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ORIGINAL RESEARCH

# Carcinogenicity Risk Assessment Supports the Chronic Safety of Dapagliflozin, an Inhibitor of Sodium–Glucose Co-Transporter 2, in the Treatment of Type 2 Diabetes Mellitus

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## ABSTRACT

***Introduction***: Dapagliflozin is a selective inhibitor of the sodium–glucose co-transporter 2 (SGLT2) that increases urinary glucose excretion to reduce hyperglycemia in the treatment of type 2 diabetes mellitus. A robust carcinogenicity risk assessment was undertaken to assess the chronic safety of dapagliflozin and SGLT2 inhibition.

***Methods***: Genotoxicity potential of dapagliflozin and its metabolites was assessed in silico, in vitro, and in vivo. Dapagliflozin was administered daily by oral gavage to mice, rats, and dogs to evaluate carcinogenicity risks, including the potential for tumor promotion. $SGLT2^{-/-}$ mice were observed to evaluate the effects of chronic glucosuria. The effects of dapagliflozin and increased glucose levels on a panel of human bladder transitional cell carcinoma (TCC) cell lines were also evaluated in vitro and in an in vivo xenograft model.

***Results***: Dapagliflozin and its metabolites were not genotoxic. In CD-1 mice and Sprague–Dawley rats treated for up to 2 years at $\geq 100\times$

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human clinical exposures, dapagliflozin showed no differences versus controls for tumor incidence, time to onset for background tumors, or urinary bladder proliferative/preneoplastic lesions. No tumors or preneoplastic lesions were observed in dogs over 1 year at >3,000× the clinical exposure of dapagliflozin or in SGLT2$^{-/-}$ mice observed over 15 months. Transcription profiling in Zucker diabetic fatty rats showed that 5-week dapagliflozin treatment did not induce tumor promoter-associated or cell proliferation genes. Increasing concentrations of glucose, dapagliflozin, or its primary metabolite, dapagliflozin 3-*O*-glucuronide, did not affect in vitro TCC proliferation rates and dapagliflozin did not enhance tumor growth in nude mice heterotopically implanted with human bladder TCC cell lines.

***Conclusion***: A multitude of assessments of tumorigenicity risk consistently showed no effects, suggesting that selective SGLT2 inhibition and, specifically, dapagliflozin are predicted to not be associated with increased cancer risk.

**Keywords:** Antidiabetic drug; Dapagliflozin; Experimental pharmacology; Glycemic control; SGLT2 inhibitor; Type 2 diabetes mellitus

## INTRODUCTION

Selective inhibitors of the sodium–glucose co-transporter 2 (SGLT2) [1, 2] are a class of antidiabetic agents with a novel mechanism of action that can be additive and complementary to existing antidiabetic therapies [3–5]. SGLT2 inhibitors promote urinary glucose excretion in an insulin-independent manner to reduce hyperglycemia and lower glycosylated hemoglobin ($HbA_{1c}$). Additional benefits associated with SGLT2 inhibition include a low risk of hypoglycemia, weight loss due to urinary caloric loss [6], and diuresis-related blood pressure lowering, with the potential for cardiovascular benefits [7]. SGLT2 inhibition is expected to be a relatively safe approach because SGLT2 is selectively expressed in the kidney [1, 8, 9]. Moreover, the limited data that exist in subjects with familial renal glucosuria due to mutations in the SGLT2 gene indicate that this condition is largely asymptomatic aside from increased glucosuria [10]. Nonetheless, drugs with novel mechanisms of action require careful examination of potential safety liabilities. In particular, elevated cancer risk is an important concern for patients with type 2 diabetes, since this patient population is already at increased risk of a variety of tumor types [11–13].

Dapagliflozin is a small-molecule inhibitor of SGLT2 [3, 4] that has been shown to be highly selective for SGLT2 compared with other SGLT family members and to have no off-target interactions in an in vitro screen of more than 330 receptors, enzymes, ion channels, and transporters [14]. Overall, dapagliflozin demonstrates good oral bioavailability, has a high volume of distribution, and has an in vivo metabolite profile that is qualitatively similar between preclinical species and humans [15]. In humans, the major circulating metabolite is a stable, non-reactive ether glucuronide [dapagliflozin 3-*O*-glucuronide (D3OG)] that has no pharmacologic activity [15]. It also has no structural alerts for mutagenicity or carcinogenicity nor any off-target effects against a similar broad panel of more than 330 receptors, enzymes, ion channels, and transporters [14]. This glucuronide metabolite is formed at a lower rate in preclinical species but plasma concentrations comparable to or higher than human exposures are achieved at

supratherapeutic doses of dapagliflozin used for toxicologic assessments [14]. Non-human species also form a pharmacologically active *O*-deethylated metabolite, which is only a minor metabolite in humans [15]. Structures for these metabolites are shown in Fig. 1. All other plasma and urinary dapagliflozin metabolites were formed at relatively low levels in animals and humans and, importantly, the animals assessed in toxicity studies formed them at sufficient levels relative to humans to assess their safety [14, 16] (Bristol-Myers Squibb and Astra Zeneca, data not shown).

Dapagliflozin shows expected pharmacodynamics (i.e., marked glucosuria)

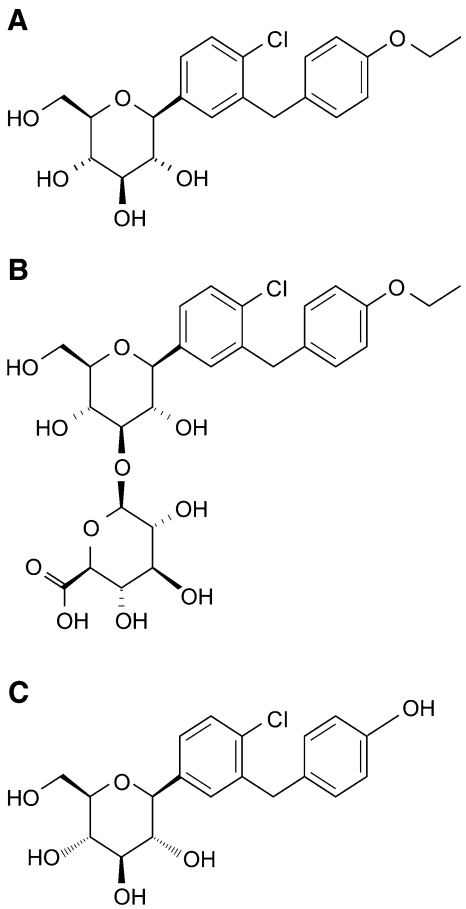


**Fig. 1** Structures of dapagliflozin (**a**), its major human metabolite, dapagliflozin 3-*O*-glucuronide (**b**), and BMS-511926, its major animal metabolite, which is *O*-deethylated (**c**)

and disease-modifying effects in a variety of animal models of diabetes [3]. In patients with type 2 diabetes mellitus, dapagliflozin treatment achieves significant reductions in $HbA_{1c}$, fasting and postprandial glucose, and body weight at the recommended human dose of 10 mg once daily [17–27]. Both non-clinical and clinical assessments have shown dapagliflozin to have a highly favorable safety profile that is consistent with its simple mechanism of action, with no off-target effects observed [14]. Despite a balanced overall incidence of malignancies and unspecified tumors between dapagliflozin and control groups (1.5% vs. 1.4%, July 15, 2011 cutoff) [28], regulatory concern was raised by a small numerical imbalance in urinary bladder tumors in the dapagliflozin global clinical program [9 in the dapagliflozin groups vs. 1 in the placebo groups (incidence rate of 0.15 vs. 0.03)] [29]. This imbalance was not supported by any preclinical data suggestive of a tumor risk [30].

Thus, we hypothesized that a robust, preclinical carcinogenic risk assessment, including the comprehensive non-clinical toxicity program for dapagliflozin, as well as additional investigative studies assessing whether dapagliflozin or its glucosuria mechanism of action could somehow act as a promoter or growth enhancer of bladder tumors, would help to de-risk any clinical tumor imbalance. The results of these studies and the overall weight of evidence support our conclusion that SGLT2 inhibition in general, and dapagliflozin specifically, are not associated with increased cancer risk.

## METHODS

The definitive studies were conducted in compliance with Good Laboratory Practice Regulations for Nonclinical Laboratory Studies

of the United States Food and Drug Administration (Code of Federal Regulations Title 21, Part 58), and all in vivo studies were approved by the Bristol-Myers Squibb Institutional Animal Care and Use Committee in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care International guidelines.

## Test Articles and Vehicles

Dapagliflozin ((2S,3R,4R,5S,6R)-2-[4-chloro-3-(4-ethoxybenzyl)phenyl]-6-(hydroxymethyl) tetrahydro-2H-pyran-3,4,5-triol; C21H25ClO6; formula weight ~409) and D3OG were synthesized by Bristol-Myers Squibb Company (New Brunswick, NJ, USA). For in vitro studies, dapagliflozin and D3OG metabolite were dissolved in a solution of dimethyl sulfoxide.

In vivo studies were conducted with a propylene glycol solvate of dapagliflozin (dapagliflozin propanediol), which is the form used in humans. The oral dosing vehicle used in the non-clinical toxicity studies consisted of 90% polyethylene glycol (PEG-400; Spectrum Quality Products, New Brunswick, NJ, USA) in 10% water.

## In Vitro Genotoxicity Studies

In vitro genotoxicity studies were conducted in accordance with International Conference on Harmonization (ICH) guidelines [31]. Dapagliflozin was assessed for the potential to induce bacterial mutation in the Ames test in *Salmonella typhimurium* stains TA98, TA100, TA1535, and TA1537 and in *Escherichia coli* strain WP2 uvrA. The potential to induce chromosome aberrations was assessed in Chinese hamster ovary (CHO) cells. In both of these in vitro systems, tests were performed up to maximum concentrations established by the ICH guidelines with and without the presence of an aroclor-induced rat S9 metabolic activation system (BioReliance; Rockville, MD, USA). Appropriate positive controls were used in each test to verify the sensitivity of the test system.

## In Vivo Rodent Cytogenetic Studies

### *Chromosomal Aberrations*

Dapagliflozin was administered to five groups of ten Sprague–Dawley (SD) rats per sex at doses of 0 (vehicle control), 25, 100, 150, or 200 mg/kg per day for 1 month. All ten rats per sex were used for toxicokinetic assessments (day 28) and a subset of five rats per sex was used for clastogenicity assessments in peripheral blood cells. Cyclophosphamide served as the positive control and was administered as a single oral dose of 60 mg/kg to ten rats per sex approximately 24 h prior to euthanasia.

### *Micronucleus Induction*

Dapagliflozin was administered to groups of five SD rats per sex at dose levels of 0 (vehicle control), 350, 700, or 1,050 mg/kg per day for 3 days to evaluate micronucleus induction. The positive control group received cyclophosphamide at 7 mg/kg per day. Bone marrow samples were obtained approximately 24 h after administration of the last dose and analyzed microscopically for the number of polychromatic erythrocytes with micronuclei. A second study was also conducted at daily doses of 0 (vehicle control), 75, 150, 200, or 250 mg/kg per day over a 2-week period in which plasma exposures were assessed in addition to bone marrow micronuclei formation.

### *DNA Repair*

The effect of dapagliflozin on unscheduled DNA repair was evaluated in groups of ten male SD

rats administered dapagliflozin at single oral doses of 0 (vehicle), 175, 350, or 700 mg/kg. An additional group of ten rats received a single oral dose of 35 mg/kg dimethylnitrosamine as the positive control. Animals were euthanized 2–4 h or 12–16 h after dosing. Primary hepatocyte cultures were established from three rats per dosing group at each time point. Cultures were incubated with medium supplemented with $^{3}$H-thymidine. After approximately 4 h, cultures were processed for autoradiographic analysis. To interpret unscheduled DNA repair responses, the net nuclear counts were averaged and the mean ± standard deviation, as well as the percent of cells in repair, was reported for each rat evaluated. A net nuclear grain count was calculated for each nucleus scored by subtracting the mean cytoplasmic area count from the nuclear area count.

## Rodent Carcinogenicity Studies

### *Mice*

CD-1 mice were obtained from Charles River Laboratories (Raleigh, NC, USA). Dapagliflozin was administered by oral gavage once daily for up to 104 weeks to groups of 60 mice per sex at doses of 0 [vehicle; PEG-400, 90% (v/v) in distilled water], 0 (distilled water), 5, 15, or 40 mg/kg per day dapagliflozin in males and at 0 (vehicle), 0 (distilled water), 2, 10, or 20 mg/kg per day in females. Different doses for males and females were chosen to achieve similar drug exposures across sexes. The doses selected for this study were based on previous findings from toxicokinetic and toxicologic studies. Relative to human area under the plasma concentration–time curve (AUC) at a dose of 10 mg, the doses in the current study were anticipated to result in exposure (AUC) multiples of approximately 4, 14, and 72× in males and 11, 52, and 105× in females. The dose volume was 4 mL/kg per day for all dose groups. Animals that died before the end of treatment were necropsied for full histologic assessment. At the end of the treatment period, all surviving animals were euthanized and necropsied. Groups of satellite mice (18/sex/group) were similarly treated and euthanized for toxicokinetic analysis during week 26. The following parameters were evaluated: viability, clinical observations, body weight, food consumption, macroscopic observations, microscopic pathology, and tumor incidence. Pathologic evaluations were conducted and peer reviewed by experienced veterinary pathologists.

### *Rats*

Harlan SD rats were obtained from Harlan Laboratories (Indianapolis, IN, USA). Dapagliflozin was administered by oral gavage once daily for up to 90 (males) or 105 weeks (females) to groups of 70 rats per sex at doses of 0 [vehicle; PEG-400, 90% (v/v) in distilled water], 0 (distilled water), 0.5, 2, or 10 mg/kg per day. The dose volume was 4 mL/kg per day for all dose groups. The doses selected for this study were based on previous findings from toxicokinetic and toxicologic studies. Relative to human AUC at a dose of 10 mg, the doses in the current study were anticipated to result in exposure (AUC) multiples of approximately 7, 25, and 130× in males and 9, 34, and 186× in females. Animals that died before the end of treatment were necropsied for full histological assessment. Those animals that survived to the end of the treatment period were euthanized and necropsied. Groups of satellite rats (10/sex/dose) were similarly treated and euthanized for toxicokinetic analyses on day 184. The following parameters were evaluated: viability, clinical observations, body weight, food

consumption, macroscopic observations, microscopic pathology and tumor incidence. Pathologic evaluations were conducted and peer reviewed by experienced veterinary pathologists.

## Additional Chronic Studies

### *SGLT2$^{-/-}$ Mice*

A description of the methods used to generate and breed SGLT2$^{-/-}$ mice has been previously published [32]. Briefly, founder mice were supplied by Lexicon Pharmaceuticals, Inc. (The Woodland, TX, USA) and back-crossed onto a C57BL/6J strain. Parental mice were homozygote offspring of in-house heterozygote mating (SGLT2$^{+/-}$ × SGLT2$^{+/-}$). Homozygote mice used for this study were offspring of next-generation in-house homozygote mating [knockout (KO) × KO, or wild-type (WT) × WT]. Pups were weaned at approximately 3 weeks of age, housed in polycarbonate boxes (one to four per box) on Alpha-dri bedding (Shepherd Specialty Papers, Watertown, TN, USA) with free access to water and feed [Teklad Global 18% Protein Rodent Diet (Harlan Laboratories)], and exposed to 12 h of continuous light per day. Genotypes (KO or WT) were confirmed by quantitative polymerase chain reaction of DNA isolated from toe snips.

The 15-month study comprised 36 (23 male, 13 female) SGLT2 KO mice and 33 (16 male, 17 female) WT mice. In-life evaluations included daily clinical observations, periodic body weight measurements, and a urine glucose measurement [Diastix® urine glucose test strips (Bayer HealthCare, Tarrytown, NY, USA)] to confirm each animal's phenotype. Mice were not subject to any therapeutic or pharmacologic interventions. Animals that were found dead prior to 15 months of age were not subjected to necropsy. Prior to euthanasia, mice were weighed and anaesthetized by isoflurane inhalation. Necropsy consisted of gross external and visceral examinations. Samples of urinary bladder, kidneys, liver, heart, pancreas, adrenal glands, thyroid, spleen, female reproductive tract, male sex glands, skin, brain, and skull were fixed in neutral buffered formalin, routinely processed for histology, and examined by light microscopy. Urinary bladder evaluations were conducted and peer reviewed by experienced veterinary pathologists.

### *12-Month Dog Study*

Beagle dogs were obtained from Marshall Farms (North Rose, NY, USA) and Covance Research Products (Cumberland, VA, USA). Dapagliflozin was administered as a solution within gelatin capsules once daily for 48 weeks, with a 36-week recovery period, to groups of seven per sex at doses of 0 [vehicle; PEG-400, 90% (v/v) in distilled water], 5, 20, or 120 mg/kg per day. The dose volume was 4 mL/kg per day for all dose groups. The following parameters were evaluated: viability, clinical observations, body weight, food consumption, macroscopic observations, and microscopic pathology. Pathologic evaluations were conducted and peer reviewed by experienced veterinary pathologists.

## Investigative Transcriptional Profiling Study

Transcription profiling was performed on RNA samples from a previously conducted efficacy study in which obese male Zucker diabetic fatty (ZDF) rats were dosed orally once daily for 1 or 5 weeks with 0 (vehicle) or 0.5 mg/kg dapagliflozin with five animals per treatment group for each of the time points. Liver, skeletal muscle, kidney, and adipose tissues were

harvested from both fasted and fed animals 48 h after the last dose of dapagliflozin treatment and immediately soaked in RNAlater (Qiagen, Valencia, CA, USA) for 24 h at 4 °C. The tissues were transferred to −80 °C for long-term storage. Tissues were homogenized in Trizol reagent (Invitrogen, Carlsbad, CA, USA) and total RNA was isolated with RNeasy mini kit (Qiagen) and subjected to off-column DNase I treatment (Qiagen). All target labeling reagents and GeneChip® HT One-Cycle Target Labeling Kits were purchased from Affymetrix (Santa Clara, CA, USA).

Double-stranded complementary DNAs (cDNAs) were synthesized from 1.2 μg total RNA from each tissue sample through reverse transcription with an oligo-dT primer containing the T7 RNA polymerase promoter using the cDNA Synthesis System (Affymetrix). Biotin-labeled cRNAs were generated from the cDNAs and were processed on a Caliper GeneChip Array Station (Affymetrix). Labeled cRNAs were hybridized on Affymetrix Rat Genome HT_RG-230PM arrays and processed according to Affymetrix's recommendations. Scanned images were subjected to visual inspection and chip quality reports were generated by Expression console (Affymetrix). The image data were processed using the Robust Multichip Average method to determine the specific hybridizing signal for each probe set. The cDNA was synthesized with a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems; Foster City, CA, USA). PCR primers and probe sets were obtained from Applied Biosystems. Ingenuity® Systems Pathway Analysis (IPA®) software version 9.0 (Ingenuity® Systems Incorporated; Redwood City, CA, USA) was used to annotate the Affymetrix probesets for the genes they represent and the functional pathways to which they belong.

## Assessment of Tumor Promotion and Tumor Growth Enhancement

### *In Vitro Cell Proliferation Studies*

Dapagliflozin and D3OG were evaluated for the ability to enhance in vitro proliferation of human bladder transitional cell carcinoma (TCC) cell lines (T24, TCCSUP, UM-UC-3, J82, SW780, and EJ-1). All cell lines were obtained from American Type Culture Collection (Manassas, VA, USA), except EJ-1, which was obtained from Japan Health Sciences Foundation (Osaka, Japan). These were a majority of the human bladder TCC cell lines that could be commercially obtained. Experiments were carried out with low-passage cell lines that were <6 months in culture from time of thaw of the original cell bank stock. Three concentrations (0.2, 2, 20 μg/mL) of each compound were evaluated, representing approximately 1.5×, 15×, and 150× the maximum observed plasma concentration ($C_{max}$; 136 ng/mL) for dapagliflozin and 1×, 10×, and 100× the $C_{max}$ (196 ng/mL) for D3OG in human plasma after a 10 mg maximum recommended human dose (MRHD) [33]. Bladder cancer cell lines were seeded into 12 well plates at 20,000 or 40,000 cells per well in Roswell Park Memorial Institute (RPMI) 1640 medium containing 10% fetal bovine serum (FBS). Cells were allowed to adhere for 24 h, at which time growth medium was replaced with medium without FBS. After 24 h in serum-free medium, vehicle control (medium only) or test compounds (0.2, 2, 20 μg/mL) were added in serum-free medium and the cultures were incubated for a further 72-h period. As a positive control, some cultures received growth medium that contained 10% FBS after the 24-h incubation in serum free medium. Another comparator control was maintained in serum-containing media throughout the duration of the experiment.

Similarly, in a separate study, increasing glucose concentrations were evaluated for the ability to enhance in vitro cellular proliferation of TCC cell lines (T24, TCCSUP, UM-UC-3, J82, and SW780). Bladder cancer cell lines were seeded into 12-well plates at 40,000 or 80,000 cells per well in RPMI 1640 medium containing 10% FBS and with the glucose concentration adjusted to 11 (control), 25, 35, or 50 mM. Cells were incubated for a 145-h period.

Cell proliferation was monitored using an IncuCyte Live-Cell Microscope Imaging System (Essen BioScience, Ann Arbor, MI, USA) that resides in a standard tissue culture incubator. Growth curves were constructed from data points (percent confluence) acquired at 2-h intervals over a 72-h test period for evaluation of dapagliflozin and D3OG, and over a 145-h test period for the evaluation of high glucose concentrations.

### *In Vivo Mouse Xenograft Studies*

Using a representative set of two human bladder TCC cell lines that had already been tested in vitro, transplantable EJ-1 or UM-UC-3 tumor xenografts were initially established by the inoculation of $5 \times 10^6$ cells harvested from exponentially growing cell culture. For tumor growth experiments, male and female athymic nude mice (24–26 g per mouse; CrTac:NCr-Foxn1$^{nu}$; obtained from Taconic) were implanted subcutaneously in the rear flank with an EJ-1 or UM-UC-3 tumor fragment (approximately 20–30 mg) using a 13-gauge trocar. Once tumors reached approximately 100 mm$^3$, animals were treated daily for up to 11 (for the EJ-1 tumor) or 15 (for the UM-UC-3 tumor) days with vehicle or dapagliflozin (4 and 20 mg/kg per day in males and 12 and 60 mg/kg per day in females; differing doses between sexes to achieve comparable exposures). Toxicokinetics of dapagliflozin and D3OG were evaluated following daily dosing on day 4 (UM-UC-3 mice) or day 5 (EJ-1 mice). Tumors were measured with calipers twice weekly and tumor volume (mm$^3$) was estimated from the formula tumor weight = (length × width$^2$)/2, where length is the larger and width is the smaller of the perpendicular diameters.

### Statistical Methods

Principal component analysis was performed using Partek Discovery Suite software (Partek Incorporated, Saint Louis, MO, USA). To identify genes with significant expression changes related to treatment in the transcriptional profiling study, a two-way analysis of variance (ANOVA) was performed across the treatment groups. A Tukey post hoc test was conducted to compare treatment versus vehicle control group within the ANOVA test. cDNA that showed a fold change of greater than 1.5 (upward or downward regulation) between treatment and vehicle groups with a $P$ value of less than 0.005 were considered significantly different in relation to treatment. Additionally, transcriptional profiles were compared with published literature of a proposed prognostic genetic signature of tumor promoters [34].

Statistical significance of the in vivo effects of dapagliflozin on tumor growth were determined using non-parametric statistics by Gehan's generalized Wilcoxon test and required a $P$ value of less than 0.05 [35].

## RESULTS

### In Vitro Genotoxicity Studies

Dapagliflozin was negative in the bacterial reverse-mutation assay in *S. typhimurium* and *E. coli* strains at maximum concentrations

(≤5,000 μg/plate) with or without metabolic activation. Dapagliflozin also was negative for chromosomal aberration induction in CHO cells in the absence of S9 stimulation. With metabolic stimulation, 4-h exposure to ≥150 μg/mL dapagliflozin increased the incidence of structural aberrations in CHO cells. These concentrations induced substantial cell growth inhibition but were not excessively cytotoxic. Importantly, these concentrations are ≥735 times greater than the total $C_{max}$ obtained in humans with the MRHD of dapagliflozin 10 mg.

### In Vivo Cytogenetics Studies in Rats

To more definitively investigate the potential for chromosomal aberrations with dapagliflozin, clastogenicity was evaluated in peripheral blood cells and in bone marrow over different durations of treatment in male and female rats. Evaluation of peripheral blood lymphocytes up to a maximum dose of 200 mg/kg per day of dapagliflozin for 1 month ($C_{max}$ ≤74 μg/mL or ≤544× MRHD; AUC ≤1,210 μg h/mL or ≤2,602× MRHD) showed no evidence of clastogenicity. When tested up to the maximum tolerated dose level for 3 days (700 mg/kg per day) or 2 weeks (250 mg/kg per day) in bone marrow micronucleus studies in rats, dapagliflozin was not genotoxic up to the maximum evaluable doses. Dapagliflozin also did not induce an increase in unscheduled DNA synthesis in male rats when tested to the maximum tolerated dose (700 mg/kg) required by ICH guidelines.

The estimated or measured $C_{max}$ achieved in the in vivo cytogenetics studies (63–74 μg/mL in the 1-month oral in vivo/in vitro cytogenetics assay and >100 μg/mL in the oral micronucleus studies as extrapolated from bridging toxicokinetics studies) approached or exceeded the 100 μg/mL level that produced cytogenetic alterations in vitro.

### Rodent Carcinogenicity Studies

#### *CD-1 Mice*

Potential carcinogenicity of orally administered dapagliflozin was assessed in CD-1 mice for a period of up to 24 months as summarized in Table 1. Systemic exposures in females were higher than in males, consistent with findings in a previous 3-month mouse study. Animals were terminated as planned from weeks 105–106, with survival ranging from 30%−43% in males and from 32%–47% in females after 24 months, which was generally within expected historical control ranges. The small reduction in the overall survival in males at 15 and 40 mg/kg per day was attributed to exacerbation of mouse urologic syndrome (MUS), a spontaneous background condition commonly seen in untreated male CD-1 mice, leading to urogenital obstruction and mortality [36]. The increased incidence of the urogenital lesions was associated with drug-related increases in macroscopic and microscopic findings of dilated renal pelves and urinary bladder distention (Table 1), which suggests that dapagliflozin caused an exacerbation of the background lesion. The incidence and/or severity of any background hyperplasia were similar between dapagliflozin and control treatment groups. Other causes of death were distributed similarly in all dose groups and/or were not dose related.

Dapagliflozin was not carcinogenic in CD-1 mice when administered by oral gavage for up to 24 months at doses ≤40 mg/kg per day [AUC from time zero to 8 h ($AUC_{0–8h}$) = 33.5 μg h/mL; 72× $AUC_{0–8h}$ MRHD] in males or at doses ≤20 mg/kg per day [AUC from time zero to 24 h ($AUC_{0–24h}$) = 48.6 μg h/mL; 105× $AUC_{0–24h}$ MRHD] in females. Neoplasms of the

**Table 1** Summary of 24-month oral carcinogenicity study with dapagliflozin in mice

| Dapagliflozin dose (mg/kg per day): | Males | | | | | Females | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0[a] (*n* = 60) | 0[b] (*n* = 60) | 5 (*n* = 60) | 15 (*n* = 60) | 40 (*n* = 60) | 0[a] (*n* = 60) | 0[b] (*n* = 60) | 2 (*n* = 60) | 10 (*n* = 60) | 20 (*n* = 60) |
| $C_{max}$ at day 182, μg/mL | NA | NA | 1.04 | 3.20 | 14.1 | NA | NA | 1.09 | 6.25 | 16.0 |
| $AUC_{(0-T)}$ at day 182[c], μg h/mL | NA | NA | 2.00 | 6.41 | 33.5 | NA | NA | 5.09 | 24.0 | 48.6 |
| No. survivors at termination, *n* (%) | 26 (43) | 24 (40) | 26 (43) | 18 (30) | 20 (33) | 28 (47) | 21 (35) | 19 (32) | 22 (37) | 25 (42) |
| Major causes of death[d], % | | | | | | | | | | |
| Urogenital system–non-neoplastic lesions[e] | 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 lesions | 0 | 11 | 15 | 2 | 5 | 0 | 3 | 2 | 0 | 0 |
| Other causes | 14 | 28 | 25 | 12 | 20 | 19 | 14 | 28 | 25 | 35 |
| Not determined | 32 | 17 | 15 | 45 | 18 | 16 | 21 | 20 | 13 | 29 |

$AUC_{(0-T)}$ area under the plasma concentration–time curve from administration to last observed concentration at *t*, $C_{max}$ maximum observed plasma concentration, *NA* not applicable

[a] Vehicle-control group

[b] Water-control group

[c] For $AUC_{(0-T)}$, $T = 8$ h (males) or 24 h (females) post dose

[d] Percentage of pretermination deaths attributed to a specific cause

[e] Non-neoplastic urogenital system lesions (compatible with male mouse urologic syndrome) comprised combinations of the following: (1) kidney lesions (pyelitis, pyelonephritis, dilated pelves); (2) urinary bladder lesions (distended, erosions/subacute ulcers/chronic inflammation; (3) prostate gland and seminal vesicles (subacute/chronic inflammation, purulent inflammation/fibrosis, abscesses). Prostate and seminal vesicle effects are included as part of mouse urologic syndrome because they are commonly affected in mouse urologic syndrome, which is a common background lesion in male mice that results in inflammatory changes in urogenital tissues (including male accessory sex glands such as prostate and seminal vesicles) and partial to full urogenital obstruction, often presenting as urinary bladder dilatation

lymphoreticular system, uterus and cervix, lung, and liver were observed in the water and vehicle control groups. Dapagliflozin did not increase the incidence of these background tumors or shorten the time to onset. Importantly, there were no indications of any tumors in the urogenital tract or urinary bladder despite some background hyperplastic effects in the bladder of male mice. Dapagliflozin did not increase urinary bladder hyperplasia over that observed in control male mice (Fig. 2). Moreover, no difference was observed in the kidney between control and dapagliflozin-treated mice, except for the aforementioned increased incidence of dilated renal pelves.

### *SD Rats*

Similar carcinogenicity studies were conducted in SD rats for a period of up to 24 months as summarized in Table 2. Female rats were

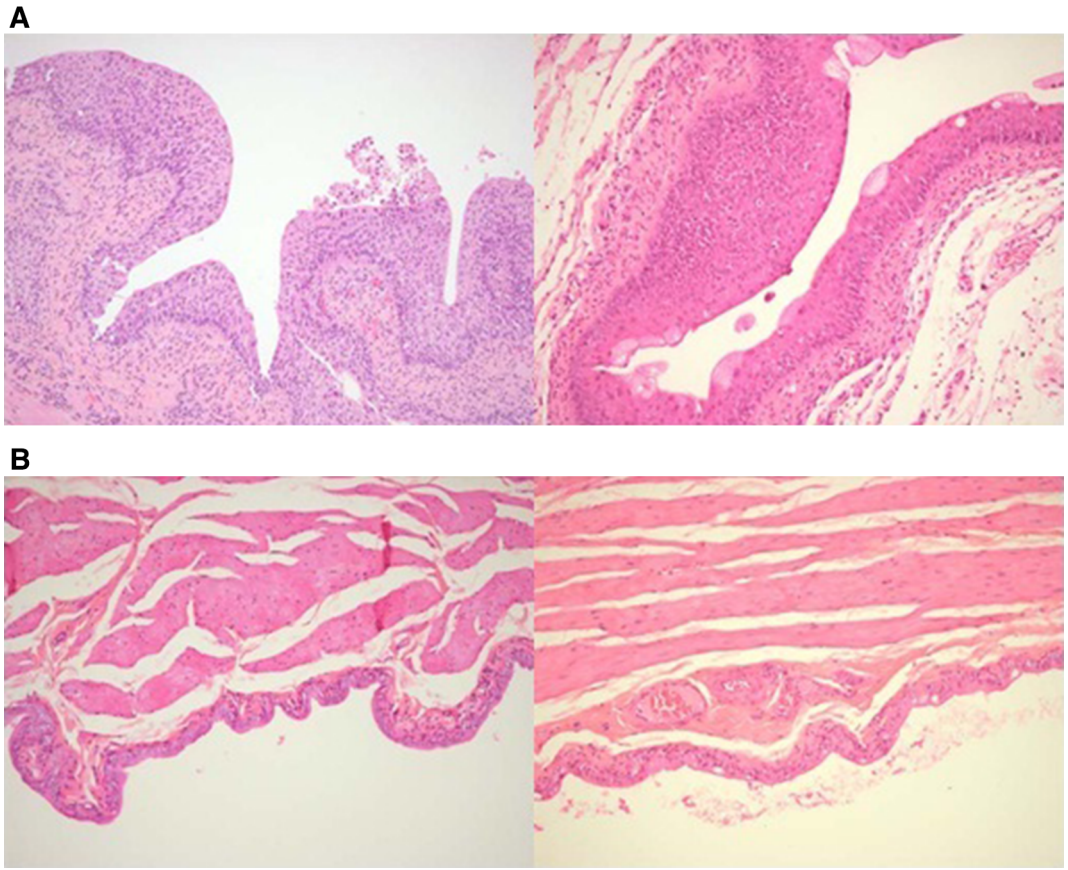


**Fig. 2** Bladder tissue histology in mice treated with vehicle control or dapagliflozin 40 mg/kg per day for >20 months and in rats treated with control or dapagliflozin 10 mg/kg per day for 90 weeks. **a** Bladder displaying incidental hyperplasia in dapagliflozin-treated (*left*) and control (*right*) male mice. In both cases, there is moderate hyperplasia of the transitional cell urothelial lining of the urinary bladder, characterized by thickening of the urothelium with an increased number of uniform cells retaining the differentiated and relatively organized character of regular epithelium, with no cellular atypia. The apparent variation in thickness is partially due to folding and/or tangential sectioning. The incidental hyperplasia is secondary to inflammation/infection, often related to mouse urologic syndrome. **b** Urinary bladder in dapagliflozin-treated (*left*) and control (*right*) male rats. In both cases, the transitional cell urothelial lining of the urinary bladder appears normal and relatively thin. ×100 magnification

**Table 2** Summary of 24-month oral carcinogenicity study with dapagliflozin in rats

| Dapagliflozin dose (mg/kg per day): | Males | | | | | Females | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0[a] ($n = 70$) | 0[b] ($n = 70$) | 0.5 ($n = 70$) | 2 ($n = 70$) | 10 ($n = 70$) | 0[a] ($n = 70$) | 0[b] ($n = 70$) | 0.5 ($n = 70$) | 2 ($n = 70$) | 10 ($n = 70$) |
| $C_{max}$ at day 184, μg/mL | NA | NA | 0.38 | 1.29 | 8.05 | NA | NA | 0.50 | 1.84 | 8.84 |
| $AUC_{(0–24)}$ at day 184[c], μg h/mL | NA | NA | 3.12 | 11.80 | 60.70 | NA | NA | 4.04 | 16.00 | 86.60 |
| No. survivors at termination, *n* (%) | 20 (29) | 22 (31) | 31 (44) | 30 (43) | 22 (31) | 28 (40) | 33 (47) | 31 (44) | 25 (36) | 27 (39) |
| Major causes of death[d], % | | | | | | | | | | |
| Chronic progressive nephropathy | 48 | 46 | 62 | 50 | 75 | 5 | 3 | 10 | 11 | 12 |
| Mammary tumor | 0 | 0 | 0 | 0 | 0 | 19 | 41 | 36 | 27 | 28 |
| Pituitary tumor | 4 | 2 | 3 | 0 | 2 | 33 | 30 | 10 | 18 | 21 |
| Other causes | 48 | 52 | 35 | 50 | 23 | 43 | 26 | 44 | 44 | 39 |

*$AUC_{(0–24)}$* area under the plasma concentration–time curve from administration to 24 h, *$C_{max}$* maximum observed plasma concentration, *NA* not applicable

[a] Vehicle-control group

[b] Water-control group

[c] Termination occurred in weeks 89–91 for males and weeks 105–106 for females

[d] Percentage of deaths attributed to a specific cause

terminated as planned at weeks 105–106. Due to declining survival in males in the two control groups and the 10 mg/kg per day group, males in all groups were terminated at weeks 89–91 to ensure sufficient numbers of rats for statistical analysis. In males, the most common cause of death was chronic progressive nephropathy (CPN), a common background finding in SD rats [37]. The higher incidence of death at 10 mg/kg per day in males was attributable to a dapagliflozin-related increase in severity of this commonly observed background condition (Fig. 3).

Dapagliflozin was not carcinogenic in rats at doses up to 10 mg/kg per day. There was a low incidence of pituitary tumors in the male rats, but the incidence of these tumors did not increase in the dapagliflozin groups. In female rats, the most common causes of death were benign and malignant mammary and pituitary tumors, but there was no dapagliflozin-related increase in the incidences or in the time to

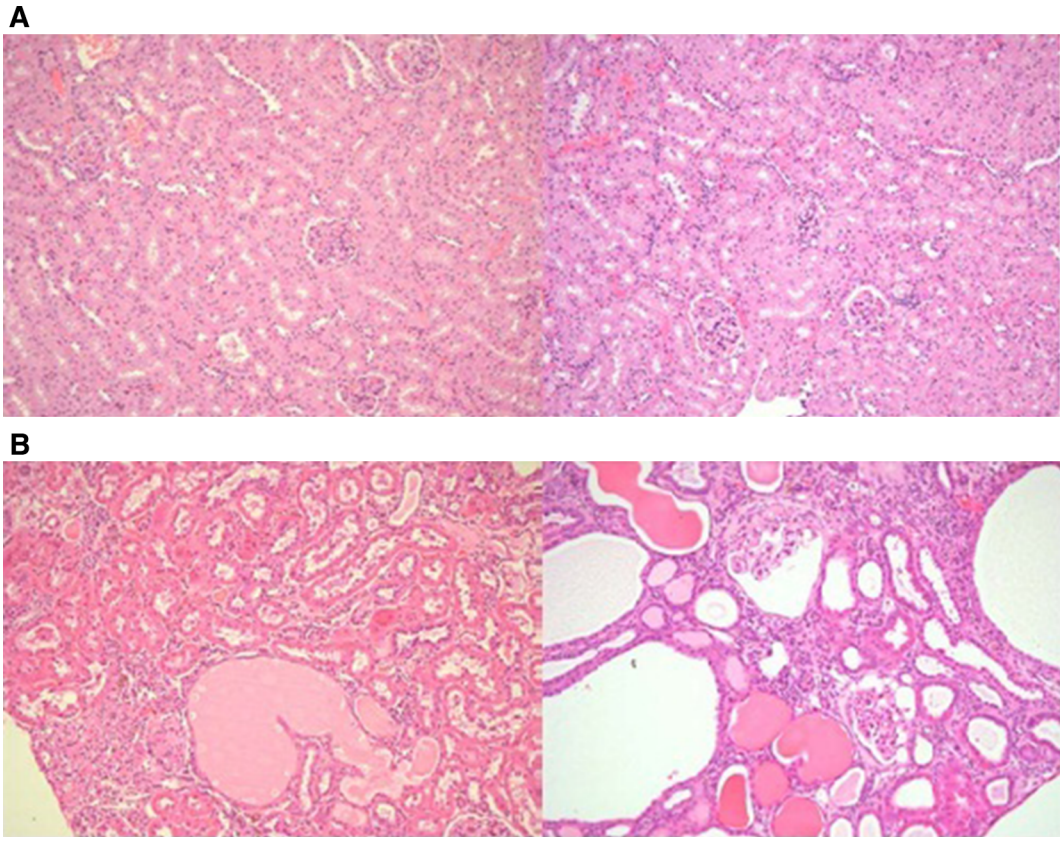


**Fig. 3** Kidney tissue histology in mice treated with vehicle control or dapagliflozin 40 mg/kg per day for >20 months and kidney pathology in rats treated with control or dapagliflozin 10 mg/kg per day for 90 weeks. **a** Kidney in dapagliflozin-treated (*left*) male mice and control (*right*). In both cases, the kidney cortex appears similar and shows normal glomeruli and renal cortical tubules. **b** Chronic progressive nephropathy (CPN) in control (*left*, moderate) and dapagliflozin-treated (*right*, severe) rats. The CPN is characterized by multifocal dilated tubules often filled with proteinaceous fluid, tubules with cytoplasmic basophilia and focally thickened tubular basement membranes, and foci of increased mononuclear cell infiltrates in the interstitium. There was exacerbation of CPN (increased severity) in the dapagliflozin-treated male rats (i.e., higher incidence of severe CPN in the dapagliflozin-treated males vs. controls). ×100 magnification

onset of these tumors. Importantly, there were no indications of any dapagliflozin-related tumors or preneoplastic lesions in the urinary bladder (Fig. 2) in rats. There were also no dapagliflozin-related neoplasms affecting the renal tubules, adrenal gland, or testicular Leydig cells as has been reported by the US Food and Drug Administration for canagliflozin [38]. In males only, there were microscopic changes in the kidney, which included exacerbation in severity of CPN and increased incidence and severity of minimal to marked atypical hyperplasia of renal cortical tubules.

## Additional Supportive Animal Studies

### *Dog Toxicology Studies*

Dogs have previously been reported to be uniquely susceptible to urinary bladder tumorigenesis [39]. Thus, dogs treated with dapagliflozin for 1 year at supratherapeutic doses and exposures >3,300× the human clinical exposure may be particularly relevant to a potential concern regarding urinary bladder tumors. Despite marked pharmacologic increases in urinary glucose excretion (8,711–32,200 mg per day) compared with control animals (5–13 mg per day), evaluation of the urinary bladder of dogs treated with dapagliflozin for 1 year did not show dapagliflozin-related proliferative or hyperplastic changes.

### *Urinary Exposure Extrapolations*

Our previous report indicated that systemic (i.e., plasma AUCs) exposures to dapagliflozin and its primary human metabolite, D3OG, in animal toxicity and carcinogenicity studies were at least equal to and often far exceeded human exposures at the MRHD [14]. Subsequently, we used bridging studies to extrapolate urinary exposures to these drug analytes (data not shown). Similar to plasma exposures, extrapolated urinary concentrations of dapagliflozin in mice, rats, and dogs were >700× relative to humans, and extrapolated urinary concentrations of the D3OG metabolite were 1–15× in rodents and 30× in dogs relative to humans.

### *SGLT2$^{-/-}$ Mice*

SGLT2 KO mice, not receiving any treatment, were used to evaluate the potential carcinogenic risk of inhibiting SGLT2, particularly in the urogenital tract chronically exposed to glucosuria and diuresis. At 15 months, 86% of the KO animals and 85% of the WT animals survived. WT animals were negative for glucosuria, and mean body weight was similar in the KO and WT mice at 15 months. As expected, KO mice exhibited substantial glucosuria (2,000 mg/dL), but there was otherwise no indication of other phenotypic differences in SGLT2 KO mice compared with WT mice, including no evidence of any renal dysfunction in SGLT2 KO mice.

Microscopic evaluation of the urinary bladder, kidneys, liver, heart, pancreas, adrenal glands, thyroids, spleen, female reproductive tract, male sex glands, skin, brain, and skull did not reveal any adverse effect attributable to SGLT2 gene deletion. Of particular note, no hyperplasia or neoplasia was observed in the urinary bladder mucosa (Fig. 4), urogenital tract, or kidneys. One SGLT2 KO male had polycystic kidney disease, a known spontaneous background condition in C57BL/6J mice [40], and, therefore, not attributable to SGLT2 gene deletion. Histopathology of this animal was consistent with this condition.

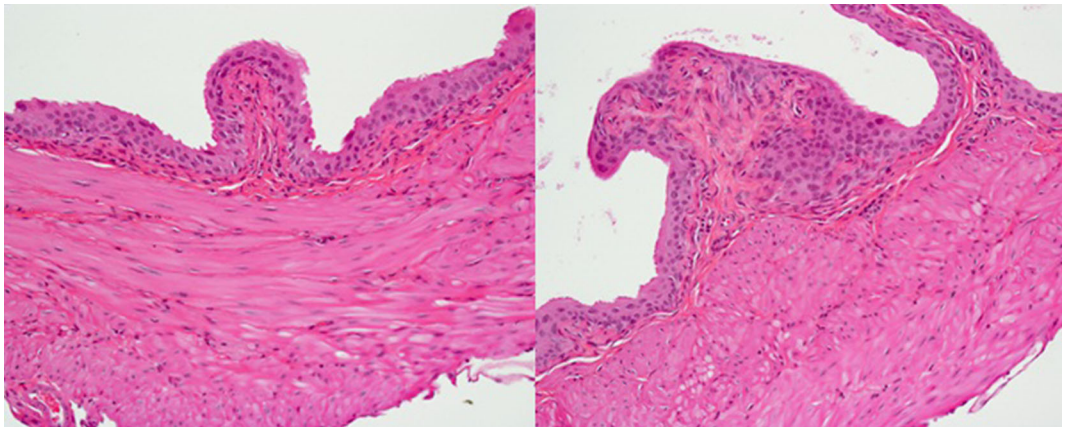

**Fig. 4** Bladder tissue histology in wild-type (*left*) and SGLT$^{-/-}$ (*right*) mice shows normal microscopic morphology for both. ×200 magnification

## Assessment of Tumor Promotion and Tumor Growth Enhancement

### *Transcriptional Profiling*

In an attempt to evaluate whether dapagliflozin was associated with any changes in the transcriptional profile of insulin-responsive and renal tissues and also any risk factors for tumor promotion, transcriptional profiling was performed in several representative tissues (although not including the bladder) following 5 weeks of dapagliflozin treatment and in the context of a diabetic state in rats. Principal Component Analysis of the entire transcription profiling data set showed that animals of each treatment group clustered together, with clear separation between fasted and fed groups and between dapagliflozin and vehicle treatment groups. Dapagliflozin produced slightly more gene expression changes in the male ZDF rats compared with vehicle-treated controls, but less than 1% of the probe sets showed significant changes. Transcriptional changes were primarily observed in the liver and related to glucose metabolism, while no major changes were observed in kidney, skeletal muscle, or adipose tissue. Importantly, very few genes involved in cell cycling, cell regulation, or cell contact were affected and none of the gene expression changes that have been reported to be prognostic indicators of tumor promoters [34, 41] were observed to occur with dapagliflozin treatment in the multiple tissues that were profiled and in the context of the diabetic disease state (see Supplemental Tables S1 and S2 in the Electronic Supplementary Material).

### *In Vitro Cell Proliferation Studies*

As expected, all six human bladder TCC cell lines showed a robust proliferative response to serum added to the culture medium. In contrast, neither dapagliflozin nor D3OG (Supplemental Figures S1 and S2 in the Electronic Supplementary Material) at concentrations up to 20 μg/mL (and ≥100× human plasma $C_{max}$ and >20× human urinary concentrations) stimulated cell proliferation relative to cells that were kept in serum-free medium throughout the 72-h period.

In the assessment of increased glucose concentrations (Supplemental Figure S3 in the Electronic Supplementary Material), increasing concentrations of glucose did not enhance TCC growth. In fact, while concentration of 25 mM did not substantially alter the growth of the T24 or the SW780 cell lines, it reduced the growth of

the J82, TCCSUP, and UM-UC-3 lines by 37%, 42%, and 73%, respectively. The growth of all cell lines was completely inhibited at both 35 mM and 50 mM glucose in the media, except for the T24 cell line, which demonstrated some growth (although reduced by 83%) in media containing 35 mM glucose, in comparison to the normal urinary glucose range of 0–0.8 mM in individuals without diabetes [42].

### *Assessment of Tumor Growth Enhancement In Vivo*

Dapagliflozin (Fig. 5) at exposures up to 75× human clinical exposures also had no effect on

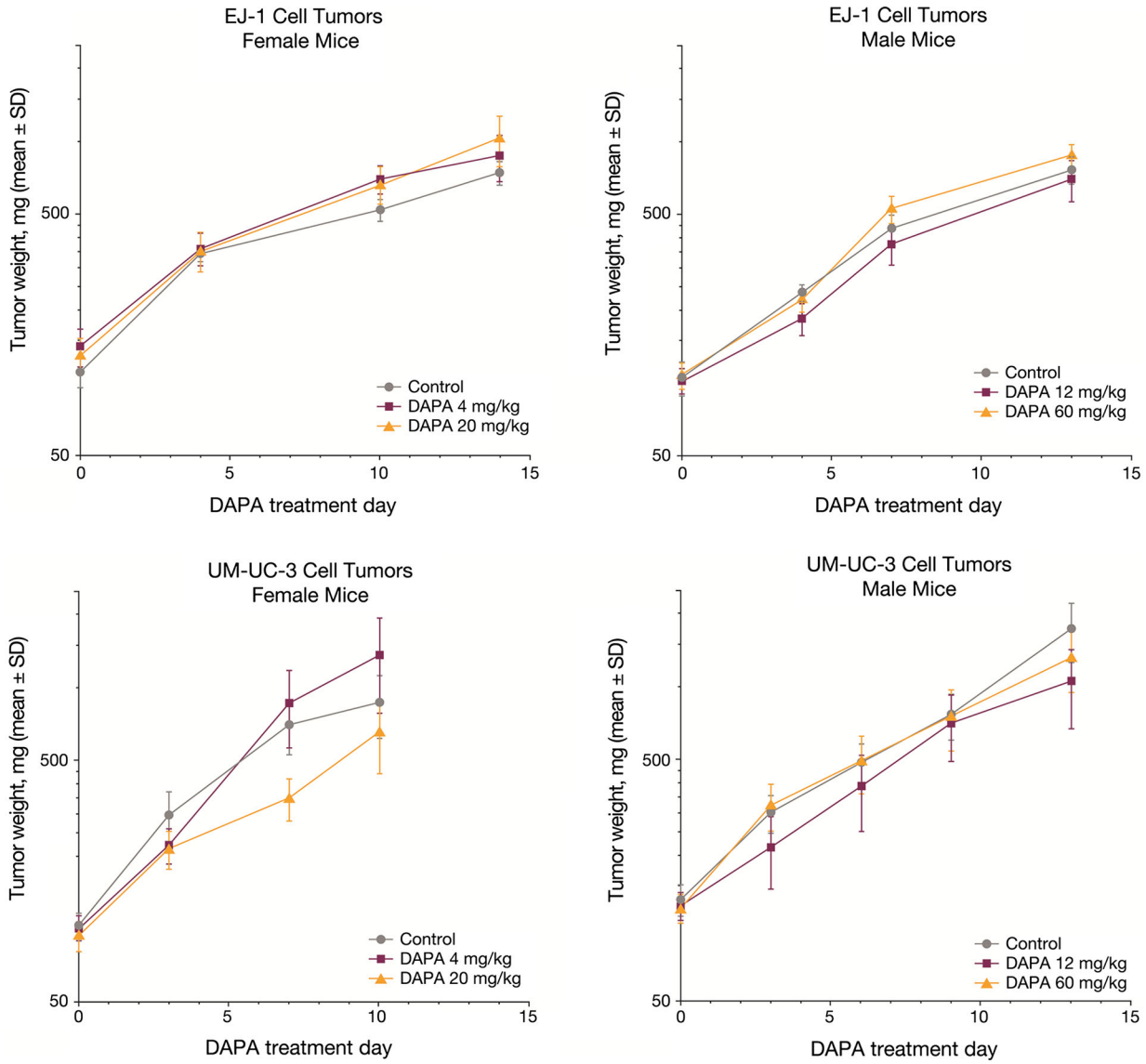


**Fig. 5** Logarithmic plot of growth of human urinary bladder transitional cell carcinoma cell line tumors in xenograft mouse models administered vehicle (control), dapagliflozin 4 mg/kg or dapagliflozin 20 mg/kg for 14 days in mice injected with the EJ-1 cell line and for 11 days in mice injected with the UM-UC-3 cell line. Tumor growth was similar in dapagliflozin-treated mice and controls. *DAPA* dapagliflozin

the growth of both the EJ-1 and UM-UC-3 tumor xenografts in either male or female nude mice. Dapagliflozin was well tolerated, producing no effect on the body weight of treated animals.

## DISCUSSION

This article provides a comprehensive preclinical evaluation of the potential carcinogenic risk for selective SGLT2 inhibition and specifically for the first drug in this new therapeutic class to reach the marketplace, dapagliflozin. While the authors acknowledge that these data cannot definitively prove the absence of any carcinogenic risk, they provide very strong weight of evidence that selective SGLT2 inhibition in general, and dapagliflozin specifically, do not present a risk for initiating, promoting, or enhancing malignancies.

Structure–activity analyses, a variety of genotoxicity assessments, and 2-year rodent carcinogenicity assays with dapagliflozin at exposures that far exceeded clinical relevance, were all negative, indicating that neither dapagliflozin nor its metabolites are tumor-initiating carcinogens. There was also no decrease in time to onset or overall increase in the incidence of common background tumor types (e.g., mammary tumors in rats) suggesting that dapagliflozin does not promote tumor growth. Furthermore, no evidence was observed of dapagliflozin-related proliferative or hyperplastic effects (i.e., neoplastic precursors) in the urinary bladder in the rodent carcinogenicity studies at >100× MRHD exposures. Although no neoplasms were observed in the kidney, there was an increase in the incidence and severity of atypical hyperplasia in the cortical tubules of male rats. As atypical hyperplasia of renal tubules is a common sequel to advanced CPN, the increased atypical hyperplasia was considered a direct result of the increased severity of CPN in these animals. While CPN-associated hyperplastic renal tubular epithelium is a potential precursor of renal tubular epithelial tumors in rats, such tumors were not observed in the current study and they are not considered a predictor of renal tumors in humans [43]. Of note, since the SGLT2 inhibitor phlorizin is pharmacologically active in the renal cortex of rats with experimental diabetes, it is also likely that dapagliflozin retains its pharmacological activity in the hyperplastic renal tubular epithelium of rats with CPN [44].

The absence of any carcinogenicity risk signals in the 2-year rodent bioassays is noteworthy as the studies presented herein assessed dapagliflozin effects at exposures >100× MRHD. Moreover, the rodent carcinogenicity assays were not confounded by the presence of any hormonal perturbation or immunotoxicologic effects, which are primarily responsible for the relatively small number of false negatives that have been reported [45]. Dapagliflozin did not bind to hormone receptors in secondary pharmacology screens nor did it induce changes in repeat-dose rat toxicity studies that would be consistent with hormonal perturbation (e.g., no microscopic changes in any sex organs, and no changes in onset of puberty, estrous cycling, or fertility) [14]. There was also no evidence of immunotoxicologic effects in repeat-dose toxicity studies conducted with dapagliflozin (e.g., no changes in lymphoid tissue or hematology parameters).

The lack of carcinogenicity findings is further supported by chronic toxicity studies conducted in rats and dogs that similarly showed no carcinogenicity [14]. The lack of

dapagliflozin-related proliferative/hyperplastic changes in the 12-month toxicity study conducted in dogs at >3,000× MRHD exposures is noteworthy as dogs are particularly sensitive to bladder carcinogenesis [39]. Although the effects observed in the rodent and dog bioassays are not a guaranteed predictor of a lack of a cancer risk for humans, the complete absence of any effects or risk factors in these and other assays provides strong evidence for the absence of a true cancer risk of dapagliflozin to humans.

The observed reduction in overall survival in male mice receiving 15 and 40 mg/kg per day dapagliflozin is most likely related to the diuretic effects of dapagliflozin in mice and not to any potential increased risk for cancer. The early deaths observed in these animals were attributable to exacerbation of MUS, a spontaneous, mouse-specific background condition commonly seen in untreated male CD-1 mice that leads to urogenital obstruction and mortality [36]. MUS was observed in CD-1 mice but not in SGLT2$^{-/-}$ mice likely due to differences among strain [46–48], age, and housing conditions. For example, CD-1 mice are inherently more susceptible to MUS, were older than the SGLT2$^{-/-}$ mice, and were housed in wire-bottom cages, which have been shown to exacerbate incidence and severity of MUS [46]. Similarly, the increased mortality in male rats observed with dapagliflozin was due to an exacerbation of CPN. CPN is also a common spontaneous lesion that has no counterpart in humans and has recently been discussed as being of little relevance to human risk assessment [49]. The non-neoplastic findings in mice and rats were not associated with other microscopic changes and were likely adaptive responses to increased urine volume secondary to osmotic diuresis due to increases in urinary glucose excretion. Similar changes in the kidney and urinary bladder of male mice and rats have also been reported in 24-month carcinogenicity studies with the diuretic furosemide [50], which were also not associated with any tumorigenic risks. Given that both MUS and rat CPN are species specific and without a counterpart in humans, these data showed no increased risk that would be relevant to humans.

The entirety of the preclinical toxicology study program was also noteworthy in terms of appropriateness for testing of on- and off-target effects from dapagliflozin or its metabolites. Dapagliflozin is pharmacologically active in all of the preclinical animal species used and the exposures in animals were in such excess of human exposures that the potential for off-target effects could be reliably tested. Moreover, the metabolic profile across species is qualitatively similar, with no unique human metabolites, and, when one considers the high doses tested in toxicology studies, is quantitatively suitable for safety evaluation of all metabolites.

The absence of any non-clinical evidence for tumor risk is consistent with results from the clinical program, in which the dapagliflozin and control groups were balanced for the overall occurrence of malignant and unspecified tumors [28, 29]. As would be expected for a drug that does not cause malignancy, the incidence rate for tumors was lower in the comparator group in some organ systems and lower in the dapagliflozin group in others, leading to the overall balance in total malignancies. However, there was a numerical imbalance in bladder cancer cases [9 of 5,501 patients (0.16%) treated with dapagliflozin and 1 of 3,184 patients (0.03%) treated with placebo/comparator in analysis performed with a July 15, 2011, cutoff] [29]. It should be noted that although the number of bladder cancer cases was small, and a number of

patients in the clinical trial had preexisting hematuria (a known risk factor for bladder cancer), there was concern that dapagliflozin and/or its associated glucosuric effect could somehow act to promote or enhance preexisting neoplastic lesions or tumors. However, the non-clinical program demonstrated that dapagliflozin does not exhibit any mechanistic characteristics of a classic tumor-promoting agent. Specifically, it did not lead to formation of crystalline solids or pH changes, was not irritating, did not cause direct cytotoxicity or inflammatory changes, and was not associated with immunosuppressive or hormonally disruptive properties. Additional studies were conducted to further evaluate tumor-initiating or -promoting potential in the context of diabetes and specific to the bladder.

To assess potential carcinogenic contributions of drug–disease interaction, an analysis of a highly predictive genetic tumor promoter signature was performed in liver, skeletal muscle, kidney, and adipose tissue from the ZDF rat model of diabetes. This analysis focused on a battery of genetic markers validated by Maeshima et al. [34, 41] to be highly correlated with in vivo tumor-promoting activities and rodent carcinogenicity and that showed 96% accuracy, sensitivity, and specificity for positive results in a two-stage cell transformation assay in Balb/c 3T3 cells. Dapagliflozin treatment did not induce transcriptional changes in the selected panel of tissues that would be characteristic of tumor promoters. Although the bladder was not specifically sampled (and histologic evaluation was not performed in this short duration study), the absence of any transcriptional changes suggestive of a tumor promoter in other tissues and the results of the additional carcinogenicity and genotoxicity assessments support the overall conclusion that dapagliflozin does not have a tumor promoter effect.

Consistent with the literature, the current study also found no evidence that elevated glucose levels stimulated cell proliferation as characterized by hyperplastic changes that are requisite for tumor promotion [51]. No evidence of dapagliflozin-related proliferative or hyperplastic effects in the bladder was observed throughout the toxicology program, in which glucosuria was a consistent finding [14]. High levels of urinary glucose on the order of 200 mM in the 2-year rodent carcinogenicity studies and up to 400–500 mM in the chronic toxicology studies, which correspond well to those observed in human subjects (mean of 166 mM at a 10 mg dose) [21], did not lead to bladder tumor development, even in male mice in which background bladder hyperplasia was observed. Additionally, an analysis of 15-month-old SGLT2$^{-/-}$ mice that had experienced a lifetime of glucosuria failed to reveal evidence of proliferative, hyperplastic, or preneoplastic type changes. If glucosuria stimulated or promoted tumor proliferation, these environments would likely have led to progression from hyperplasia to preneoplastic lesions.

Having found no evidence that dapagliflozin or glucosuria exert hyperplastic or tumorogenic effects, we explored the possibility that glucose itself, dapagliflozin, or its primary human metabolite could enhance or accelerate growth of bladder tumor cells. We used an experimental strategy similar to the one that was used to analyze the antithrombotic drug prasugrel [52]. Prasugrel also led to numerical imbalances in specific tumor types during phase 3 clinical trials despite the absence of any other preclinical cancer signals associated with the drug. In the current study, neither glucose,

dapagliflozin, nor its primary human metabolite enhanced urinary bladder TCC tumor cell growth in vitro or heterotopically in a mouse xenograft model system at exposures up to 75× and 0.9× (dapagliflozin and its primary metabolite, respectively) human clinical exposures.

The findings of these studies, showing no bladder cancer signal, are further supported when placed in the context of what is known regarding SGLT2. SGLT2 is expressed almost entirely in the kidney, not the bladder; therefore any on-target effects would be expected to occur in the kidney [8, 9]. Moreover, dapagliflozin is highly selective for SGLT2, which makes off-target effects unlikely [6, 14]. To date, there is no published report of cancer risk associated with selective SGLT2 inhibition. To the contrary, some published data suggest that inhibition of SGLT2 may actually decrease growth in tumor cells expressing this transporter by inhibiting glucose uptake into the malignant cells [53, 54]. In vitro data from this study have shown that supraphysiologic urinary glucose concentrations above 20 mM, which is still well below the levels caused by dapagliflozin in the clinic (166 mM) and in animal studies (up to 400–500 mM) [14, 21], actually inhibited tumor cell growth of a variety of human bladder TCC tumor cell lines.

Recently, carcinogenicity results for canagliflozin, an SGLT2 inhibitor that, compared with dapagliflozin, is less selective for SGLT2 than for SGLT1 (canagliflozin, 200×; dapagliflozin, 1,200×) [55], were publically disclosed [38]. Canagliflozin did not increase the incidence of neoplasms or preneoplastic lesions in CD-1 mice in a 2-year carcinogenicity study at exposures 14× clinical exposures [38]. In rats, canagliflozin increased the incidence of neoplasms of the renal tubules (≥12×), adrenals (≥12×), and testicular Leydig cells of SD rats (≥1×). These events have been hypothesized to be related to carbohydrate malabsorption and calcium imbalance due to SGLT1 inhibition, which may explain why similar tumors were not observed with dapagliflozin. In neither rodent species was there any evidence of an increased risk of bladder tumors. Publically disclosed clinical results for canagliflozin also did not suggest any malignancy signal (with 8,000 person-years exposure), further supporting the safety of SGLT2 inhibition in humans [38].

## CONCLUSION

The studies presented here were undertaken because it is well recognized that even the most extensive clinical development programs can at best only provide safety screening signals related to human malignancies, especially for risks involving specific cancer types. Using a multitude of different approaches and at high multiples of human exposures, this comprehensive non-clinical safety program provided a substantial body of evidence suggesting no indication of tumor initiation or promotion or enhancement of tumor progression associated with selective SGLT2 inhibition in general or with dapagliflozin specifically. Therefore, these results support the finding of no overall imbalance in malignancy observed in clinical trials and add to the weight of evidence regarding the overall safety of dapagliflozin in humans.

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***Conflict of interest.*** Timothy P. Reilly is an employee of Bristol-Myers Squibb. Michael J. Graziano is an employee of Bristol-Myers Squibb. Evan Janovitz is an employee of Bristol-Myers Squibb. Thomas Dorr is an employee of Bristol-Myers Squibb. Craig Fairchild is an employee of Bristol-Myers Squibb. Francis Lee is an employee of Bristol-Myers Squibb. Jian Chen is an employee of Bristol-Myers Squibb. Tai Wong is an employee of Bristol-Myers Squibb. Jean Whaley is an employee of Bristol-Myers Squibb. Mark Tirmenstein is an employee of Bristol-Myers Squibb.

***Compliance with ethics guidelines.*** The definitive studies were conducted in compliance with Good Laboratory Practice Regulations for Nonclinical Laboratory Studies of the United States Food and Drug Administration (Code of Federal Regulations Title 21, Part 58), and all in vivo studies were approved by the Bristol-Myers Squibb Institutional Animal Care and Use Committee in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care International guidelines.



## REFERENCES

1. Kanai Y, Lee WS, You G, Brown D, Hediger MA. The human kidney low affinity Na+ /glucose cotransporter SGLT2. Delineation of the major renal reabsorptive mechanism for D-glucose. J Clin Invest. 1994;93:397–404.

2. Wallner EI, Wada J, Tramonti G, Lin S, Kanwar YS. Status of glucose transporters in the mammalian kidney and renal development. Ren Fail. 2001;23:301–10.

3. Han S, Hagan DL, Taylor JR, et al. Dapagliflozin, a selective SGLT2 inhibitor, improves glucose homeostasis in normal and diabetic rats. Diabetes. 2008;57:1723–9.

4. Meng W, Ellsworth BA, Nirschl AA, et al. Discovery of dapagliflozin: a potent, selective renal sodium-dependent glucose cotransporter 2 (SGLT2) inhibitor for the treatment of type 2 diabetes. J Med Chem. 2008;51:1145–9.

5. Oku A, Ueta K, Arakawa K, et al. T-1095, an inhibitor of renal $Na^+$–glucose cotransporters, may provide a novel approach to treating diabetes. Diabetes. 1999;48:1794–800.

6. Jabbour SA, Whaley JM, Tirmenstein M, et al. Targeting renal glucose reabsorption for the treatment of type 2 diabetes mellitus using the SGLT2 inhibitor dapagliflozin. Postgrad Med. 2012;124:62–73.

7. Bhartia M, Tahrani AA, Barnett AH. SGLT-2 inhibitors in development for type 2 diabetes treatment. Rev Diabet Stud. 2011;8:348–54.

8. Chen J, William S, Ho S, et al. Quantitative PCR tissue expression profiling of the human SGLT2 gene and related family members. Diabetes Ther. 2010;1:57–92.

9. Sabolic I, Vrhovac I, Eror DB, et al. Expression of $Na^+$–D-glucose cotransporter SGLT2 in rodents is kidney-specific and exhibits sex and species

differences. Am J Physiol Cell Physiol. 2012;302:C1174–88.

10. Santer R, Calado J. Familial renal glucosuria and SGLT2: from a Mendelian trait to a therapeutic target. Clin J Am Soc Nephrol. 2010;5:133–41.

11. Bansal D, Bhansali A, Kapil G, Undela K, Tiwari P. Type 2 diabetes and risk of prostate cancer: a meta-analysis of observational studies. Prostate Cancer Prostatic Dis. 2013;16:151–8.

12. Hardefeldt PJ, Edirimanne S, Eslick GD. Diabetes increases the risk of breast cancer: a meta-analysis. Endocr Relat Cancer. 2012;19:793–803.

13. Newton CC, Gapstur SM, Campbell PT, Jacobs EJ. Type 2 diabetes mellitus, insulin-use and risk of bladder cancer in a large cohort study. Int J Cancer. 2013;132:2186–91.

14. Tirmenstein M, Dorr TE, Janovitz EB, et al. Nonclinical toxicology assessments support the chronic safety of dapagliflozin, a first-in-class sodium–glucose contransporter 2 inhibitor. Int J Toxicol. 2013;32:336–50.

15. Obermeier M, Yao M, Khanna A, et al. In vitro characterization and pharmacokinetics of dapagliflozin (BMS-512148), a potent sodium–glucose cotransporter type II inhibitor, in animals and humans. Drug Metab Dispos. 2010;38:405–14.

16. Kasichayanula S, Liu X, Lacreta F, Griffen SC, Boulton DW. Clinical pharmacokinetics and pharmacodynamics of dapagliflozin, a selective inhibitor of sodium–glucose co-transporter type 2. Clin Pharmacokinet. 2014;53:17–27.

17. Bailey CJ, Gross JL, Pieters A, Bastien A, List JF. Effect of dapagliflozin in patients with type 2 diabetes who have inadequate glycaemic control with metformin: a randomised, double-blind, placebo-controlled trial. Lancet. 2010;375:2223–33.

18. Bolinder J, Ljunggren O, Kullberg J, et al. Effects of dapagliflozin on body weight, total fat mass, and regional adipose tissue distribution in patients with type 2 diabetes mellitus with inadequate glycemic control on metformin. J Clin Endocrinol Metab. 2012;97:1020–31.

19. Ferrannini E, Ramos SJ, Salsali A, Tang W, List JF. Dapagliflozin monotherapy in type 2 diabetic patients with inadequate glycemic control by diet and exercise: a randomized, double-blind, placebo-controlled, phase 3 trial. Diabetes Care. 2010;33:2217–24.

20. Komoroski B, Vachharajani N, Feng Y, Li L, Kornhauser D, Pfister M. Dapagliflozin, a novel, selective SGLT2 inhibitor, improved glycemic control over 2 weeks in patients with type 2 diabetes mellitus. Clin Pharmacol Ther. 2009;85:513–9.

21. List JF, Woo V, Morales E, Tang W, Fiedorek FT. Sodium–glucose cotransport inhibition with dapagliflozin in type 2 diabetes. Diabetes Care. 2009;32:650–7.

22. Nauck MA, Del PS, Meier JJ, et al. Dapagliflozin versus glipizide as add-on therapy in patients with type 2 diabetes who have inadequate glycemic control with metformin: a randomized, 52-week, double-blind, active-controlled noninferiority trial. Diabetes Care. 2011;34:2015–22.

23. Rosenstock J, Vico M, Wei L, Salsali A, List JF. Effects of dapagliflozin, an SGLT2 inhibitor, on HbA(1c), body weight, and hypoglycemia risk in patients with type 2 diabetes inadequately controlled on pioglitazone monotherapy. Diabetes Care. 2012;35:1473–8.

24. Strojek K, Yoon KH, Hruba V, Elze M, Langkilde AM, Parikh S. Effect of dapagliflozin in patients with type 2 diabetes who have inadequate glycaemic control with glimepiride: a randomized, 24-week, double-blind, placebo-controlled trial. Diabetes Obes Metab. 2011;13:928–38.

25. Wilding JP, Norwood P, T'joen C, Bastien A, List JF, Fiedorek FT. A study of dapagliflozin in patients with type 2 diabetes receiving high doses of insulin plus insulin sensitizers: applicability of a novel insulin-independent treatment. Diabetes Care. 2009;32:1656–62.

26. Wilding JP, Woo V, Soler NG, et al. Long-term efficacy of dapagliflozin in patients with type 2 diabetes mellitus receiving high doses of insulin: a randomized trial. Ann Intern Med. 2012;156:405–15.

27. Zhang L, Feng Y, List J, Kasichayanula S, Pfister M. Dapagliflozin treatment in patients with different stages of type 2 diabetes mellitus: effects on glycaemic control and body weight. Diabetes Obes Metab. 2010;12:510–6.

28. Ptaszynska A, Johnsson KM, Apanovitch AM, Sugg JE, Parikh SJ, List JF. Safety of dapagliflozin in clinical trials for T2DM [abstract]. Diabetes 2012;61(suppl 1):A258 (Abstract 1011-P).

29. Johnsson K, Ptaszynska A, Apanovitch AM, Sugg J, Parikh S, List J. Safety of dapagliflozin in clinical trials for T2DM. Presented at: European Association for the Study of Diabetes, Berlin; 2012 (Abstract 743).

30. US Food and Drug Administration. FDA briefing document for the July 19, 2011 Meeting of the

Endocrinologic and Metabolic Drugs Advisory Committee. NDA 202293; Dapagliflozin tablets. US Food and Drug Administration. http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/EndocrinologicandMetabolicDrugsAdvisoryCommittee/UCM262994.pdf (Accessed January 9, 2014).

31. International Conference on Harmonisation of Technical Requirement for Registration of Pharmaceuticals for Human Use. ICH harmonised tripartite guideline: guidance on genotoxicity testing and data interpretation for pharmaceuticals intended for human use S2(R1). International Conference on Harmonisation Web site. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S2_R1/Step4/S2R1_Step4.pdf (Accessed July 2, 2013).

32. Vallon V, Platt KA, Cunard R, et al. SGLT2 mediates glucose reabsorption in the early proximal tubule. J Am Soc Nephrol. 2011;22:104–12.

33. Kasichayanula S, Liu X, Zhang W, Pfister M, LaCreta FP, Boulton DW. Influence of hepatic impairment on the pharmacokinetics and safety profile of dapagliflozin: an open-label, parallel-group, single-dose study. Clin Ther. 2011;33:1798–808.

34. Maeshima H, Ohno K, Tanaka-Azuma Y, Nakano S, Yamada T. Identification of tumor promotion marker genes for predicting tumor promoting potential of chemicals in BALB/c 3T3 cells. Toxicol In Vitro. 2009;23:148–57.

35. Gehan EA. A generalized Wilcoxon test for comparing arbitrarily singly-censored samples. Biometrika. 1965;52:203–23.

36. Gaillard ET. Ureter, urinary bladder, and urethra. In: Maronpot RR, Boorman GA, Gaul BW, editors. Pathology of the mouse—reference and atlas. Vienna: Cache River Press; 1999. p. 251–3.

37. Baylis C, Corman B. The aging kidney: insights from experimental studies. J Am Soc Nephrol. 1998;9:699–709.

38. Janssen Pharamecuticals I. FDA briefing document. NDA 204042. Invokana (canagliflozin) Tablets. FDA Web site. http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/EndocrinologicandMetabolicDrugsAdvisoryCommittee/UCM334550.pdf (Accessed April 13, 2013).

39. Clayson DB, Cooper EH. Cancer of the urinary tract. Adv Cancer Res. 1970;13:271–381.

40. Mandell J, Koch WK, Nidess R, Preminger GM, McFarland E. Congenital polycystic kidney disease. Genetically transmitted infantile polycystic kidney disease in C57BL/6J mice. Am J Pathol. 1983;113:112–4.

41. Maeshima H, Ohno K, Nakano S, Yamada T. Validation of an in vitro screening test for predicting the tumor promoting potential of chemicals based on gene expression. Toxicol In Vitro. 2010;24:995–1001.

42. Dugdale DC. Glucose test-urine. MedlinePlus. http://www.nlm.nih.gov/medlineplus/ency/article/003581.htm (Accessed June 24, 2013).

43. Hard GC, Banton MI, Bretzlaff RS, et al. Consideration of rat chronic progressive nephropathy in regulatory evaluations for carcinogenicity. Toxicol Sci. 2013;132:268–75.

44. Osorio H, Bautista R, Rios A, et al. Effect of phlorizin on SGLT2 expression in the kidney of diabetic rats. J Nephrol. 2010;23:541–6.

45. Sistare FD, Morton D, Alden C, et al. An analysis of pharmaceutical experience with decades of rat carcinogenicity testing: support for a proposal to modify current regulatory guidelines. Toxicol Pathol. 2011;39:716–44.

46. Everitt JI, Ross PW, Davis TW. Urologic syndrome associated with wire caging in AKR mice. Lab Anim Sci. 1988;38:609–11.

47. Maita K, Hirano M, Harada T, et al. Mortality, major cause of moribundity, and spontaneous tumors in CD-1 mice. Toxicol Pathol. 1988;16:340–9.

48. Son WC. Factors contributory to early death of young CD-1 mice in carcinogenicity studies. Toxicol Lett. 2003;145:88–98.

49. Hard GC, Khan KN. A contemporary overview of chronic progressive nephropathy in the laboratory rat, and its significance for human risk assessment. Toxicol Pathol. 2004;32:171–80.

50. Bucher JR, Huff J, Haseman JK, Eustis SL, Davis WE Jr, Meierhenry EF. Toxicology and carcinogenicity studies of diuretics in F344 rats and B6C3F1 mice. 2. Furosemide. J Appl Toxicol. 1990;10:369–78.

51. Tannock IF, Kopelyan I. Influence of glucose concentration on growth and formation of necrosis in spheroids derived from a human bladder cancer cell line. Cancer Res. 1986;46:3105–10.

52. Buckley LA, Sanbuissho A, Starling JJ, Knadler MP, Iversen PW, Jakubowski JA. Nonclinical assessment of carcinogenic risk and tumor growth enhancement potential of prasugrel, a platelet-inhibiting therapeutic agent. Int J Toxicol. 2012;31:317–25.

53. Nelson JA, Falk RE. The efficacy of phloridzin and phloretin on tumor cell growth. Anticancer Res. 1993;13:2287–92.

54. Nelson JA, Falk RE. Phloridzin and phloretin inhibition of 2-deoxy-D-glucose uptake by tumor cells in vitro and in vivo. Anticancer Res. 1993;13:2293–9.

55. Washburn WN. Evolution of sodium glucose co-transporter 2 inhibitors as anti-diabetic agents. Expert Opin Ther Pat. 2009;19:1485–99.