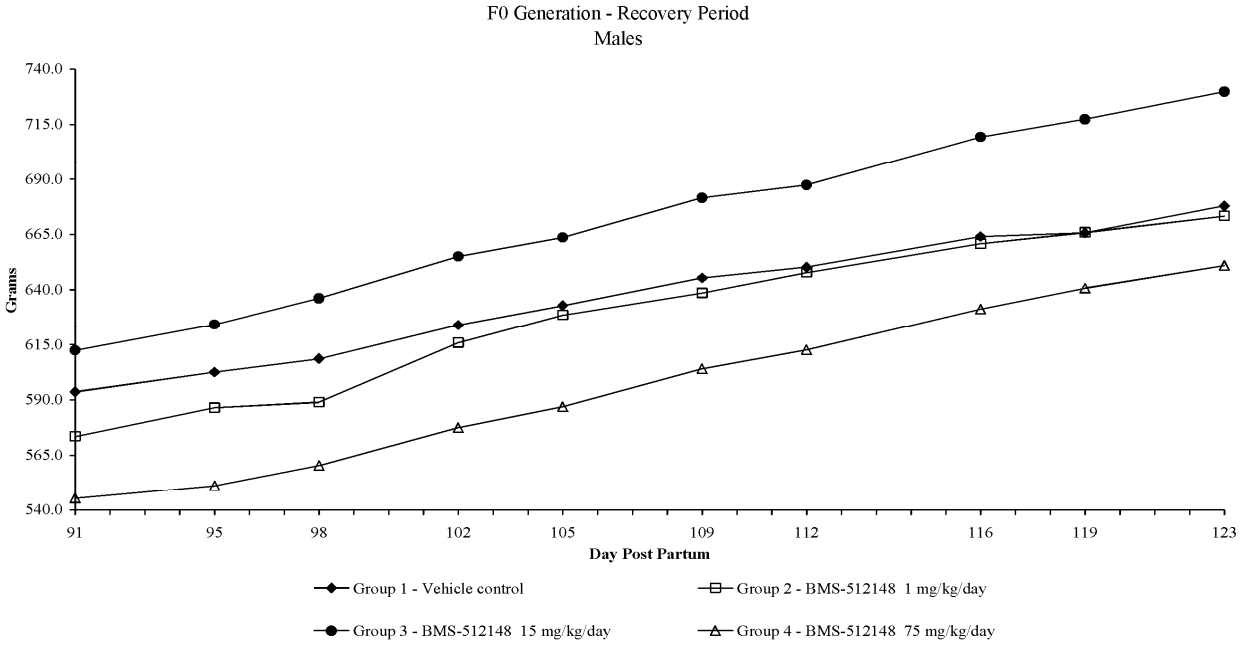
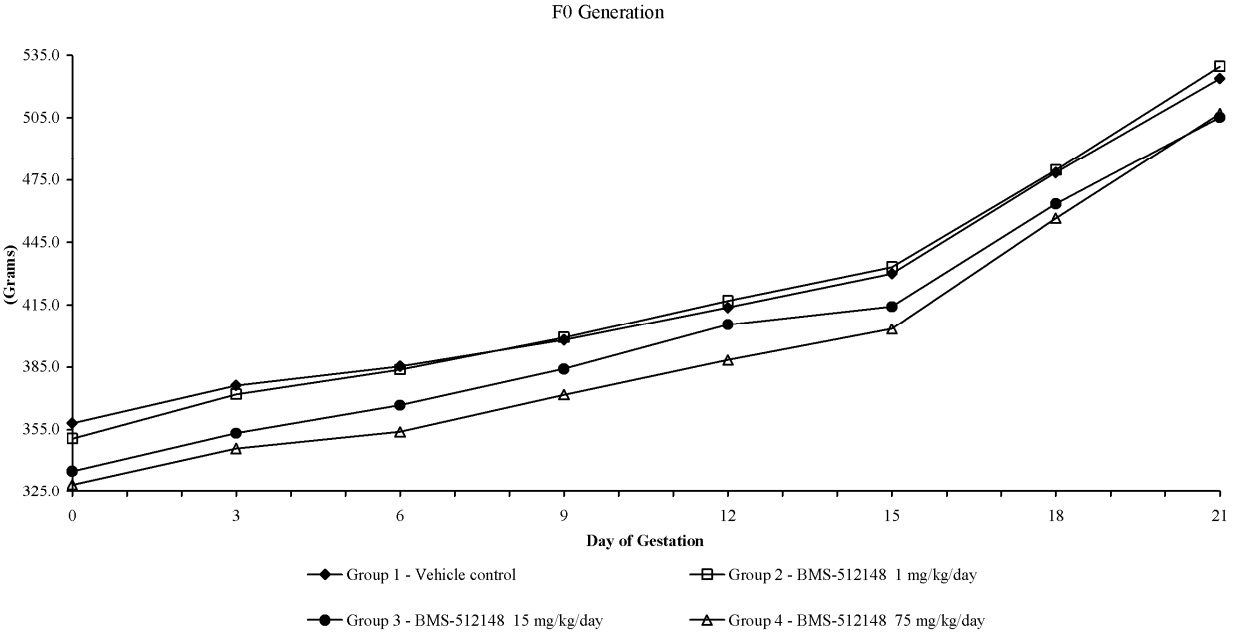


Figure 29. F_0 Body weight in Recovery Females



Food Consumption: From SD 28-91 food consumption (FC) was statistically significantly (ss) increased 7-32% and 12-20% in the 1 mg/kg males and females respectively. FC was also ss increased 15-49% and 22-38% in the 15 mg/kg males and females, respectively; and ss increased 17-48% and 21-38% in the 75 mg/kg males and females, respectively. FC was not increased in the dams during gestation.

$\mathbf{F_0}$ Growth: The crown-to-rump length was statistically significantly reduced 3-5% from PND 35-90 (during treatment) in the 75 mg/kg males. Similarly, the crown-to-rump length was statistically significantly reduced 2-4% from PND 39-90 in the 75 mg/kg females. The reduced growth in the 75 mg/kg animals correlated with reduced body weights and body weight gain. The crown-to-rump length was statistically significantly reduced 2-3% from PND 60-88 in the 1 mg/kg males; and 2-3% from PND 70-77 in the 15 mg/kg males. However, final crown-rump length at the end of the dosing period was significantly reduced in males and females only at 75mg/kg.

Table 81. Males: Crown-Rump Length

Group	Summary Information	21	90
1	Mean	9.19	22.29
	SD	0.72	0.49
	N	21	21
2	Mean	9.26	21.89
	SD	0.80	0.49
	N	21	19
3	Mean	9.33	22.02
	SD	0.97	0.54
	N	21	21
4	Mean	9.29	21.48 ***
	SD	0.58	0.66
	N	21	21

Table 82. Females: Crown-Rump Length

Group	Summary Information	21	90
1	Mean	8.79	19.21
	SD	0.56	0.49
	N	21	21
2	Mean	9.21	18.98
	SD	0.60	0.64
	N	21	21
3	Mean	9.14	18.98
	SD	0.59	0.43
	N	21	21
4	Mean	9.12	18.64 **
	SD	0.72	0.42
	N	21	21

F_0 Physical Exam: The age of preputial separation was statistically significantly delayed in each of the dapagliflozin-treated groups. When corrected for body weight at the age when the criterion was met, there remained a ss delay (1-2 days). The 1-2 day delay was within the historical control range and had no adverse effect on mating or fertility. Sponsor's tables:

Table 83. F_0 Physical Development in the Males

Summary Data: Preputial Separation

F0 Generation

Group 1 - Vehicle control
Group 2 - BMS-512148 1 mg/kg/day
Group 3 - BMS-512148 15 mg/kg/day
Group 4 - BMS-512148 75 mg/kg/day

Group	Summary Information	Day of Development	Body Weights on Day of Development
1	Mean	42.0	258.9
	SD	1.2	20.6
	N	21	21
2	Mean	42.9 §	259.0
	SD	1.3	19.3
	N	21	21
3	Mean	43.0 §	262.8
	SD	1.1	22.0
	N	21	21
4	Mean	44.0 §§§	264.5
	SD	1.5	24.2
	N	21	21

Significantly different from control group (group 1) value: § - P ≤ 0.05 §§ - P ≤ 0.01 §§§ - P ≤ 0.001 (Wilcoxon) (Day of Development)
Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 *** - P ≤ 0.001 (Dunnett) (Body Weight Day of Development)
† - P ≤ 0.05 †† - P ≤ 0.01 ††† - P ≤ 0.001 (Dunn)

F0 Generation

Group 1 - Vehicle control
Group 2 - BMS-512148 1 mg/kg/day
Group 3 - BMS-512148 15 mg/kg/day
Group 4 - BMS-512148 75 mg/kg/day

Group	Summary Information	Day of Development
1	LSMean	42.0
	SELSM	0.3
	N	21
2	LSMean	43.0 *
	SELSM	0.3
	N	21
3	LSMean	42.9 *
	SELSM	0.3
	N	21
4	LSMean	43.9 ***
	SELSM	0.3
	N	21

Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 *** - P ≤ 0.001 (Dunnett)

F_0 Estrous Cycles: There were no dapagliflozin-related effects on estrous cyclicity.

F_0 Mating and Fertility: The mating and fertility indices, mean number of days to mating and the conception rate of the F_0 adult males and females when placed for mating with untreated counterparts was comparable to the controls.

F_0 Maternal Performance: The length of gestation, duration of parturition, number of implant scars, number of live/dead pups, malformed pups at birth, sex ratio, liver birth index was unaffected by treatment with dapagliflozin.

F_0 Reproductive Performance: There were no treatment-related effects on reproductive performance.

F_0 Clinical pathology:
RBC and Clotting Parameters: Minimal but statistically significant (ss) reductions in red blood cells (RBC) (↓6%) and hematocrit (Hct) (↓7%) were observed in the high dose females. Mean platelet volume (MPV) was ss increased approximately 11% in the mid and high dose males and also the high dose females. APPT was ss increased 9% and 11%, respectively, in the mid and high recovery males.

Table 84. Treatment Period RBC Parameters

RBC PARAMETERS AT END OF DOSING PERIOD								
Parameter (units)	**Males** (mg/kg/day)				**Females** (mg/kg/day)			
	0	1	15	75	0	1	15	75
RBC (10^6/uL)	8.45	8.71	8.71	8.22	8.01	7.90	7.57	**7.52** *
Hct (%)	46.61	46.54	47.44	45.23	43.42	42.68	41.58	**40.33** **
MPV (fL)	6.71	6.90	**7.42** **	**7.48** **	6.58	6.84	6.87	**7.35** ***
APTT (sec) Recovery	15.94	16.50	**17.35** *	**17.66** **	13.21	14.18	14.00	15.24

WBC Parameters: Dose-dependent reductions in white blood cells (WBC) that were ss reduced were observed in the mid (↓36%) and high dose (↓45%) males and the mid (↓20%) and high dose females (↓37%). WBC remained ss reduced in the high dose female recovery animals (↓34%). There was also a dose-dependent reduction in lymphocyte counts (LYMPH) that were ss reduced in the mid (↓40%) and high dose (↓47%) males and the mid (↓24%) and high dose (↓44%) females. Monocytes (MONO) were dose-dependently and ss reduced in the mid (↓40%) and high dose (↓63%) males. Eosinophils (EOS) and basophils (BASO) were also dosed-dependently and ss reduced approximately 44-66% in the high dose males and females. Leukocytes (LUC) were dose-dependently and ss reduced in the high dose males (↓46%). Lymphocytes and monocytes remained dose-dependently and ss reduced (↓35% and 46%, respectively) in the high dose recovery females. WBC were ss reduced 34% in the high dose recovery females. The reduction in the WBC and differential WBC is suggestive of bone marrow suppression/damage or could be a stress response. However, there was no correlative bone marrow pathology.

Table 85. Treatment Period WBC Parameters

WBC PARAMETERS AT END OF DOSING PERIOD								
Parameter (units)	**Males** (mg/kg/day)				**Females** (mg/kg/day)			
	0	1	15	75	0	1	15	75
WBC (10^3/uL)	13.59	13.53	**8.66** *	**7.53** *	9.03	8.63	**7.19** *	**5.65** **
LYMPH (10^3/uL)	11.43	11.41	**6.83** **	**5.96** **	7.76	7.09	**5.91** **	**4.34** **
MONO (10^3/uL)	0.41	0.37	**0.24** *	**0.15** *	0.2	0.23	0.17	0.2
EOS (10^3/uL)	0.18	0.14	0.11	**0.06** **	0.1	0.1	0.07	**0.06** *
BASO (10^3/uL)	0.05	0.04	**0.02** *	**0.01** *	0.01	0.01	0.01	**0.007** **
LUC (10^3/uL)	0.24	0.22	0.14	**0.13** *	0.13	0.11	0.11	0.08
WBC Recovery (10^3/uL)	12.78	12.2	11.51	10.95	10.93	9.6	12.25	7.24 *
LYMPH Recovery (10^3/uL)	10.43	9.82	9.18	8.44	7.30	7.05	6.92	**4.76** *
MONO Recovery (10^3/uL)	0.50	0.51	0.39	0.40	0.36	0.32	0.26	**0.19** **

** p ≤ 0.05, **p<0.01 (from sponsor's assessment).*

CBC Parameters: Serum ALT was ss increased in the low (↑36%), mid (↑62%) and high dose males (↑63%). Serum AST was ss increased in the mid (↑40%) and high dose (↑58%) males and the high dose females (↑42%). Elevations in serum transaminases were without histopathology correlates. Total protein (T PROT) and albumin (ALB) were ss reduced in both high dose males (↓7%) and females (↓9%). Total bilirubin (TBIL) was also ss reduced in the high dose males (↓7%). Blood urea nitrogen (UREA) was ss and dose-dependently increased 47%, 95% and 121% in the low, mid and high dose males and ss increased 58%, 125% and 159% in the low, mid and high dose females, respectively. In contrast, serum creatinine (CREAT) was ss reduced (↓25%) in the high dose males. The increased UREA suggests increased protein catabolism and/or renal damage and the decreased creatinine suggests reduced muscle mass. Histopathologically in the kidney, there was an increased incidence and severity of kidney tubular and pelvic dilatation that was sometimes associated with cortical tubular mineralization.

Serum glucose (GLUC) was ss reduced in the mid dose male (↓42%) and female (↓22%) animals and also the high dose male (↓48%) and female (↓39%) animals, respectively. Serum calcium (CA) was dose-dependently and ss reduced in the mid (↓4%) and high (↓6%) dose males and also ss reduced in the high dose females (↓4%). Serum chloride (CL) was also ss reduced in the mid (↓3%) and high (↓2%) dose males and the mid dose (↓2%) females.

All serum chemistry parameters returned to baseline in the recovery animals.

Table 86. Treatment Period Serum Chemistry Parameters

SERUM CHEMISTRY AT END OF DOSING PERIOD								
Parameter (units)	**Males** (mg/kg/day)				**Females** (mg/kg/day)			
	0	1	15	75	0	1	15	75
ALT (U/L)	40.3	**54.8***	**65.4*****	**65.9*****	42.3	47.6	50.1	55.4
AST (U/L)	116.8	133.6	**163.8***	**185*****	106.2	132.4	119	**150.4***
T PROT (U/L)	6.86	6.53	6.59	**6.35***	6.8	6.71	6.66	**6.22****
ALB (g/dL)	4.43	4.25	4.34	**4.16 ***	4.83	4.63	4.61	**4.41 ****
TBIL (mg/dL)	0.109	0.101	0.096	**0.076*****	0.137	0.14	0.125	0.111
UREA (mg/dL)	14.16	**20.8*****	**27.61*****	**31.27*****	14.23	**22.47*****	**32.02*****	**36.96*****
CREAT (mg/dL)	0.4	0.37	0.38	**0.3****	0.42	0.42	0.39	0.36
GLUC (mg/dL)	105.5	96.4	**61.6*****	**54.7*****	102.8	102	**79.9*****	**62.8*****
CA (mg/dL)	10.99	10.7	**10.55***	**10.35****	10.8	10.54	10.53	**10.34****
CL (mg/dL)	99.2	98.9	**96.5***	**97.3***	100.9	100.5	**98.6***	99.1

** p ≤ 0.05, **p ≤ 0.01 and ***p≤ 0.001 (from sponsor's assessment).*

Urinalysis: A dose-dependent increase in urinary volume (UVOL) that was ss was observed in the mid (↑142%) and high dose (↑150%) dapagliflozin-treated males and the mid (↑98%) and high dose (96%) dapagliflozin-treated females. Urinary glucose (GLUC) was also ss increased 533-597% in the dapagliflozin-treated males and 657-940% in the dapagliflozin-treated females, respectively. The specific gravity (SG) of the urine was ss increased 1-2% in the dapagliflozin-treated females but not in the males. Urinary sodium (NA) and phosphorous (PHOS) excretion were both ss increased 93-205% in the mid dose males and females. Urinary calcium (CA) excretion was ss

increased 163-411% in the dapagliflozin-treated males and females. Urinary glucose (GLUC) excretion was also ss increased 657-2734% in all dapagliflozin-treated animals except the low dose females, where GLUC excretion was also increased 1306% but was not ss.

Table 87. Treatment Period Urinalysis Parameters

URINALYSIS, END OF DOSING PERIOD								
Parameter (units)	**Males** (mg/kg/day)				**Females** (mg/kg/day)			
	0	1	15	75	0	1	15	75
UVOL (mL)	15.25	23.52	**36.94*** **	**38.14*** **	13.8	19.07	**27.29** **	**27.06** **
GLUC (mg/dL)	12.3	**7356.1 *** **	**6573.1 ** **	**7228.9 *** **	7.3	**4801.9 * **	**6866 *** **	**6301.9 *** **
SG	1.0327	1.0493	1.0425	1.0452	1.0269	**1.0391 * **	**1.0451 ** **	**1.0427 ** **
NA exc (mmol/collection period)	0.52	1.006	**1.142 * **	**1.587 *** **	0.48	0.811	**1.148 ** **	**1.156 ** **
PHOS exc (mg/collection period)	16.817	20.52	**35.147 ** **	**37.445 *** **	12.106	17.086	**26.717 *** **	**28.606 *** **
CA exc (mg/collection period)	0.66	**1.739 * **	**1.972 ** **	**3.373 *** **	0.926	**2.441 * **	**3.093 *** **	**3.647 *** **
GLUC exc (mg/collection period)	1.48	1572.21	**2399.35 *** **	**2751.17 *** **	0.64	836.28	**1750.31 *** **	**1584.94 *** **

** p ≤ 0.05, **p ≤ 0.01 and ***p≤ 0.001 (from sponsor's assessment).*

F_0 Organ Weights: Absolute kidney weight, kidney to body weight ratio and kidney to brain weight ratio were all statistically significantly and dose-dependently increased (15-55%) in both male and female dapagliflozin-treated rats (sponsor's table below). This correlated macroscopically with kidney enlargement and pelvic dilatation and microscopically with minimal to moderate tubular dilatation and minimal to marked pelvic dilatation. The increased absolute and relative kidney weights failed to reverse in the recovery animals and correlated microscopically with minimal to moderate tubular dilatation and minimal to marked pelvic dilatation in the recovery animals (sponsor's table below).

Table 88. Dapagliflozin Organ Weight Changes in the Treatment Period

Dose (mg/kg/day):		1		15		75	
	Sex:	M	F	M	F	M	F
Body weight		↓13	↓7	↓12	↓3	↓16	↓14
Kidney							
	Absolute	↑15*	↑21**	↑17*	↑32***	↑27***	↑33***
	% body	↑33***	↑32***	↑33***	↑36***	↑50***	↑55***
	% brain	↑19**	↑21**	↑21***	↑34***	↑33***	↑40***

Note: The numerical values in the table represent the respective percent increase [↑] or percent decrease [↓] from control mean, relative to body and relative to brain values [(treated group mean - control group mean) ÷ control group mean] × 100.

Table 89. Dapagliflozin Organ Weight Changes in the Recovery Period

Dose (mg/kg/day):		1		15		75	
	Sex:	M	F	M	F	M	F
Body weight		—	↓1	↑9	↓3	↓7	↓5
Kidney							
	Absolute	↑13	↑3	↑24***	↑4	↑13	↑11
	% body	↑13*	↑5	↑16*	↑8	↑23***	↑17**
	% brain	↑14*	↑6	↑23***	↑5	↑15*	↑14**

A dash (-) indicates absence of change in group; * = P ≤ 0.05; ** = P ≤ 0.01; *** = P ≤ 0.001 for absolute values

Note: The numerical values in the table represent the respective percent increase [↑] or percent decrease [↓] from control mean, relative to body and relative to brain values [(treated group mean - control group mean) ÷ control group mean] × 100.

The absolute male and female spleen weights and spleen to brain weight ratios were significantly reduced (↓approx. 17-22%) in the mid and high males and high dose female rats. The reduction in spleen weight did not have histopathological correlates. Spleen weights were unremarkable in recovery animals.

Absolute adrenal weight was ss increased 16% in the high dose males and adrenal to body weight ratios were ss increased 20%, 37% and 25% in the mid and high dose males and the high dose females, respectively. The adrenal to brain weight ratio was also ss increased 22% in the high dose males. The increase in adrenal gland weight in the high dose males correlated macroscopically, in some individual animals, with adrenal gland enlargement and microscopically with adrenal gland cortical vacuolation and cortical hypertrophy. Absolute adrenal weight and adrenal to body/brain weight ratios were unremarkable in recovery animals.

The absolute liver weight and the liver to brain weight ratio were decreased 10-14% in the dapagliflozin-treated males. The liver to body weight ratio was increased 17-23% in the mid and high dose females and remained elevated 14% in the high dose female recovery animals. Changes in the liver weight /body weight ratio did not correlate to liver histopathology.

F_0 necropsy: At the terminal necropsy kidney enlargement and dilatation of the pelvis was observed in the dapagliflozin-treated animals. This correlated microscopically with kidney tubular dilatation and pelvic dilatation. In the glandular stomach there were dark areas and foci in some of the dapagliflozin-treated animals that correlated microscopically with minimal hemorrhage. Furthermore in one or two dapagliflozin-treated males there were depressed areas of the stomach that that correlated microscopically with minimal to slight erosion/ulceration. In recovery animals pelvic dilation was present in the dapagliflozin-treated males and the mid and high dose females that correlated with pelvic dilation and tubular dilatation. Two high doses females had pale discoloration of the adrenal gland that correlated microscopically with slight vacuolation of the adrenal zona glomerulosa (sponsor's tables):

Table 90. Gross Necropsy Findings During the Treatment Period

Incidence of Noteworthy Gross Findings

Dose (mg/kg/day):	0	1	15	75
No. of rats (M/F):	10/10	11/10	10/10	10/10
Sex:	M/F	M/F	M/F	M/F
Kidney:				
Dilatation pelvis	4/1	9/1	8/3	8/5
Enlargement	1/0	4/0	5/2	5/2
Stomach:				
Area dark	—	—	2/3	0/4
Area depressed	—	1/0	2/0	1/0
Foci dark	—	0/1	1/3	3/2

A dash (—) indicates absence of finding in group

Table 91. Gross Necropsy Findings During the Recovery Period

Incidence of Noteworthy Gross Findings in Post-dose Recovery Animals

Dose (mg/kg/day):	0	1	15	75
No. of rats (M/F):	11/11	10/11	11/11	11/11
Sex:	M/F	M/F	M/F	M/F
Kidney:				
Dilatation pelvis	3/3	7/1	7/5	6/6
Enlargement	—	—	1/0	1/0

A dash (—) indicates absence of finding in group

F_0 microscopy: In all dapagliflozin-treated animals there is an increased incidence and severity of kidney tubular dilatation and pelvic dilatation. These changes correlated with kidney enlargement observed grossly and increased kidney weight and kidney body/brain weight ratios. Microscopically these findings were associated with cortical tubular mineralization that increased in incidence in the high dose males. The cortical tubular mineralization differed from the collecting duct mineralization and medullary mineralization observed in the 6 month rat and 1 year dog nonclinical studies, respectively. **Reviewer note**: For all treatment group males, the incidence of renal pelvic dilatation is above the historical control incidence range of renal pelvic dilatation (82-100% vs 0-40%).

Minimal to slight adrenal gland cortical vacuolation (zona glomerulosa) was observed in the dapagliflozin-treated rats. In the stomach there was an increased incidence of minimal hemorrhage and minimal to slight ulceration/erosion of the glandular mucosa in the mid and high dose dapagliflozin-treated animals. The hemorrhage correlated to the gross necropsy finding of depressed stomach areas.

In the pancreas there was a dose-related increase in the (minimal to slight) reduction of zymogen granules observed in the mid and high dose dapagliflozin-treated animals. In the high dose animals minimal to slight increased trabecular bone was observed (sponsor's tables):

Table 92. Microscopic Findings During the Treatment Period

Incidence of Noteworthy Microscopic Findings in Dosing Period Animals

Dose (mg/kg/day):	0	1	15	75
No. of rats (M/F):	10/10	11/10	10/10	10/10
Sex:	M/F	M/F	M/F	M/F
Adrenal:				
Vacuolation: cortical	—	6/5	6/6	9/8
Minimal	—	5/3	3/3	6/4
Slight	—	1/2	3/3	3/4
Kidney:				
Dilatation: tubular	1/0	9/5	9/8	10/9
Minimal	1/0	4/4	4/4	4/6
Slight	—	4/1	5/4	6/3
Moderate	—	1/0	—	—
Dilatation: pelvis	4/1	9/2	10/3	10/5
Minimal	—	2/0	3/1	4/0
Slight	3/1	4/1	3/0	5/1
Moderate	1/0	3/1	4/1	1/2
Marked	—	—	0/1	0/2
Mineralization: tubular	0/1	4/0	6/1	8/4
Minimal	0/1	4/0	6/1	8/4

Dose (mg/kg/day):	0	1	15	75
No. of rats (M/F):	10/10	11/10	10/10	10/10
Sex:	M/F	M/F	M/F	M/F
Stomach:				
Hemorrhage	0/1	0/2	3/6	4/5
Minimal	0/1	0/2	3/6	4/5
Ulceration/erosion: glandular mucosa	—	—	4/4	2/5
Minimal	—	—	3/2	1/0
Slight	—	—	1/2	1/5
Pancreas:				
Decreased zymogen granules	—	—	5/8	8/10
Minimal	—	—	4/5	6/3
Slight	—	—	1/3	2/7

Sternum:					
Increased trabecular bone		—	—	—	6/7
	Minimal	—	—	—	2/4
	Slight	—	—	—	4/3

A dash (-) indicates absence of finding in group

Minimal to slight vacuolation of adrenal gland (zona glomerulosa) was present in all female recovery animals with a slightly higher incidence in dapagliflozin-treated females. In the kidney, there is an increased incidence and severity (minimal to moderate) of kidney tubular dilatation and pelvic dilatation in the dapagliflozin-treated recovery animals. These findings correlated with increased kidney body weight and body weight ratios and also gross necropsy observations of kidney pelvic dilatation. Kidney tubular mineralization was also observed, particularly in the mid and high dose dapagliflozin-treated recovery males (sponsor's table):

Table 93. Microscopic Findings During the Recovery Period

Incidence of Noteworthy Microscopic Findings in Post-Dose Recovery Animals

Dose (mg/kg/day):		0	1	15	75
No. of rats (M/F):		11/11	10/11	11/11	11/11
Sex:		M/F	M/F	M/F	M/F
Adrenal:					
Vacuolation: cortical		1/6	1/11	1/9	3/10
	Minimal	1/4	1/6	1/1	3/5
	Slight	0/2	0/5	0/8	0/5
Kidney:					
Dilatation: tubular		1/1	4/2	9/8	7/10
	Minimal	1/1	4/2	5/8	4/10
	Slight	—	—	3/0	3/0
	Moderate	—	—	1/0	—
Dilatation: pelvis		4/3	7/2	9/7	7/8
	Minimal	2/0	2/1	3/3	3/3
	Slight	2/2	4/1	4/3	2/5
	Moderate	0/1	1/0	1/1	2/0
	Marked	—	—	1/0	—
Mineralization: tubular		0/1	1/0	3/0	7/0
	Minimal	0/1	1/0	3/0	7/0

F_0 Toxicokinetics

At PND 21 (first dose) the $AUC_{0\text{-}24h}$ and C_{max} increased in a dose proportional manner. Mean T_{max} was 2 or 4 hours at 1 and 15 mg/kg and 4 hours at 75 mg/kg. The systemic exposure to dapagliflozin was similar in the males and females. At PND 83 the $AUC_{0\text{-}24h}$ and C_{max} increased in an approximate dose proportional manner. Mean T_{max} was 2 or 1 hours at 1 and 15 mg/kg and 4 hours at 75 mg/kg. The systemic exposure to dapagliflozin was approximately 28-35% lower in the males than in the females. The exposure to dapagliflozin was slightly lower in the mature animals at PND 83 than that at PND 21, and suggests an increase in metabolic capability of the adult animals (sponsor's table):

Table 94. Toxicokinetic Summary

Parameter	Day	Dapagliflozin					
		1 mg/kg/day		15 mg/kg/day		75 mg/kg/day	
		Male	Female	Male	Female	Male	Female
Cmax (µg/mL)	21	0.935	0.921	14.1	14.3	56.4	67.1
	83	0.772	1.12	11.3	15.9	39.9	51.4
AUC(0-24 h) (µg•h/mL)	21	9.92	11.7	167	176	849	937
	83	6.97	9.63	97.0	135	505	779
Tmax (h)	21	4.0	2.0	4.0	2.0	4.0	4.0
	83	2.0	2.0	1.0	1.0	4.0	4.0

F_1 Weaning period

F_1 Mortality & Viability: No differences were noted in the number of pups born dead or found dead or dying and there were no differences in average litter sizes or viability.

F_1 Clinical Signs: No treatment related differences were observed though LD 4.

F_1 Body Weight: Pup weight was unremarkable.

F_1 Gross Pathology: Unremarkable.

11 Integrated Summary and Safety Evaluation

The proposed dapagliflozin film-coated tablet was submitted in accordance with 21 USC 505(b)(1) for the treatment of type 2 diabetes mellitus. Dapagliflozin is the first in class SGLT2 inhibitor that has been submitted to the Agency for approval.

A comprehensive battery of nonclinical studies were conducted to support the development of dapagliflozin for chronic use. All pivotal nonclinical studies were conducted using oral administration of the drug, which is the clinical exposure route, and in accordance with US FDA GLP regulations (21CFR58) as stated by the sponsor. Most nonclinical studies were reviewed in the course of drug development and are summarized in the NDA review.

Safety margins to expected human exposure were estimated using C_{max} = 136 ng/mL and AUC_{0-24} = 465 ng.h/mL plasma exposure in healthy subjects at the proposed maximum recommended human dose (MRHD) of 10 mg dapagliflozin.

Pharmacology
Dapagliflozin (BMS-512148 (b) (4)) is a selective inhibitor of sodium glucose co-transporter (SGLT) 2. SGLT2 is selectively expressed in the kidney S1 proximal tubule and is responsible for the renal reabsorption of glucose. Inhibition of SGLT2 by dapagliflozin results in the excretion of glucose thereby producing glucosuria. In in vitro studies dapagliflozin was a potent and selective inhibitor of human (h) SGLT2 relative to the closely related hSGLT1 with a selectivity of 1242-1600-fold. The glucose transporters (GLUT) play a critical role in tissue glucose uptake in tissues such as the skeletal muscle and adipose tissue. Dapagliflozin did not inhibit human or mouse adipocyte GLUT1 or GLUT4 transporters when tested at 20 μM which is greater than the human C_{max} of approximately 1 μM.

In nonclinical models of diabetes dapagliflozin promoted glucose excretion, polyuria and lowered plasma glucose in diabetic and non-diabetic animal models under conditions of hyperglycemia (oral glucose tolerance test).

Safety pharmacology assessment of cardiovascular, neurological and pulmonary effects of dapagliflozin did not identify significant liabilities.

Absorption, Distribution, Metabolism and Excretion
An oral dose of dapagliflozin was rapidly absorbed and is approximately 80% bioavailable in the rat and dog but only 25% bioavailable in the non-human primate. Dapagliflozin distributes rapidly to most rat tissues with low amounts distributing to the brain and bone. The steady state volume of distribution for dapagliflozin was greater than that of plasma suggestive of extravascular distribution. Dapagliflozin has a longer half-life in humans (13 hours) than in the rat, monkey or dog (3-7 hours), suggestive of different rates of renal elimination. Plasma protein binding was high (91-95%) in humans and in all nonclinical species.

Dapagliflozin undergoes low (10%) oxidative metabolism in vitro by numerous human cytochrome P450 enzymes. The major metabolites in hepatocyte preparations were the glucuronide conjugates and dapagliflozin 3-O-glucuronide was the major human metabolite formed in the kidney and also the liver. UGT1A9 was the major human UDP-glucuronosyltransferase responsible for the formation of dapagliflozin 3-O-glucuronide and this enzyme is predominantly found in the kidney. No unique dapagliflozin human metabolites were identified. Dapagliflozin excretion is predominately via metabolism to the dapagliflozin 3-O-glucuronide followed by excretion in the urine. The parent is also found to a much lower extent in the urine, feces and bile. Dapagliflozin was also found to be excreted in the milk of lactating rats.

Dapagliflozin was a weak substrate for p-glycoprotein and did not inhibit OAT1 or OCT2 and was weak inhibitor of hOAT3. These results suggest a low probability for drug-drug interactions except for potential inducers/inhibitors of UGT1A9 which may effect the elimination of dapagliflozin.

General Toxicology
Pivotal repeat dose studies were conducted in the Sprague-Dawley rat and Beagle dogs up to 6 and 12 months duration, respectively. Findings in the pivotal rat and dog studies were generally consistent with pharmacodynamic activity of dapagliflozin, including dose-dependent increases in urinary glucose. Pharmacodynamic action also resulted in reduced body weight (BW), increased food consumption (FC), increased urinary volume, calcium (Ca), phosphorus, protein and decreased urinary osmolality.

In the 6 month rat study, rats were exposed to dapagliflozin at 85-3097x MRHD. Multiple mortality was observed at the high dose (HD). As the cause of death was undetermined this defined the NOAEL as the mid dose which was 346x and 675x MRHD in male and females, respectively. Major target organs with toxicity included the kidney (chronic progressive nephropathy (CPN), mineral deposits, tubule epithelial hyperplasia and urothelial hyperplasia), sternum and femur (increased trabecular bone), heart, vessels and trachea (mineralization), adrenal glands (vacuolation/hypertrophy) and spleen and liver (extramedullary hematopoiesis). Generally most findings were at the HD, except for adrenal vacuolation which showed a dose-dependent trend. Increased bone mass and strength was also noted in the HD animals. The target organs identified were likely the result of exaggerated pharmacology due to inhibition of SGLT2 (e.g. glucosuria) or were the result of off-target effects or were due to the osmotic and/or diuretic effect of enhanced glucose excretion (e.g. polyuria). Adrenal gland vacuolation could be a compensatory response of aldosterone production due to increased sodium excretion. Off-target effects include the increased trabecular bone and tissue mineralization likely due to modulation of calcium homeostasis and increased urinary calcium excretion. The sponsor has proposed off-target inhibition of rat SGLT1 in the intestine that results in increased calcium absorption and increased serum calcium levels. This in turn leads to increased trabecular bone and tissue mineralization as down stream events of excess calcium. The propensity for dapagliflozin to cause off-target inhibition of SGLT1 in humans is reduced due to the lower affinity of dapagliflozin for human SGLT1 compared to the rat. Overall, target organ toxicities in adult rats

occurred at high exposure multiples (≥3097x MRHD) and the safety margins to the final clinical dose are high suggesting low clinical risk.

In the 12 month study, treated dogs were exposed to dapagliflozin at 128-3269x MRHD. Dose-dependent increases in absolute or relative weights of the kidney, adrenal and liver were observed with histopathology correlates. Increased adrenal and liver weights were irreversible. Pharmacodynamic action also resulted in reduced body weight (BW) and a dose-dependent increase in urinary volume, glucose, calcium (Ca), phosphorus, protein and decreased urinary osmolality. Due to renal toxicity and moribundity at the high dose, the NOAEL was the mid dose which was 516x and 619x MRHD in males and females, respectively.

A summary of the sub-chronic and chronic toxicity studies is given in the table below.

Reproductive Toxicology
Reproductive and developmental toxicity were assessed in fertility, early embryonic development, pre- and post-natal development and juvenile animal studies. In the fertility study significant mortality was observed in the males at the high dose (1707x MRHD) and reduced body weight gain (≥20%) was observed in the mid and high dose males. No effects were seen on mating and fertility indices in the males except for altered spermatogenesis at the high dose (1707x MRHD), resulting in a NOAEL of 160x MRHD. In the fertility study resorptions were increased in females at ≥ 188x MRHD, but a NOAEL for this finding was established at 39x MRHD. No effects were seen on mating and fertility indices in the females and a NOAEL for female fertility was 188x MRHD due to reduced weight gain at higher doses.

Dapagliflozin was not teratogenic at up to 75 mg/kg (1441x MRHD) in the rat. Due to maternal toxicity (reduced body weight gain) a NOAEL was not established in the dams. Higher exposures resulted in late gestational fetal deaths and malformations and skeletal variations at ≥ 1441x MRHD. Dapagliflozin was also not teratogenic in the rabbit at up to 1191x MRHD. Again reduced body weight gain resulted in maternal toxicity in the rabbit, but effects on the litters and malformations and variations were not observed.

Exposure to dapagliflozin at 19-1415x MRHD in a pre- and post-natal development study in the rat had no pathological effects in the dams, yet showed renal pelvic dilatation in the in utero and lactationally exposed pups at the high dose (1415x MRHD). The NOAEL for this renal pathology was 249x MRHD. However, due to reduced growth the NOAEL for the pups was <19x MRHD. In the dams the NOAEL was also 249x MRHD due to reduced body weight gain at higher doses. Treatment of juvenile rat pups until maturity at identical exposures replicated the renal pelvic dilatation pathology but at a substantially lower drug exposure (1mg/kg, ~15x MRHD). A ‘no effect” dose was not identified so it is possible that dapagliflozin exposure causing this adverse effect occurs very near clinical exposure. The susceptible period in 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 gestation, with functional renal development continuing until ~2yrs of age. In addition, the renal pelvic dilatation also showed irreversibility in recovery animals, suggesting dapagliflozin is a renal pelvic development toxicant. The cause of renal pelvic and tubular dilation is not known. Consequently the sponsor has recommended against the use of dapagliflozin during the second and third trimesters of pregnancy and during nursing. The FDA agrees with the sponsor that dapagliflozin should not be used during pregnancy or nursing.

Genetic Toxicology
Dapagliflozin was not mutagenic or clastogenic in an in vitro Ames assay or in the in vivo assays: rat bone marrow micronucleus assay or peripheral blood lymphocyte chromosomal aberration assay or the hepatocyte unscheduled DNA synthesis (UDS) assay. However, dapagliflozin was clastogenic in the presence of S9 in multiple in vitro chromosomal aberration assays. The necessity of S9 (rat liver microsomes) to elicit clastogenicity indicates that an unidentified metabolite or metabolites of dapagliflozin were causative, not the intact parent molecule. The inability to reproduce the positive *in vitro* clastogenic effect in the intact rat might be explained by the presence of chromosomal reparative pathways in the intact rat, potentially absent generation of the clastogenic metabolite *in vivo*, or by an insufficient drug concentration tested in the rat study. All metabolites of dapagliflozin identified in human subjects have also been identified in mice and rats *in vivo*, and would have been evaluated for genotoxic potential in these studies. The weight of evidence supports the view that dapagliflozin and its identified metabolites are unlikely to be clastogenic in human subjects.

Carcinogenicity
Dapagliflozin was assessed for its potential to induce tumors in two-year bioassays conducted in rats and mice. The two-year bioassays are intended to detect drug-induced tumors that arise from genotoxic as well as non-genotoxic mechanisms of action after approximately life-time exposure to an investigational drug. Dapagliflozin did not increase the incidence of any tumor in rats and mice at drug exposures reaching 131x and 72x the clinical dose, respectively. Hyperplastic lesions that could be viewed as pre-neoplastic alterations were not observed in any tissue, including the mammary and bladder tissues, with the potential exception of the kidney tubules. An increased incidence and severity of atypical hyperplasia of the renal tubules was observed at all doses of dapagliflozin in male rats, though there was no increase in renal tubule adenoma or carcinoma.

Special Toxicology Studies
Exposure of rats in utero and during lactation to dapagliflozin at up to 918x MRHD resulted in excretion of dapagliflozin in the breast milk of lactating rats at a milk to plasma ratio of 0.49x. The fetal exposure to dapagliflozin was 2-142x MRHD, suggesting that pups were exposed to pharmacologically and toxicologically relevant levels of the drug.

Table 95. Summary of Sub-Chronic and Chronic Toxicology Studies.

SPECIES TOXICOLOGY STUDIES			
SPECIES/ STUDY	**NOAEL**	**MULTIPLE OF MRHD 10 mg: 0.465 ng.hr/mL AUC basis***	**BASIS**
Mouse 3 month: 0, 50, 150, 250 and 400 mg/kg	150 mg/kg/day	M: 653x F: 1058x	-Mortality at 250 and 400 mg/kg. -Increased prostatic weight (≥20%) at 150 and 250 mg/kg.
Rat 3 month: 0, 5, 50 and 200 mg/kg	5 mg/kg/day	M: 13x F: 35x	-Mortality at 250 mg/kg. -Significantly reduced body weight gain (≥19%) at 50 and 200 mg/kg. -Marked reactive hyperplasia of the renal collecting duct, dilatation of the cortical and/or medullary tubules and CPN at 200 mg/kg. -Increased trabecular bone in the sternum and femur at 200 mg/kg. -Mineralization in multiple tissues including the heart at 200 mg/kg.
Rat 6 month: 0, 5, 25 and 150 mg/kg	25 mg/kg/day	M: 346x F: 675x	-Mortality at 150 mg/kg. At 150 mg/kg: -Adrenal gland hypertrophy/vacuolation and increased organ weight. -Kidney CPN, mineralization and increased organ weight. -Increased trabecular bone (sternum and femur) -Heart and vessels mineralization.
Dog 12 month: 0, 5, 20 and 120 mg/kg	20 mg/kg/day	M: 561x F: 619x	-Mortality at 120 mg/kg. -Dose-dependent increased adrenal, kidney and liver weights. -Reduced mean body weight (1-17%).

*AUC in human: 0.465ng.hr/ml at 10 mg/day.

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/s/

MUKESH SUMMAN
08/30/2011

TODD M BOURCIER
08/31/2011
Pharm/tox supports AP

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 202293 **Applicant: BMS/AZ** **Stamp Date: December 27th 2010**

Drug Name: Dapagliflozin/BMS-512148 **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		
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		Oral dose administration (gavage for animal studies and tablets in the clinic)
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		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

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		Human dose equivalents are expressed as MRHD multiples.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)			Not applicable. No PT impurity issues were identified.
11	Has the applicant addressed any abuse potential issues in the submission?		X	
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable

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.

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

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

/s/

MUKESH SUMMAN
02/14/2011

TODD M BOURCIER
02/15/2011
NDA fileable for pharm/tox