Clinical Trial Protocol: THR-1442-C-454

Study Title:	A Phase 1, Open-Label, Non-Randomized, Fixed-Sequence Composite Study to Evaluate the Effects of Probenecid, Rifampin, and Verapamil on the Pharmacokinetics and Pharmacodynamics of Bexagliflozin in Healthy Subjects
Study Number:	THR-1442-C-454
Study Phase:	1
Product Name:	Bexagliflozin tablets
Indication:	Type 2 Diabetes Mellitus
Investigators:	Single center
Sponsor:	Theracos Sub, LLC
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	Date
Version 1	28 August 2017

Confidentiality Statement

The information contained in this protocol is confidential and provided only to the investigators, clinical study collaborators, investigational drug managers, study sites and institutional review boards participating in the study. The information may, therefore, not be, disclosed to any third party except for subjects when receiving their consent, or used for purposes other than this study without the written consent of Theracos Sub, LLC.

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SYNOPSIS

Sponsor: Theracos Sub, LLC

Name of Finished Product: Bexagliflozin tablets, 20 mg

Name of Active Ingredient: Bexagliflozin

Name of Inactive Ingredients :

Polyethylene oxide, glyceryl behenate, lactose monohydrate, micronized poloxamer, microcrystalline cellulose, colloidal silicon dioxide, and magnesium stearate.

Name of Reference Therapies:

Probenecid tablets, rifampin capsules, and verapamil tablets

Study Title:

A Phase 1, Open-Label, Non-Randomized, Fixed-Sequence Composite Study to Evaluate the Effects of Probenecid, Rifampin, and Verapamil on the Pharmacokinetics and Pharmacodynamics of Bexagliflozin in Healthy Subjects

Study Number:THR-1442-C-454

Study Phase: 1

Primary Objective:

- To evaluate the effects of probenecid, rifampin, and verapamil on the pharmacokinetics (PK) and pharmacodynamics (PD) of bexagliflozin in healthy subjects

Secondary Objective:

- To assess the safety and tolerability of bexagliflozin co-administered with probenecid, rifampin, or verapamil

Study Design:

In this study, a total of 48 healthy subjects will be enrolled and assigned to one of three groups of sixteen. Each group will participate in one of three phase 1, open-label, non-randomized, fixed-sequence studies:

Study 1: Bexagliflozin/probenecid (n=16)

This is an open-label study of bexagliflozin and probenecid taken in a sequential order by healthy subjects. Sixteen healthy subjects will take one bexagliflozin tablet, 20 mg, once daily (qd) and/or probenecid tablets, 500 mg, twice daily (bid) in a sequential order as follows: on Day 1 subjects will take one bexagliflozin tablet, 20 mg, on Days 3 to 4 subjects will take probenecid tablets, 500 mg (bid), on Day 5 subjects will take one bexagliflozin tablet, 20 mg (qd) + probenecid tablets, 500 mg (bid), and on Day 6 subjects will take probenecid tablets, 500 mg (bid).

Blood samples to characterize the PK profile of bexagliflozin and its principal (3'-*O*-glucuronide) metabolite EGT0002149 will be collected at pre-dose and at 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 36, and 48 h after administration of bexagliflozin on Day 1 and Day 5.

Urine samples for PD measurement will be collected at pre-dose (h -12 to 0), and at 0 to 12 h, 12 to 24 h, 24 to 36 h, and 36 to 48 h after oral administration of bexagliflozin on Day 1 and Day 5.

Plasma concentrations of bexagliflozin and EGT0002149 will be determined by validated LC-MS/MS assays.

Study 2: Bexagliflozin/rifampin (n=16)

This is an open-label study of bexagliflozin and rifampin taken in a sequential order by healthy subjects. Sixteen healthy subjects will take bexagliflozin tablets, 20 mg, and/or 600 mg of rifampin (2 x 300 mg capsules) daily in sequential order as follows: on Day 1 subjects will take one bexagliflozin tablet, 20 mg, on Days 3 to 5, subjects will take 600 mg of rifampin (2 x 300 mg capsules) once daily, on Day 6 subjects will take one bexagliflozin tablet, 20 mg and 600 mg of rifampin, and on Day 7 subjects will take 600 mg of rifampin.

Blood samples for the characterization of the PK profile of bexagliflozin and its principal metabolite EGT0002149 will be collected at pre-dose and at 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 36, and 48 h after administration of bexagliflozin on Day 1 and Day 6.

Urine samples for PD (UGE) will be collected at pre-dose (h -12 to 0), and at 0 to 12 h, 12 to 24 h, 24 to 36 h, and 36 to 48 h after administration of bexagliflozin on Day 1 and Day 6.

Plasma concentrations of bexagliflozin and EGT0002149 will be determined by validated LC-MS/MS assays.

Study 3: Bexagliflozin/verapamil (n=16)

This is an open-label study of bexagliflozin and verapamil taken in a sequential order by healthy subjects. Sixteen healthy subjects will be administered bexagliflozin tablets, 20 mg and/or verapamil tablets, 120 mg, in sequential order as follows: on Day 1 subjects will take one bexagliflozin tablet, 20 mg, on Day 4 subjects will take one verapamil tablet, 120 mg one hour before taking one bexagliflozin tablet, 20 mg.

Blood samples for characterization of the PK profile of bexagliflozin will be collected at pre-dose and at 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 36, and 48 h after administration of bexagliflozin on Day 1 and Day 3.

Urine samples for PD (UGE) analysis will be collected at pre-dose (h -12 to 0), and at 0 to 12 h, 12 to 24 h, 24 to 36 h, and 36 to 48 h after administration of bexagliflozin on Day 1 and Day 3.

Plasma concentrations of bexagliflozin will be determined by validated LC-MS/MS assay.

Clinical laboratory tests and safety monitoring will be conducted during the study for all subjects.

For study events table, see Appendix 1.

Study Population: 48 subjects are planned to be enrolled, 16 in each of the 3 studies.

Diagnosis and Main Criteria for Inclusion

1. Healthy subjects who are between the ages of 18 and 55 years, inclusive.
2. Subjects with body-mass index (BMI) between 18.0 kg/m^2 and 32.0 kg/m^2, inclusive.
3. Subjects who are not surgically sterile must agree to refrain from donating sperm and use appropriate birth control such as the use of condoms when engaging in sexual intercourse for a period of 30 days after discharge from the clinic.
4. Subjects who are non-smokers for at least 3 months prior to screening.
5. Subjects with adequate venous access at multiple sites in both arms.

Test Product, Dose and Mode of Administration: Bexagliflozin tablets, 20 mg qd, po.

Reference Therapy, Dose and Mode of Administration:

Probenecid tablets, 500 mg bid, po

Rifampin capsules, 600 mg qd, po

Verapamil tablets, 120 mg qd, po

Duration of Treatment:

A total of 48 healthy subjects will be enrolled in three groups of sixteen. Individuals with histories incompatible with enrollment in some groups may be assigned to alternate groups. Each group will participate in one of three fixed-sequence studies:

Study 1: Bexagliflozin/probenecid (n=16)

Study 2: Bexagliflozin/rifampin(n=16)

Study 3: Bexagliflozin/verapamil (n=16)

Subjects in Study 1 (probenecid) will check into the clinic on the day before dosing, and void their bladders at 12 h prior to anticipated first dose. They will remain domiciled in clinical research unit (CRU) until the final plasma sample is collected at 48 h post-dose on the morning of study Day 7.

Subjects in Study 2 (rifampin) will check into the clinic on the day before dosing and void their bladders at 12 h prior to the anticipated first dose. They will remain domiciled in CR) until the final plasma sample is collected at 48 h post-dose on the morning of study Day 8.

Subjects in Study 3 (verapamil) will check into the clinic on the day before dosing and void their bladders at 12 h prior to the anticipated first dose. They will remain domiciled in CR) until the final plasma sample is collected at 48 h post-dose on the morning of study Day 6.

Screening will be taken place within 21 days before the first intake of study drug. The duration of the overall study from screening until study termination is estimated to be a maximum of 30 days. For details of the schedule and nature of the investigations, see the Schedule of Events in Appendix 1.

Pharmacokinetic Variables:

The following PK parameters will be determined after each subject is dosed with

bexagliflozin alone or with bexagliflozin and the potential interacting agent (probenecid, rifampin or verapamil). The analytes measured will be bexagliflozin (in all studies) and its 3'-*O*-glucuronide (EGT0002149; in study 1 and 2.)

C_{max} Maximum observed plasma concentration

T_{max} Time of maximum observed plasma concentration

λ_z Terminal elimination phase rate constant

$T_{1/2}$ Apparent terminal elimination half-life

CL/F Apparent oral clearance

V_z/F Apparent volume of distribution

AUC_{0-t} Area under the plasma concentration-time curve from Time 0 to Time t (time of last quantifiable plasma concentration)

$AUC_{0-\infty}$ Area under the plasma concentration-time curve from Time 0 to infinity

AUC_{extr} % of $AUC_{0-\infty}$ due to extrapolation from T_{last} to infinity

M/P AUC Ratio Metabolite-to-parent ratio for AUC (AUC_{0-t} and $AUC_{0-\infty}$)

M/P C_{max} Ratio Metabolite-to-parent ratio for C_{max}

Pharmacodynamics Assessments:

PD parameters include:

- Urinary glucose excretion (UGE)
- Urinary creatinine

Safety Assessments:

- Physical examinations
- Vital signs
- 12-lead electrocardiograms
- Clinical laboratory tests including blood chemistry and hematology parameters
- Urinalysis
- Adverse events

Statistical Methods:

An adequate number of subjects will be enrolled to obtain approximately 16 evaluable subjects in each study.

Statistical analysis will be performed using Statistical Analysis Software SAS for Windows® (SAS Institute Inc., USA). PK parameters will be calculated using non-compartmental analyses of plasma concentration data. To assess the effect of co-administration of the interacting agent (probenecid, rifampin and verapamil) on the PK of bexagliflozin, analyses of variance (ANOVA) using a linear mixed-effects model will be fitted to the natural logarithmic transformation of the PK parameters (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$). The linear mixed-effects model will include subject as a random effect, and treatment as fixed effects.

The 90% confidence intervals will be constructed for the ratio of geometric means of PK parameters (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$) inferred from bexagliflozin dosing in combination with the interacting drug (probenecid, rifampin or verapamil) divided by the values for bexagliflozin dosing as a sole agent, with 80-125% defined as the lack of interaction boundaries.

Descriptive statistics for the PK parameters C_{max}, T_{max}, $AUC_{0-\infty}$, AUC_{0-t}, AUC_{extr}, M/P C_{max} and AUC Ratio (AUC_{0-t} and $AUC_{0-\infty}$) (for Study 1 and Study 2), CL/F, V_z/F, λ_z, and $T_{1/2}$ will be tabulated by treatment. Means, standard deviations, medians, ranges (min, max) and geometric means and coefficients of variation will be presented for all PK parameters with the exception of T_{max}. Medians and ranges will be presented for T_{max}.

The amount of UGE, urinary creatinine, and creatinine normalized UGE (nUGE) will be calculated. The descriptive statistics will be used to describe any differences in these PD parameters between treatments.

Date of Protocol V.1.0: 28 August 2017

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	adverse event
ALT	alanine aminotransferase
ANOVA	analysis of variance
AST	aspartate aminotransferase
AUC	area under the plasma concentration-time curve
AUC_{extr}	% of $AUC_{0-\infty}$ due to extrapolation from T_{last} to infinity
$AUC_{0-\infty}$	area under the plasma concentration-time curve from time 0 to infinity
AUC_{0-t}	area under the plasma concentration-time curve from time 0 to time t (time of last quantifiable plasma concentration)
bid	twice daily
BMI	body mass index
BLOQ	below limit of quantification
BP	blood pressure
C_{max}	maximum observed plasma concentration
C_{last}	concentration corresponding to T_{last}
CL/F	apparent oral clearance
CRF	case report form
CRU	Clinical research unit
CRO	contract research organization
DDI	drug-drug interaction
DPP-4	dipeptidyl peptidase 4
ECG	electrocardiogram
eGFR	estimated glomerular filtration rate
FPG	fasting plasma glucose
GCP	Good Clinical Practice
GMI	genital mycotic infection
HBsAg	hepatitis B surface antigen
HCV	hepatitis C virus
HIV	human immunodeficiency virus
ICH	International Council on Harmonisation
IEC	Independent Ethics Committee
IRAE	immediately reportable adverse event
IRB	Institutional Review Board
IR	Immediate release
K_2EDTA	potassium ethylenediaminetetraacetic acid
LS	least squares
CLR	renal clearance
λ_z	terminal elimination phase rate constant
MedDRA	Medical Dictionary for Regulatory Activities
NCA	non-compartmental analysis
OHA	oral hypoglycemic agent
OTC	over-the-counter

PD	pharmacodynamic
PE	physical examination
po	by mouth
Pgp	p-glycoprotein
PK	pharmacokinetic
SAE	serious adverse event
SD	standard deviation
SFU	Sulfonylurea
SGLT1	sodium glucose cotransporter 1
SGLT2	sodium glucose cotransporter 2
SOP	standard operating procedure
SR	slow release
TEAE	treatment emergent adverse event
$T_{1/2}$	apparent terminal elimination half life
T_{last}	time of last measurable concentration
T_{max}	time of maximum observed plasma concentration
T2DM	type 2 diabetes mellitus
UGE	urine glucose excretion
ULN	upper limit of normal
UGT	uridine diphosphate glucuronosyltransferases
UTI	urinary tract infection
V_z/F	apparent volume of distribution
WBC	white blood cells
WHO-DD	World Health Organization Drug Dictionary
WOCBP	women of child-bearing potential

1 INTRODUCTION

Bexagliflozin is a potent and selective sodium glucose linked transporter 2 (SGLT2) inhibitor being developed as a treatment for type 2 diabetes mellitus and hypertension. The transport of glucose from the renal tubule lumen into tubular epithelial cells is facilitated by SGLT2, which is expressed primarily in the S1 and S2 segments of the proximal tubule (Washburn, 2009).Bexagliflozin blocks the reabsorption of filtered glucose by inhibiting SGLT2, promotes urinary glucose excretion and reduces plasma glucose (Zhang et al., 2011).

Extended release bexagliflozin tablet formulations have been developed that provide greater than 75% release in approximately 8 h *in vitro*. Clinical pharmacokinetic (PK) studies have shown that the peak plasma bexagliflozin concentrations occur between 3 to 5 h and thereafter decline in a biphasic manner with mean elimination half-life values ranging from 7.80 to 9.71 h. Bexagliflozin is cleared predominantly by metabolism to an inactive metabolite, bexagliflozin 3'-*O*-glucuronide by uridine diphosphate glucuronosyltransferase isoform 1A9 (UGT1A9). Exposure to the 3'-*O*-glucuronide occurs at 26%-32% of exposure (as AUC) to the parent drug. Although phase 2 metabolism is largely considered to have high capacity, the possible consequences of systemic inhibition of UGT1A9, or UGTs in general, on bexagliflozin metabolism would be helpful to understand. Both probenecid and valproic acid have been suggested to be medically relevant model UGT inhibitors ((FDA), 2012). Selective inhibition of UGT1A9 can be achieved with the NSAID niflumic acid (Miners et al., 2011) but this agent is not frequently encountered in clinical practice, whereas probenecid is more widely prescribed. In this study probenecid will be used to explore possible consequences of UGT inhibition.

In vitro studies have shown that bexagliflozin is not a potent inhibitor for transporters including human breast cancer resistance protein (BCRP), organic anion transporting polypeptide OATP1B1 and OATP1B3, organic anion transporter 1 (OAT1) and OAT3, bile salt export pump (BSEP), or human organic cation transporters OCT1 and OCT2 ($IC_{50} \geq 34.8$ μM). Bexagliflozin is a substrate for P-glycoprotein (P-gp) and inhibits P-glycoprotein (P-gp) efflux ratio with an IC_{50} of 3.7 μM or 1720 ng/mL, about 11 × the C_{max} expected for the expected marketed dose of 20 mg. Although bexagliflozin was found to produce changes in efflux ratio at relatively low concentrations, much higher concentrations were required to produce changes in P-gp-mediated net transport. It is unknown whether an inhibitor of P-gp would increase the availability of bexagliflozin in humans. Among the inhibitors of P-gp that are available for use as probes, verapamil is attractive for the degree of characterization of its action in antecedent studies and for its favorable safety profile.

Bexagliflozin is neither a significant inducer nor inhibitor of cytochrome P450 (CYP) isoenzymes and is a substrate of CYP3A4 *in vitro*. Phase 1 metabolites EGT0001301, EGT0001663, and EGT0001494 have been observed after incubation with CYP3A4 *in vitro* (THR-1442-N-440). Although the contribution of oxidative metabolism to clearance of bexagliflozin has been found to be low, the potential for a clinically meaningful decrease in plasma concentration following exposure to a potent inducer of xenobiotic metabolism cannot be excluded. For the most relevant CYP isoform, CYP3A4, rifampin is known to be a strong inducer ((FDA), 2012).

The overall investigational plan for assessing potential drug-drug interactions of bexagliflozin has been designed to comply with the relevant Guidance for Industry addressing drug interaction studies ((FDA), 2012). The substrate drug, bexagliflozin, will be dosed at the maximum expected planned dose, 20 mg ((FDA), 2012) . The shortest dosing interval of the potential interacting drug has been chosen in each case ((FDA), 2012).This interval varies according to the interacting drug. For verapamil, previous studies have indicated that a maximal effect on absorption can be achieved with a single dose of 120 mg administered an hour in advance of the dosing of the target compound. Although the bexagliflozin formulation chosen for development has a gastroretentive mechanism, T_{max} is reached after dosing in the fasted state relatively promptly, and a timing adjustment for the dosing of verapamil is not thought to be necessary. The relevant site of action of verapamil for its effect on bexagliflozin absorption is the intestine, and hence the lumenal concentration of verapamil is arguably more important than the plasma level.

Assessment of the potential for interaction of bexagliflozin metabolism with probenecid and rifampin requires a longer dosing period to allow the latter agents, or their actions, to reach steady state. Two prior consecutive days of dosing of probenecid are anticipated to be required to attain approximately asymptotic plasma profiles. Although rifampin has a relatively short elimination half-life, its effects on induction of xenobiotic metabolizing enzymes requires chronic dosing to develop, and based on literature modeling, bexagliflozin administration is planned for day four of dosing. Because large increases in elimination half-life are not expected to be encountered in any of these studies, the bexagliflozin sampling will cease at 48 h post-dose for both the initial administration and co-administration phases of the study.

1.1 Bexagliflozin for the Treatment of Type 2 Diabetes Mellitus

Bexagliflozin is a highly specific inhibitor of SGLT2 with an *in vitro* IC_{50} of 2 nM or 0.9 ng/mL and a 2435-fold selectivity for human SGLT2 over human SGLT1. Details of the nonclinical and clinical findings can be found in the Investigator's Brochure.

1.2 Summary of Nonclinical Data with Bexagliflozin

Bexagliflozin exhibits high permeability and is a potential Pgp (p-glycoprotein) substrate and inhibitor. It is not a significant inducer or inhibitor of cytochrome P450 isozymes and other transporters relevant for drug-drug interactions.

1.3 Summary of Clinical Data with Bexagliflozin

Following administration of bexagliflozin tablets in extended release formulations, T_{max} was reached between 3 to 5 h. The tablets produced dose proportional exposure with a C_{max} of 4 or 8 ng/mL per mg bexagliflozin and an AUC_{0-24} of 40 or 50 ng·h/mL per mg bexagliflozin in healthy subjects when dosing was before or after a meal, respectively.

The principal metabolites in humans are similar to those found in monkeys and are dominated by glucuronides of the parent compound, for which the AUC is >30% relative to parent bexagliflozin. Following oral administration of radiolabeled bexagliflozin, >90% of

the ingested radioactivity was recovered, 51% as fecal excretion and 41% as urinary excretion. In urine, bexagliflozin accounts for 1.5% of the dose; most of the radioactivity is excreted as bexagliflozin 3'-*O*-glucuronide. The largest fraction of the radioactivity in feces is bexagliflozin, accounting for about 30% of the administered dose.

The safety and tolerability of bexagliflozin in healthy or diabetic subjects was initially evaluated in a single-dose study of up to 100 mg, followed by a 4-week treatment study in which doses ranged from 5 mg/d to 50 mg/d. The most frequently occurring adverse events (AEs) were mild to moderate headache, pollakiuria, nausea, and fatigue. No symptomatic hypoglycemia was reported and no treatment-related adverse event led to discontinuation of treatment. Extended release tablets of 3 mg to 90 mg strengths were well tolerated in a Japanese population with few adverse events reported.

In study THR-1442-C-481, a delayed T_{max} of 5 h was observed under fed conditions compared to 3.5 h under fasted conditions and an approximately 30% increase in C_{max} under fed conditions was seen compared to the C_{max} in the fasted state, without significant changes in AUC. The 90% CI for the ratios of geometric means between fed and fasted conditions in both AUC_{0-t} and $AUC_{0-\infty}$ were contained within 80% to 125% demonstrating that bexagliflozin bioavailability was not affected by co-administering with food.

Detailed information on prior clinical experience with bexagliflozin and potential risks for study subjects are provided in the Investigator's Brochure.

2 STUDY OBJECTIVES

2.1 Primary Objective

To evaluate the effects of probenecid, rifampin, and verapamil on the pharmacokinetics (PK) and pharmacodynamics (PD) of bexagliflozin in healthy subjects

2.2 Secondary Objective

To assess the safety and tolerability of bexagliflozin when it is co-administered with probenecid, rifampin, or verapamil

3 INVESTIGATIONAL PLAN

3.1 Overall Study Design and Plan

In this study, a total of 48 healthy subjects will be enrolled and assigned to three groups of sixteen subjects per group. Each group will participate in one of three open-label, non-randomized, fixed-sequence studies (Figure 1):

Study 1: Bexagliflozin/probenecid (n=16)

Study 2: Bexagliflozin/rifampin (n=16)

Study 3: Bexagliflozin/verapamil (n=16)

Study 1: Bexagliflozin/probenecid (n=16)

Sixteen healthy subjects will take bexagliflozin tablets, 20 mg, qd and/or probenecid tablets, 500 mg, bid, in sequential order as follows: on Day 1 subjects will take one bexagliflozin tablet, 20 mg. on Days 3 and 4 subjects will take probenecid tablets, 500 mg, bid, on Day 5 subjects will take one bexagliflozin tablet, 20 mg and probenecid tablets, 500 mg, bid, and on Day 6 subjects will take probenecid tablets, 500 mg, bid.

Blood samples to characterize the PK profile of bexagliflozin and its principal (3'-*O*-glucuronide) metabolite EGT0002149 will be collected at pre-dose and at 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 36, and 48 h after administration of bexagliflozin on Day 1 and Day 5.

Urine samples for PD measurement will be collected at pre-dose (h -12 to 0), and at 0 to 12 h, 12 to 24 h, 24 to 36 h, and 36 to 48 h after oral administration of bexagliflozin on Day 1 and Day 5.

Plasma concentrations of bexagliflozin and EGT0002149 will be determined by validated LC-MS/MS assays.

Study 2: Bexagliflozin/rifampin(n=16)

Sixteen healthy subjects will take bexagliflozin tablets, 20 mg, and/or 600 mg of rifampin (2 x 300 mg capsules) daily in sequential order as follows: on Day 1 subjects will take one bexagliflozin tablet, 20 mg, on Days 3 to 5, subjects will take 600 mg of rifampin (2 x 300 mg capsules) once daily, on Day 6 subjects will take one bexagliflozin tablet, 20 mg and 600 mg of rifampin, and on Day 7 subjects will take 600 mg of rifampin.

Blood samples for the characterization of the PK profile of bexagliflozin and its principal metabolite EGT0002149 will be collected at pre-dose and at 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 36, and 48 h after administration of bexagliflozin on Day 1 and Day 6.

Urine samples for PD (UGE) will be collected at pre-dose (h -12 to 0), and at 0 to 12 h, 12 to 24 h, 24 to 36 h, and 36 to 48 h after administration of bexagliflozin on Day 1 and Day 6.

Plasma concentrations of bexagliflozin and EGT0002149 will be determined by validated LC-MS/MS assays.

Study 3: Bexagliflozin/verapamil (n=16)

This is an open-label study of bexagliflozin and verapamil taken in a sequential order by healthy subjects. Sixteen healthy subjects will be administered bexagliflozin tablets, 20 mg and/or verapamil tablets, 120 mg, in sequential order as follows: on Day 1 subjects will take one bexagliflozin tablet, 20 mg, on Day 4 subjects will take one verapamil tablet, 120 mg one hour before taking one bexagliflozin tablet, 20 mg.

Blood samples for characterization of the PK profile of bexagliflozin will be collected at pre-dose and at 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 36, and 48 h after administration of bexagliflozin on Day 1 and Day 3.

Urine samples for PD (UGE) analysis will be collected at pre-dose (h -12 to 0), and at 0 to 12 h, 12 to 24 h, 24 to 36 h, and 36 to 48 h after administration of bexagliflozin on Day 1 and Day 3.

Plasma concentrations of bexagliflozin will be determined by validated LC-MS/MS assays.

For study events table, see Appendix 1.

A. Study 1: Bexagliflozin/probenecid

Days	1	2	3	4	5	6	7
Bexagliflozin (20 mg), qd	X				X		
Probenecid (500 mg), bid			X	X	X	X	
Blood sample	*	*	*		*	*	*

B. Study 2: Bexagliflozin/rifampin

Days	1	2	3	4	5	6	7	8
Bexagliglozin (20 mg)	X					X		
Rifampin (600 mg)			X	X	X	X	X	
Blood sample	*	*	*			*	*	*

Study 3: bexagliflozin/verapamil

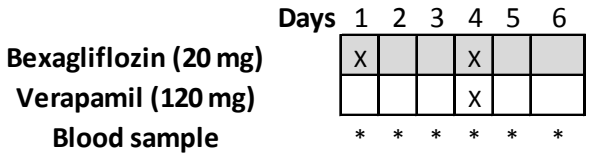

Figure 1 Study design of drug-drug interaction between bexagliflozin and probenecid (A), rifampin (B) or verapamil (C)

Clinical laboratory tests and safety monitoring will be conducted during the study as is outlined in Appendix 1 Schedule of Events.

3.2 Rationale for Study Design and Control Group

3.2.1 Rationale for Study Design

Previous studies have indicated that bexagliflozin is cleared predominantly by metabolism to an inactive metabolite, bexagliflozin 3'-*O*-glucuronide (EGT0002149), by uridine diphosphate glucuronosyltransferase isoform 1A9 (UGT1A9). Additionally, phase 1 metabolites EGT0001301, EGT0001663, and EGT0001494 have been observed after incubation with CYP3A4 (study THR-1442-N-440). *In vitro* metabolism studies have shown that bexagliflozin is neither a potent inhibitor nor inducer of CYP450 enzymes. *In vitro* studies have shown that bexagliflozin is a substrate for P-glycoprotein (P-gp) and inhibits the efflux ratio for P-glycoprotein (P-gp) mediated transport with an IC_{50} of 3.7 μM.

The uricosuric agent probenecid is commonly used to manage gout (Pui et al., 2013). and is a relatively subtype-non-selective inhibitor of UGTs that has been recommended as an interacting agent for drugs that are metabolized by glucuronidation ((FDA), 2012). Probenecid inhibition of UGTs does not show delayed kinetics(Uchaipichat et al., 2004) and following oral dosing an elimination half-life of 4 to 12 hours has been observed for the doses contemplated here (Cunningham et al., 1981).

The effects of verapamil on dabigatran have been studied by Hartter (Hartter et al., 2013), who concluded that a single dose of immediate release verapamil 120 mg, 1 hour before dosing with dabigatran produced the greatest effects. A similar study design is appropriate to the present context because the proposed mechanism of interaction is the same, and posits that verapamil may increase availability of bexagliflozin by reducing intestinal basolateral to apical transport by P-gp. Verapamil, 120 mg, was also delivered one hour before dosing with the SGLT2 inhibitor empagliflozin in a similar drug interaction study (Macha et al., 2013).

Rifampin is a strong inducer of CYP3A4 and moderate inducer of CYPs 2C19, 2B6, 2C8 and 2C9. It also potently induces several UGT enzymes (UGT1A1, UGT1A4, UGT1A9,

UGT2B4, and UGT2B7) (Chen and Raymond, 2006; Soars et al., 2003). Physiologically based pharmacokinetic modeling of rifampin effects have been conducted by Yamashita (Yamashita et al., 2013) and by Baneyx (Baneyx et al., 2014), who predicted that three and five days of dosing of rifampin, 600 mg, qd, respectively, would suffice for maximal or appropriate induction, respectively. A four day dosing period has been chosen for this investigation.

3.2.2 Rationale for Dose Selection

Bexagliflozin produces a dose-related, saturable increase in UGE in healthy volunteers and diabetic subjects. Population pharmacodynamic modeling has indicated that the UGE is well fit by a simple logistic model. The ED_{50} values for healthy and diabetic adults are 3.6 mg and 1.2 mg, respectively and the maximum dose intended to be submitted for approval is 20 mg. FDA guidance recommends that the interaction target be dosed at the maximum proposed dosage ((FDA), 2012).

Conversely, FDA guidance also recommends that the shortest dosing interval of the interacting agent be used. The dosage of 500 mg probenecid orally twice a day is the recommended adult maintenance dose for gout and after four dose the daily pharmacokinetics are expected to be close to asymptotic Physiologically based pharmacokinetic modeling studies of rifampin induction kinetics have been predicated on once daily administration of 600 mg rifampin. As discussed above three and five days have been predicted or recommended and the present treatment has adopted four days as the duration for induction. Studies on the effect of verapamil on drug absorption have concluded that a single 120 mg dose delivered in advance of dosing produces the greatest effect (Hartter et al., 2013), and that course has been adopted here.

3.2.3 Rationale for PK and PD Sampling Time Points

The PK samples for combination treatment will be taken hourly up to hour 6, semi-hourly to hour 12, and at hours 16, 24, 36 and 48 post-dose. The times of collection are anticipated to allow accurate estimates of any change in T_{max} and C_{max} and provide sufficient data points for measurement of the elimination half-life.

Urine samples for PD will be collected at pre-dose (h -12 to 0), and at 0 to 12 h, 12 to 24 h, 24 to 36 h, and 36 to 48 h after administration of bexagliflozin.

3.3 Study Duration and Dates

A total of 48 healthy subjects will be enrolled in three groups of sixteen. Individuals with histories incompatible with enrollment in some groups may be assigned to alternate groups. Each group will participate in one of three fixed-sequence studies:

Study 1: Bexagliflozin/probenecid (n=16)
Study 2: Bexagliflozin/rifampin (n=16)
Study 3: Bexagliflozin/verapamil (n=16)

Subjects in Study 1 (probenecid) will check into the clinic on the day before dosing, and void their bladders at 12 h prior to anticipated first dose. They will remain domiciled in CRU until the final plasma sample is collected at 48 h post-dose on the morning of study Day 7.

Subjects in Study 2 (rifampin) will check into the clinic on the day before dosing and void their bladders at 12 h prior to the anticipated first dose. They will remain domiciled in CRU until the final plasma sample is collected at 48 h post-dose on the morning of study Day 8.

Subjects in Study 3 (verapamil) will check into the clinic on the day before dosing and void their bladders at 12 h prior to the anticipated first dose. They will remain domiciled in CRU until the final plasma sample is collected at 48 h post-dose on the morning of study Day 6.

Screening will be taken place within 21 days before the first intake of study drug. The duration of the overall study from screening until study termination is estimated to be a maximum of 30 days. For details of the schedule and nature of the investigations, see the Schedule of Events in Appendix 1.

4 STUDY POPULATION SELECTION

4.1 Study Population

Forty-eight eligible healthy male and female subjects who consent to participate in this study will be enrolled.

4.2 Inclusion Criteria

To be enrolled in this study a subject must:

1. be between 18 and 55 years of age at screening, inclusive, and in good health based on medical history, physical examination (PE), electrocardiogram (ECG) and routine laboratory tests.
2. have a body-mass index (BMI) between 18.0 kg/m^2 and 32.0 kg/m^2 at screening, inclusive.
3. have abstained from tobacco consumption for at least 3 months prior to screening.
4. have adequate venous access at multiple sites in both arms.
5. be willing and able to be confined to the clinical research facility as required by the protocol.
6. be able to comprehend the explanation of the informed consent and be willing to provide written informed consent in accordance with institutional and regulatory guidelines.

4.3 Exclusion Criteria

Subjects who exhibit any of the following characteristics will be excluded from the study.

1. A clinically significant history of allergy to drugs or latex (at the investigator's discretion.)
2. A history of alcohol or drug dependence in the last 12 months.
3. A history of donation of 400 mL of whole blood within two months, 200 mL of whole blood within one month, or blood components within 14 days prior to the first dose.
4. A history of prescription or over-the-counter (OTC) drug use within 14 days prior to the first dose.
5. A history of vitamin preparation or supplement use (including St. John's Wort and ginseng) within 14 days prior to the first dose.
6. A history of strenuous physical activity within 72 hours prior to dosing.
7. A history of exposure to an investigational drug within 30 days or 7 half-lives of the investigational drug, whichever is longer, prior to the first dose of investigational drug in this trial.
8. A history of prior exposure to EGT0001474 or bexagliflozin at any time, or of exposure to any other SGLT2 inhibitors within 3 months from screening or of participation in previous bexagliflozin clinical trials.

9. A history of consumption of probenecid, rifampin or verapamil within 3 months of screening.
10. A screening ECG that demonstrates any one of the following: heart rate > 100 bpm, QRS > 120 msec, QTc > 470 msec (corrected by Bazett's formula), PR > 220 msec (a subject with PR > 220 msec will generally be excluded but exceptions may be allowed at the discretion of the investigator), or any clinically significant arrhythmia.
11. A sitting blood pressure that is above 140/90 mmHg at screening. If the sitting blood pressure at screening is above 140/90 mmHg, one repeat measurement can be taken. Subjects will be excluded if the repeated sitting blood pressure is above 140/90 mmHg but exceptions may be allowed at the discretion of the investigator.
12. A positive result for hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV) antibody, or for urinary drug or cotinine tests.
13. A history of human immunodeficiency virus (HIV) infection.
14. A history of febrile illness within 5 days prior to the first dose of investigational drug.
15. A history of vaccination (with the exception of the flu vaccine) within 30 days prior to the first dose of investigational drug.
16. An estimated glomerular filtration rate (eGFR) < 80 mL/min/1.73 m^2 or a history of kidney transplant.
17. If male who are not surgically sterile, unwilling to refrain from donating sperm and unwilling to use appropriate birth control when engaging in sexual intercourse for a period of 30 days after discharge from the clinic. Appropriate birth control methods include condoms with spermicide, female partner's use of diaphragm with spermicide, female partner's use of stable oral, implanted, or injected contraceptive hormones, or of an intrauterine device.
18. If female of childbearing potential, unwilling to use an adequate method of contraception and/or to avoid pregnancy for the duration of the study. Hormonal contraceptive methods are inadequate for subjects enrolled in the rifampin study. Appropriate methods of contraception for female subjects of childbearing potential in the rifampin study include bilateral tubal ligation, intrauterine device, diaphragm with spermicide and male partner's use of male condom with spermicide. Female subjects who are surgically sterile (i.e. have undergone partial or full hysterectomy, or bilateral oophorectomy) or postmenopausal (absence of menses greater than 12 months and age > 45 years) are eligible if they test negative on a urine pregnancy test.
19. Evidence of anemia if selected for probenecid study.
20. Evidence of abnormal liver function tests (total bilirubin > 1.5 x upper limit of normal (ULN)); or alanine aminotransferase (ALT) or aspartate aminotransferase (AST) > 2.5 x ULN.
21. If selected for the rifampin study, unwilling to refrain from the use of soft contact lenses during the study.
22. Unwilling to forgo consumption of alcohol 72 hours pre admission and throughout the study.
23. Unwilling to forgo consumption of grapefruit and grapefruit products from 7 days prior to dosing through discharge from the clinic.

24. A history of recurrent yeast or urinary tract infections or any such infections in the 6 months prior to first dose.
25. A history of gout, glucose-6-phosphate dehydrogenase deficiency or nephrolithiasis if a candidate for the probenecid study.

5 STUDY PROCEDURES

5.1 Description of Investigational Products

5.1.1 Bexagliflozin

Bexagliflozin tablets, 20 mg, are blue caplet-shaped, film-coated tablets that are intended for use in investigational studies in humans. The tablets contain excipients designed to promote extended release through a gastroretentive mechanism. The active tablets exhibit a greater than 75% release of drug substance by 8 hours in simulated gastric fluid *in vitro*.

5.1.2 Probenecid, Rifampin and Verapamil

Probenecid tablets, 500 mg, rifampin capsules, 300 mg and verapamil hydrochloride tablets, 120 mg, will be procured by Covance pharmacy.

Probenecid is a competitive inhibitor of organic acid transport in the kidney and other organs, primarily used as a uricosuric agent for the treatment of chronic gout. It has also been used to enhance plasma levels of β-lactam antibiotics. Absorption of probenecid is essentially complete following oral administration. The drug is extensively metabolized by glucuronide conjugation and by oxidation of the alkyl side chains. The half-life of probenecid in plasma is dose-dependent and falls in the range of 4 to 12 hours for the 500 mg dose planned here (Cunningham et al., 1981).

Rifampin is an inhibitor of bacterial RNA polymerase typically used to treat mycobacterial infections. Common side effects include nausea, vomiting, diarrhea, and loss of appetite. Rifampin is a red substance and may cause the appearance of a red or orange color in urine, sweat, and tears. Soft (hydrogel) contact lenses may become discolored as a result. Liver enzyme elevation or allergic reactions have been observed.

Oral delivery of rifampin results in peak plasma concentrations in about two to four hours. Only about 7% of the administered drug is excreted unchanged in urine, though urinary elimination accounts for only about 30% of the drug excretion. About 60% to 65% is excreted through feces. The half-life of rifampin ranges from 1.5 to 5.0 hours, though hepatic impairment significantly increases it. Food consumption inhibits its absorption from the GI tract, and the drug is more quickly eliminated. When rifampin is taken with a meal, its peak blood concentration falls by 36%.

Verapamil is an inhibitor of cardiac L-type calcium channels used predominantly for the management of hypertension and arrhythmias. Common side effects include headache, hypotension, nausea, and constipation. Other side effects include allergic reactions and arthralgia.

More than 90% of verapamil is absorbed following oral administration, but due to high first-pass metabolism, the absolute bioavailability is 10–35%. T_{max} is 1 to 2 hours post-dose. It undergoes extensive hepatic metabolism.

Verapamil hydrochloride tablets, 120 mg (Mylan) will be provided for this study.

5.2 Substances Administered

Subjects who consent to participation will be assigned to 1 of 3 groups. Each subject in Study 1 will receive a single bexagliflozin tablet, 20 mg, alone, 4-days dosing of probenecid capsules, 500 mg (bid), including the combination of both (bexagliflozin tablet and probenecid) on Day 5.

Every subject in Study 2 will receive a single bexagliflozin tablet, 20 mg, alone, 5-days dosing of 600 mg of rifampin (2 x 300mg capsules) qd including the combination of both (bexagliflozin tablet and rifampin capsules) on Day 6.

Every subject in Study 3 will receive a single bexagliflozin tablet, 20 mg, alone, and a combination of a single bexagliflozin tablet, 20 mg and one verapamil tablet, 120 mg on Day 4.

5.3 Selection and Timing of Dose for Each Subject

Dosing order with bexagliflozin alone, or bexagliflozin in combination with probenecid, rifampin or verapamil will be based on a fixed-sequence schedule.

For subjects in Study 1, on Day 1 each subject will receive a single dose of bexagliflozin tablets, 20 mg (~30 minutes after breakfast, standard low-fat meal) with approximately 240 mL water. PK samples will be collected from Days 1 to 3 at pre-specified time points. On Days 3 to 4 subjects will take probenecid tablets, 500 mg, bid, about 30 min after standard meal with approximately 240 mL water. On Day 5, subjects will take bexagliflozin tablets, 20 mg and probenecid tablets, 500 mg, ~30 min after breakfast with approximately 240 mL water. And 10-12 h later, subjects will receive probenecid tablets, 500 mg, ~30 min after night meal with approximately 240 mL water. On Day 6 subjects will take probenecid tablets, 500 mg, bid, with approximately 240 mL water. Subjects will be discharged after the 48-h PK sample collection on day 7.

For subjects in Study 2, on Day 1, subjects will receive a single dose of bexagliflozin tablets, 20 mg, after an overnight fast of at least 10 h with approximately 240 mL water. PK samples will be collected from Days 1 to 3 at pre-specified time points. On Days 3 to 5, subjects will take daily dose of two 300-mg rifampin capsules alone after overnight fast with approximately 240 mL water. On Day 6 subjects will take bexagliflozin tablets, 20 mg and two 300-mg rifampin capsules after overnight fast with approximately 240 mL water. On Day 7, subjects will take a single dose of two 300-mg rifampin capsules alone after overnight fast with approximately 240 mL water. Subjects will be discharged after the 48-h PK sample collection on Day 8.

For subjects in study 3, on Day 1 each subject will receive a single dose of bexagliflozin tablets, 20 mg (~30 minutes after breakfast, standard low-fat meal) with approximately 240 mL water. PK samples will be collected from Days 1 to 3 at pre-specified time points. On Day 4, subjects will take one verapamil tablet, 120 mg one hour before taking one

bexagliflozin tablet, 20 mg, about 30 min after breakfast with approximately 240 mL water. Subjects will be discharged after the 48-h PK sample collection on Day 6.

5.4 Method of Assigning Subjects to Treatment Groups and Sequences

A total of 48 healthy subjects will be enrolled and assigned to one of three groups of sixteen. Each group of sixteen will receive a fixed-sequence treatment. Subjects who discontinue participation due to safety reasons will not be replaced.

5.5 Blinding

This is an open-label study.

5.6 Concomitant Therapy

Participants will be prohibited from taking any prescription or non-prescription drugs, vitamins or dietary supplements at any time during the 14 days prior to first drug administration and for the duration of the study. Medications may be prescribed for management of an AE. Subjects may receive any medications for AEs that are necessary to treat the event or minimize the likelihood of more serious adverse events in the investigators' judgment.

Concomitant medications administered during the study are to be recorded on the case report form (CRF). The medication name, dose, frequency, route of administration, date(s) of administration and reason for administration shall be recorded. This documentation shall continue until the end of the study.

5.7 Restrictions

5.7.1 Prior Therapy

No study subject shall have been dosed with any SGLT2 inhibitor within 3 months prior to screening, nor with an investigational drug within 30 days or 7 half-lives, nor consumed any medication, vitamin or herbal supplement within 14 days prior to the first dose of study medication.

5.7.2 Fluid and Food Intake

For subjects in Study 1, bexagliflozin and probenecid will be administered ~30 minutes after standard meal with approximately 240 mL water. Water can be allowed as desired except for one hour before and after drug administration.

For subjects in Study 2, bexagliflozin and rifampin will be administered to subjects after an overnight fast of at least 10 h with approximately 240 mL water. Water can be allowed as desired except for one hour before and after drug administration.

For subjects in Study 3, bexagliflozin and verapamil will be administered ~30 minutes after standard meal with approximately 240 mL water. Water can be allowed as desired except for one hour before and after drug administration.

5.7.3 Subject Activity Restrictions

Subjects should not perform strenuous or repetitive activities that could result in elevations of muscle creatine kinase levels. Smoking during the study is prohibited. Alcohol consumption should be avoided for 72 hours prior to each clinic admission check-in.

5.8 Treatment Compliance

To ensure compliance, all medication dosing will be supervised by the Investigator or qualified staff in the clinic. The exact times of dosing will be recorded in the CRFs, including a record of hand and mouth inspections.

5.9 Packaging and Labeling

5.9.1 Bexagliflozin

Bexagliflozin tablets, 20 mg, are packaged in high density polyethylene bottles sealed with a child resistant closure. The product is packaged in 90 count bottles.

Investigational product bottles will be labeled with protocol number, drug name and strength, lot number, sponsor's name, storage condition, and the investigational drug caution statement.

5.9.2 Probenecid, Rifampin and Verapamil

Probenecid tablets USP 500 mg (Watson) are bisected, capsule-shaped, yellow, film-coated tablets imprinted DAN DAN and 5347 supplied in bottles of 100. Probenecid Tablets USP 500 mg contain the following inactive ingredients: colloidal silicon dioxide, D&C Yellow No. 10, FD&C Blue No. 1, FD&C Yellow No. 6, hypromellose, magnesium stearate, microcrystalline cellulose, polyethylene glycol, polysorbate 80, sodium lauryl sulfate, sodium starch glycolate and titanium dioxide.

Rifampin capsules 300 mg (Lannett) are maroon/red capsules imprinted with LANNETT on the cap and 1315 on the body. The capsules are supplied in bottles of 30. The inactive ingredients include pregelatinized starch, colloidal silicon dioxide, talc, magnesium stearate, D&C Red # 28, FD&C Blue # 1, FD&C Red # 40, gelatin, and titanium dioxide.

Verapamil hydrochloride tablets, 120 mg (Mylan), are white, film-coated, round tablets debossed with MYLAN above the score and 772 below the score on one side of the tablet and blank on the other side. The tablets are supplied in bottles of 100. The inactive ingredients include calcium sulfate, colloidal silicon dioxide, croscarmellose sodium, hydroxypropyl cellulose, hypromellose, magnesium stearate, microcrystalline cellulose,

polyethylene glycol, pregelatinized starch (corn), sodium lauryl sulfate, talc and titanium dioxide.

5.10 Storage and Reconciliation

Bexagliflozin will be stored below 30 °C (86 °F) in a secure area with access limited to authorized personnel. Verapamil tablets, probenecid tablets and rifampin capsules will be stored at controlled room temperature 20-25 °C (between 68 and 77 °F), protected from moisture and heat. The pharmacist shall maintain an inventory of bexagliflozin product and the number of tablets dispensed per study subject. A reconciliation of drug inventory will be performed at the end of the study and the results of this inventory shall be recorded in the Drug Consumption Form. Empty and partially used bottles may be discarded after use according to the study site's regulations for the disposal of investigational drug substances at study close out, after the Drug Accountability Form is completed.

6 STUDY PROCEDURES

6.1 Informed Consent

Before each subject is enrolled, written informed consent will be obtained according to the applicable regulatory and legal requirements. As part of this procedure, the investigator or designee shall explain orally and in writing the nature, duration, and purpose of the study, and the action of the drug in such a manner that the study subject is aware of the potential risks, inconveniences, or AEs that may occur. The investigator shall educate potential subjects about the scientific importance of their data and the vital role that their participation has for the outcome of the entire study. The subject shall be informed that he/she is free to withdraw from the study at any time.

The informed consent document shall be signed and dated. One copy will be given to the subject, and the investigator will retain a copy as part of the clinical study records. The investigator will not undertake any investigation specifically required for the clinical study until written consent has been obtained. The terms of the consent and the time it was obtained shall also be documented.

6.2 Medical History

The following information will be collected at the screening visit:

- Demographic information including age, sex, and race.
- Significant medical and surgical history and with documentation of the dates relative to the time of study screening, if applicable.
- History of clinically significant allergies including to drugs and latex.
- History of smoking in the 6 months prior to first dose, of alcohol or drug dependence, or abuse of substances in the 12 months prior to first dose.
- History of blood donation within 2 months or blood component donation in the 14 days prior to first dose.
- Medication history including OTC drugs, dietary supplements and vitamins to span the 14 days prior to first dose.
- History of vaccination within 30 days prior to first dose.
- History of diagnosis of HIV, hepatitis B or hepatitis C infection.
- History of exposure to any investigational drug in the previous 30 days or 7 half-lives prior to first dose, whichever is longer.
- History of exposure to bexagliflozin (or EGT0001474), or any other SGLT2 inhibitors in the last 3 months prior to first dose.
- History of exposure to probenecid, rifampin or verapamil in the 3 months prior to first dose.
- History of recurrent yeast or urinary tract infections or any such infections in the 6 months prior to first dose.

6.3 Physical Examination

The investigator or designated qualified individual will perform the PEs. A complete PE will be performed at screening and on the last day of clinic prior to discharge. Partial PEs will be performed on scheduled days as described in Appendix 1.

A complete PE will include measurement of body weight and height (height will be measured only at screening), general assessment of all body systems including the skin, head, eyes, ears, nose, throat, neck, lungs, heart, abdomen, lymph nodes, and extremities. A partial PE will include body weight and an update of the general assessment of the skin, heart, lungs and abdomen.

6.4 Vital Signs

Vitals signs, including pulse, systolic and diastolic blood pressure (BP), respiration rate, and oral cavity temperature, will be measured on scheduled visits as described in Appendix 1.

Vital signs shall be measured prior to blood draws and after a subject has been sitting for 5 minutes.

Pulse, systolic and diastolic BP should be measured in a seated position after a subject has been sitting for 5 minutes.

Respiration rate should be measured after at least 5 minutes of rest.

BP measurements will be obtained using a calibrated manual or oscillometric sphygmomanometer.

6.5 Electrocardiography

A 12-lead electrocardiography (ECG) will be conducted as listed in Appendix 1 and whenever clinically indicated.

This procedure shall be performed in the supine position after at least 5 minutes of rest. ECG parameters measured will be the RR interval, PR interval, QRS duration, and QT. Each ECG shall also be assessed by the investigator for signs of ischemia, clinically significant hypertrophy, and clinically significant T-wave abnormalities.

It is the investigator or designee's responsibility to review the results of the ECG as they become available. For each abnormal ECG result, the investigator shall ascertain if the abnormality represents a clinically significant change from the screening ECG for that individual subject. This determination, however, need not be made the first time an abnormal result is observed. The investigator may repeat the ECG to verify the results of the original result. If the ECG result is determined to be a clinically significant and abnormal change from baseline for that subject, the finding shall be considered an AE.

6.6 Clinical Laboratory Tests

6.6.1 Laboratory Parameters

Subjects will be in a seated, semi-recumbent or supine position during blood collection. Clinical blood chemistry and hematology tests will be performed at the scheduled visits (Appendix 1). Blood samples shall be drawn after overnight fasting (at least 8 h). The details of the required laboratory tests are listed in Table 1.

Table 1. Required Laboratory Tests

Test Name		mL (sample)
Hematology		4.0 (blood)
Hematocrit	Mean corpuscular volume	
Hemoglobin	Platelet count	
Mean corpuscular hemoglobin	Red blood cell count	
Mean corpuscular hemoglobin concentration	White blood cell count with differential	
Serum Chemistry, Electrolytes and Lipids		8.5 (blood)
Albumin	Calcium	
Alanine aminotransferase (ALT)	Magnesium	
Aspartate aminotransferase (AST)	Phosphorus	
Blood urea nitrogen (BUN)	Potassium	
Glucose	Sodium	
Bicarbonate	Total bilirubin	
Creatinine	Direct bilirubin	
Chloride	Uric acid	
Total protein	Total cholesterol	
Low-density lipoprotein cholesterol, calculated	High-density lipoprotein cholesterol	
Triglycerides		
Urinalysis		20 (urine)
Appearance	Nitrite	
Bilirubin	pH	
Color	Protein	
Glucose	Specific gravity	
Ketones	Urobilinogen	
Microscopic examination of sediment	Leukocyte esterase	
Urine Collection		
Glucose Creatinine		All (urine)
Urine Drug Screen		10 (urine)
Amphetamines	Opiates	
Barbiturates	Benzodiazepines	
Cocaine Metabolites	Cannabinoids	

Table 1. Required Laboratory Tests

Test Name		mL (sample)
Cotinine		
Urine Pregnancy Test (Female only)		5 (urine)
Infectious Disease Testing		3.5 (blood)
Hepatitis B surface antigen (HBsAg)	Hepatitis C virus (HCV)	

6.6.2 Sample Collection, Storage, and Shipping

6.6.2.1 Hematology and Blood Chemistry

Blood samples for hematology and chemistry will be collected. Timing of the collection is described in Appendix 1.

6.6.2.2 Urinalysis

Clean-catch, midstream urine samples will be collected according to the schedule outlined in Section 7 and in Appendix 1. Dipstick urinalysis will be conducted. Microscopic examination will be performed if a subject has a positive result on any of the dipstick tests that require microscopic follow-up to clarify their significance. If the urine is dipstick positive for leukocyte esterase or nitrites, a sample will be sent for microscopic evaluation and culture. In addition, urinalysis will be performed from a clean-catch urine sample at any time in subjects with symptoms of UTI or pyelonephritis.

6.6.2.3 Urine Collection for PD

Pre-dose urine samples shall be collected from -12 to 0 h for baseline measurement. Subjects shall empty their bladders 12 h prior to dosing with bexagliflozin and accumulate their urine output into separate containers at 12 h intervals beginning at the time of dosing and ending 48 h after dosing. Urine must be refrigerated at 2-8 °C during collection. A 20 mL aliquot of urine will be prepared for analysis from well mixed urine collections. The samples will be analyzed for urinary glucose and creatinine.

6.6.2.4 Plasma Sample Collection for PK

Whole venous blood samples of 3 mL will be collected from a peripheral vein at pre-dose and at 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 36, and 48 h after administration of bexagliflozin . Blood samples will be collected in tubes containing K_2EDTA and stored on ice until processing by centrifugation under refrigeration for at least 10 min at 3,000 rpm. After centrifugation, plasma will be removed, divided into 2 aliquots of approximately 0.5 mL, frozen and stored at or below -20 °C. Plasma shall be processed and frozen within 2 h of blood collection. Frozen plasma samples will be transferred on dry ice to the analytical laboratory and will be stored at or below -20 °C until analysis.

6.7 Adverse Events Assessments

Adverse Event (AE*):* Any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product. An AE does not necessarily have to have a causal relationship with this treatment.

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product, whether or not it is considered related to exposure to the investigational product.

Serious Adverse Event (SAE): A serious adverse event (experience) or reaction is any untoward medical occurrence that at any dose:

- results in death,
- is life-threatening *(NOTE:* The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event and does not refer to an event which hypothetically might have caused death if it were more severe.),
- requires inpatient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity,
- is a congenital anomaly/birth defect,
- is an important medical event.

An important medical event is an event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

Non-serious adverse events are all events that do not meet the criteria for a "serious" adverse event.

Immediately Reportable Adverse Event (IRAE): Any serious adverse event or any adverse event that necessitates discontinuation of investigational product.

Adverse Clinical Laboratory Change: An abnormal laboratory test result can be an adverse event if it is a clinically significant and deleterious change from baseline (at predose on Day 1) for that individual subject. This determination, however, does not necessarily need to be made the first time an abnormal value is observed. The investigator may repeat the laboratory test or request additional tests to verify the results of the original laboratory tests. If this laboratory value is determined to be a clinically significant deleterious change from baseline for that subject, this is considered an AE.

Hypoglycemia will be defined as any fasting plasma glucose (FPG) value < 70 mg/dL and documented as described in Section 6.7.4.4.

Any increase in liver function tests (aspartate aminotransferase (AST), alanine aminotransferase (ALT), or bilirubin) greater than 3 times the upper limit of normal (ULN) for the testing laboratory will be considered a clinical laboratory adverse event.

An increase in creatinine from baseline by 0.5 mg/dL or more will be reported as a laboratory adverse event.

Severity: Adverse events will be graded on a 3-point scale and reported as indicated on the CRF. The intensity of an adverse experience is defined as follows:

1 = Mild: discomfort noticed, but no disruption to daily activity

2 = Moderate: discomfort sufficient to reduce or affect normal daily activity

3 = Severe: inability to work or perform normal daily activity

Investigational Product Causality: A determination of potential dependence on study compound administration shall be performed. Relationship of an adverse event to dosing will be assessed as follows:

Definite: There is a reasonable causal relationship between the investigational product and the AE when the event responds to withdrawal of the investigational product (dechallenge), and recurs with administration of the investigational product (rechallenge).

Probable: There is a reasonable causal relationship between the investigational product and the AE. The event responds to dechallenge. Rechallenge is not required.

Possible: There is a reasonable causal relationship between the investigational product and the AE. Dechallenge is lacking or unclear.

Not Likely: There is a temporal relationship to investigational product administration, but there is not a reasonable causal relationship between the investigational product and the event.

Unrelated: There is no temporal or causal relationship to investigational product administration.

6.7.1 Collecting and Reporting Adverse Events

Adverse event recording will begin from the time of signing the informed consent form. The investigator will periodically assess subjects for the occurrence of adverse events. To avoid bias in collecting information about adverse events, the investigator should ask subjects the following question: "How have you felt since you were last checked?" All adverse events (serious and non-serious) reported by the subject shall be recorded on the source documents and CRFs provided by the sponsor.

In addition, Theracos' Medical Monitor or its designated personnel shall be notified immediately by telephone or fax of any immediately reportable adverse events according to the procedure outlined below. Special attention shall be paid to recording hospitalizations and concomitant medications.

6.7.2 Immediately Reportable Adverse Events

The investigator shall report any SAE, by telephone, email, or fax, to Theracos or its representative immediately after the investigator becomes aware of the event. An IRAE form shall be completed and sent by email or fax or overnight courier to the sponsor within 24 h of knowledge of the event.

Non-serious events that require discontinuation of investigational product (including laboratory abnormalities) shall be reported to Theracos within 3 working days. The IRAE form shall be completed and sent by email or fax or overnight courier to the sponsor.

Subjects experiencing an SAE shall be followed clinically until their health has returned to baseline status or until all parameters have returned to normal, or have otherwise been explained. It is expected that the investigator will provide or arrange appropriate supportive care for the subject.

6.7.3 Follow-up of Adverse Events

6.7.3.1 Follow-up of Non-serious Adverse Events

Non-serious adverse events that are identified on the last scheduled contact shall be recorded on the AE CRF with the current status noted. All non-serious events that are ongoing at the time shall be recorded as ongoing on the CRF.

6.7.3.2 Follow-up of Post-Study Serious Adverse Events

Any new SAEs reported by the subject to the investigator that occur after discharge, and are determined by the investigator to be reasonably associated with the use of the investigational product, shall be reported to the Medical Monitor or the sponsor's designated personnel. These may include SAEs that are captured on follow-up telephone contact or at any other time point after discharge. The investigator shall follow subjects with SAEs identified after discharge until the events are resolved, or the subject is lost to follow-up. The investigator shall continue to report any significant follow-up information to the Sponsor until the event has been resolved.

6.7.3.3 Hepatotoxicity

Any clinically significant increase in hepatic enzymes and specifically ALT or AST $\geq$ 3x ULN requires immediate repeat test within 48 to 72 h to confirm the hepatic enzyme elevation. Study medication shall be stopped and the event shall be reported as an adverse event within the CRF if the enzyme elevation is confirmed or worsening. Potential contributors to hepatic enzyme elevation shall be evaluated by the investigator. The

investigator is encouraged to consult with the Medical Monitor regarding ongoing diagnostic workup.

Investigational product shall be permanently discontinued if any of the following criteria is met:

- ALT or AST > 8 x ULN,
- ALT or AST > 3 x ULN and (total bilirubin > 2 x ULN or INR > 1.5),
- ALT or AST > 3 x ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (> 5%).

6.7.3.4 Hypoglycemia

Hypoglycemia will be recorded under 5 categories:

- Critical hypoglycemia: An event requiring assistance of another person to actively administer carbohydrates, glucagon, or other resuscitative actions. These episodes may be associated with sufficient neuroglycopenia to induce seizure or coma. Plasma glucose measurements may not be available during such an event, but neurological recovery attributable to the restoration of plasma glucose to normal is considered sufficient evidence that the event was induced by a low plasma glucose concentration. All such events shall be recorded as serious adverse events in the CRF.
- Documented symptomatic hypoglycemia: An event during which typical symptoms of hypoglycemia are accompanied by a measured plasma glucose concentration ≤ 70 mg/dL (3.9 mmol/L).
- Asymptomatic hypoglycemia: An event not accompanied by typical symptoms of hypoglycemia but with a measured plasma glucose concentration ≤ 70 mg/dL (3.9 mmol/L).
- Probable symptomatic hypoglycemia: An event during which symptoms of hypoglycemia are not accompanied by a plasma glucose determination but that was presumably caused by a plasma glucose concentration ≤ 70 mg/dL (3.9 mmol/L).
- Relative hypoglycemia: An event during which the person reports any of the typical symptoms of hypoglycemia, and interprets those as indicative of hypoglycemia, but with a measured plasma glucose concentration > 70 mg/dL (3.9 mmol/L).

If a subject experiences symptomatic hypoglycemia, confirmed by point-of-care glucose monitoring at the time of symptoms with a blood sugar level < 70 mg/dL (3.9 mmol/L), the subject should be treated with 15 to 20 grams of oral glucose or simple carbohydrate (glucose tablets, raisins, orange juice, glucose-containing soda, or soft sugar candies).

If a subject with symptomatic hypoglycemia is unable to self-administer glucose tablets, candies, or glucose-containing solutions, then the appropriate study staff shall administer

50% IV Dextrose as soon as possible. If IV Dextrose is unable to be administered, an injection of 1.0 mg of glucagon shall be administered intramuscularly or subcutaneously, and the injection may need to be repeated after 15 minutes depending on the response.

Blood glucose monitoring shall be done by appropriate study staff using a point-of-care glucose monitor beginning at the time of hypoglycemia detection and continue every fifteen minutes, or more frequently if required, until that time that the subject's level of alertness has returned to appropriate levels and point-of-care glucose levels are above 70 mg/dL.

6.8 Concomitant Medication Assessments

A concomitant medication is any medication the subject takes during the course of the trial. All prescription and non-prescription medications, vitamins and herbal supplements that subjects receive during the trial shall be documented on the CRF. This documentation shall continue until the subjects are discharged.

Concomitant medications shall be coded using the World Health Organization Drug Dictionary (WHO-DD). A table of concomitant medications based on the anatomic therapeutic chemical classification and preferred name shall be produced. A listing of concomitant medications shall include all medications taken by any subjects during the course of the study.

Concomitant medications administered during the study shall be recorded on the CRF. The medication name, dose, frequency, route of administration, date(s) of administration and reason for administration shall be recorded. This documentation shall continue until discharge from the study.

6.9 Removal of Subjects from the Trial or Discontinuation of Investigational Product

The investigator shall emphasize to potential subjects the importance of continued participation for the full duration of the trial during the informed consent process. Participation in the study is voluntary. A participant has the right to withdraw from the study at any moment for any reason. The investigator shall be informed immediately.

The investigator has the right to terminate participation of a subject in case it is difficult to obtain blood samples, in case of violation of the protocol or in case of severe or serious adverse events.

In case a subject withdraws from the study, the study monitor shall be informed immediately. If there is a medical reason for withdrawal, the volunteer shall remain under the supervision of the medical investigator until satisfactory health returns or the condition has evolved to the point that further meaningful change is unlikely.

Subjects who discontinue the active dosing phase of the study due to adverse event(s) or other safety concerns shall not be replaced.

If the decision is made to discontinue a subject's participation in the study, no further investigational product medication shall be administered. Every attempt shall be made to complete all required study evaluations and procedures. Reasons for all withdrawals shall be recorded on the CRF.

The investigator may withdraw a subject from the THR-1442-C-454 trial for any of the following reasons:

- A protocol violation occurs, or
- A serious or intolerable adverse event occurs, or
- A clinically significant change in a laboratory parameter occurs, or
- The sponsor or investigator terminates the study, or
- The subject requests to be discontinued from the study.

Subjects who do not complete the study but who have received investigational product should have a follow-up examination, including a complete physical examination, vital signs, and clinical laboratory tests if clinically indicated and feasible according to Section 7.

6.10 Appropriateness of Measurements

PK and safety parameters in this protocol are standard assessments and are widely used and generally recognized as reliable, accurate, and relevant measurements.

Determination of urinary glucose is a non-invasive and quantitative method that allows immediate assessment of the PD effects of an SGLT2 inhibitor.

7 STUDY ACTIVITIES

7.1 Study Activities for Study 1

7.1.1 Screening (Day -21 to Day -1)

During the screening period, the following information will be gathered and the indicated procedures will be performed:

- Content of informed consent will be explained to the subject and the signed informed consent collected.
- Medical history and demographic information will be obtained as described in Section 6.2.
- Physical examination will be conducted, including height and weight measurements as described in Section 6.3.
- Vital signs will be measured, including pulse, temperature, respiratory rate, and blood pressure taken in the sitting position after at least 5 min of rest as described in Section 6.4.
- 12-lead ECG will be taken in the supine position after at least 5 min of rest as described in Section 6.5.
- Clean-catch, mid-stream urine will be collected for urinalysis as described in Section 6.6.2.2. If urine is dipstick positive for leukocyte esterase or nitrites, sample is to be sent for microscopic evaluation and culture.
- Urine screen will be conducted for drug abuse and cotinine. If the drug screen is conducted more than 5 days pre-dose, the drug screen shall be repeated prior to dosing. Drug abuse testing includes amphetamines, barbiturates, cocaine metabolites, opiates, benzodiazepines, cotinine, and cannabinoids.
- All female subjects will receive urine pregnancy test.
- Blood will be drawn for hematology, serum chemistry, and serology as detailed in Section 6.6.2.1.
- Inclusion/exclusion criteria will be evaluated based on the information collected at the screening examination.

7.1.2 **Day 0 (Clinic Admission)**

The following information will be gathered and the indicated procedures will be performed:

- Partial PE will be performed as described in Section 6.3.
- If screening was conducted more than 5 days prior to dosing, the inclusion and exclusion criteria shall be confirmed and a urine drug screen performed.
- All female subjects will receive urine pregnancy test.

- Subjects will void their bladders at 12 h prior to dosing and begin the collection of the -12 h to 0 h batch for baseline analysis of UGE and creatinine (PD) as described in Section 6.6.2.3.
- Concomitant medications and adverse event information will be collected as appropriate.
- If the subject is still eligible based on the study inclusion and exclusion criteria, the subject will be admitted into the study.

7.1.3 Day 1 (Dosing Day): Pre-dose

The following information will be gathered and the indicated procedures will be performed pre-dose:

- Vital signs will be recorded pre-dose.
- Pre-dose urine will be collected until 0 h (-12 h to 0 h batch) for baseline UGE and creatinine (PD) analysis as described in Section 6.6.2.3.
- Urine sample will be collected for pre-dose urinalysis as described in Section 6.6.2.2. If urine is dipstick positive for leukocyte esterase or nitrites, sample is to be sent for microscopic evaluation and culture. If screening is conducted more than 5 days prior to dosing, drug screening shall be repeated.
- Pre-dose blood will be drawn for hematology, and serum chemistry as detailed in Section 6.6.2.1.
- Pre-dose plasma samples will be drawn for PK analysis as detailed in Section 6.6.2.4.
- Pre-dose concomitant medications and adverse event information will be collected as appropriate.

7.1.4 Day 1 (Dosing Day): Dosing

- Bexagliflozin tablets, 20 mg, will be administered ~30 minutes after breakfast (standard low-fat meal), as detailed in Section 5.3.

7.1.5 Day 1: Post-dose

- Vital signs will be recorded at 4 h post-oral dose
- Urine samples for PD (UGE and creatinine) analysis will be at specified time interval after oral administration of bexagliflozin, as described in Section 6.6.2.3.
- Blood samples for PK samples will be collected at 1, 2, 3, 4, 5, 6, 8, 10, 12, 16 h after oral administration of bexagliflozin.
- Post-dose concomitant medications and adverse event information will be collected as appropriate.

For subjects who are terminated from the study for any reason after dosing, all activities for the clinic day except for PK and PD sample collection shall be completed. The reason for termination shall be entered onto the case report form.

7.1.6 Day 2

- Urine samples for PD (UGE and creatinine) analysis will be collected at specified time interval after oral administration of bexagliflozin, as described in Section 6.6.2.3.
- Plasma samples will be drawn for PK analysis as detailed in Section 6.6.2.4 at 24 and 36 h post-dose.
- Post-dose concomitant medications and adverse event information will be collected as appropriate.

7.1.7 Day 3 (Dosing Day): Pre-dose

- Partial PE will be performed as described in Section 6.3.
- Vital signs will be recorded at 48 h post-dose (from day 1).
- Urine samples for PD (UGE and creatinine) analysis will be collected at specified time interval after oral administration of bexagliflozin, as described in Section 6.6.2.3.
- Plasma samples will be drawn for PK analysis
- Concomitant medications and adverse event information will be collected as appropriate.
- Urine will be collected for urinalysis as described in Section 6.6.2.2. If urine is dipstick positive for leukocyte esterase or nitrites, sample is to be sent for microscopic evaluation and culture.
- Blood will be drawn for hematology and serum chemistry as detailed in Section 6.6.2.1.

7.1.8 Day 3 (Dosing Day): Dosing

- Probenecid tablets, 500 mg, will be administered twice a day about 30 min after standard meal with approximately 240 mL water as detailed in Section 5.3.

7.1.9 Day 3 (Dosing Day): Post-dose

- Vital signs will be recorded at 4 h post-oral dose
- Post-dose concomitant medications and adverse event information will be collected as appropriate.

For subjects who are terminated from the study for any reason after dosing, all activities for the last clinic day except for PK and PD sample collection shall be completed. The reason for termination shall be entered onto the case report form.

7.1.10 Day 4 (Dosing Day): Dosing

- Probenecid tablets, 500 mg, will be administered twice a day about 30 min after standard meal with approximately 240 mL water as detailed in Section 5.3.

7.1.11 Day 4: Post-dose

- Post-dose concomitant medications and adverse event information will be collected as appropriate.
- Pre-dose urine will be collected (-12 h to 0 h batch) for PD (UGE and creatinine) analysis as described in Section 6.6.2.3.

For subjects who are terminated from the study for any reason after dosing, all activities for the last clinic day except for PK and PD sample collection shall be completed. The reason for termination shall be entered onto the case report form.

7.1.12 Day 5: Pre-dose

- Partial PE will be performed as described in Section 6.3.
- Vital signs will be recorded at pre-dose.
- 12-lead ECG will be taken in the supine position after at least 5 min of rest as described in Section 6.5.
- Pre-dose urine will be collected until 0 h (-12 h to 0 h batch) for PD (UGE and creatinine) analysis as described in Section 6.6.2.3.
- Urine sample will be collected for pre-dose urinalysis as described in Section 6.6.2.2. If urine is dipstick positive for leukocyte esterase or nitrites, sample is to be sent for microscopic evaluation and culture.
- Blood will be drawn for hematology and serum chemistry as detailed in Section 6.6.2.1.
- Pre-dose plasma samples will be drawn for PK analysis as detailed in Section 6.6.2.4.
- Pre-dose concomitant medications and adverse event information will be collected as appropriate.

7.1.13 Day 5: Dosing

- Subjects will take bexagliflozin tablets, 20 mg and probenecid tablets, 500 mg, ~30 min after breakfast with approximately 240 mL water. And 10-12 h later, subjects will receive 500 mg probenecid ~30 min after night meal with approximately 240 mL water, as detailed in Section 5.3.

7.1.14 Day 5: Post-dose

- Vital signs will be recorded at 4 h post-oral dose
- 12-lead ECG will be taken at 4 h post-dose in the supine position after at least 5 min of rest.
- Urine samples for PD (UGE and creatinine) will be collected at specified time interval after oral administration of bexagliflozin, as described in Section 6.6.2.3.
- Blood samples for PK analysis will be collected as detailed in Section 6.6.2.4 at 1, 2, 3, 4, 5, 6, 8, 10, 12, 16 h after oral administration of bexagliflozin
- Post-dose concomitant medications and adverse event information will be collected as appropriate.

For subjects who are terminated from the study for any reason after dosing, all activities for the last clinic day except for PK and PD sample collection shall be completed. The reason for termination shall be entered onto the case report form.

7.1.15 Day 6: Dosing

- Subjects will take probenecid tablets, 500 mg, ~30 min after breakfast with approximately 240 mL water. And 10-12 h later, subjects will receive 500 mg probenecid ~30 min after night meal with approximately 240 mL water, as detailed in Section 5.3.

7.1.16 Day 6: Post-dose

- Plasma samples will be drawn for PK analysis as detailed in Section 6.6.2.4 at 24 and 36 h post-dose of bexagliflozin.
- Urine samples for PD (UGE and creatinine) analysis will be collected at specified time interval after oral administration of bexagliflozin, as described in Section 6.6.2.3.
- Post-dose concomitant medications and adverse event information will be collected as appropriate.

For subjects who are terminated from the study for any reason after dosing, all activities for the last clinic day except for PK and PD sample collection shall be completed. The reason for termination shall be entered onto the case report form.

7.1.17 Day 7

The following information will be gathered and the indicated procedures will be performed pre-dose:

- Complete PE will be performed as described in Section 6.3
- Vital signs will be recorded 48 h post-dose of bexagliflozin (from day 5)

- Plasma samples will be drawn for PK analysis as detailed in Section 6.6.2.4 at 48 h post-dose (from Day 5)
- Urine samples for PD (UGE and creatinine) analysis will be collected at specified time interval after oral administration of bexagliflozin, as described in Section 6.6.2.3.
- Urine will be collected for urinalysis as described in Section 6.6.2.2.
- Blood will be drawn for hematology and serum chemistry as detailed in Section 6.6.2.1.
- Post-dose concomitant medications and adverse event information will be collected as appropriate.
- Subject will be discharged after completion of all activities.

7.2 Study Activities for Study 2

7.2.1 Screening (Day -21 to Day -1)

During the screening period, the following information will be gathered and the indicated procedures will be performed:

- Content of informed consent will be explained to the subject and the signed informed consent collected.
- Medical history and demographic information will be obtained.
- Physical examination will be conducted, including height and weight measurements as described in Section 6.3.
- Vital signs will be measured, including pulse, temperature, respiratory rate, and blood pressure taken in the sitting position after at least 5 min of rest as described in Section 6.4.
- 12-lead ECG will be taken in the supine position after at least 5 min of rest as described in Section 6.5.
- Clean-catch, mid-stream urine will be collected for urinalysis as described in Section 6.6.2.2. If urine is dipstick positive for leukocyte esterase or nitrites, sample is to be sent for microscopic evaluation and culture.
- Urine screen will be conducted for drug abuse and cotinine. If the drug screen is conducted more than 5 days pre-dose, the drug screen shall be repeated prior to dosing. Drug abuse testing includes amphetamines, barbiturates, cocaine metabolites, opiates, benzodiazepines, cotinine, and cannabinoids.
- All female subjects will receive urine pregnancy test.
- Blood will be drawn for hematology, serum chemistry, and serology as detailed in Section 6.6.2.1.
- Inclusion/exclusion criteria will be evaluated based on the information collected at the screening examination.

7.2.2 Day 0 (Clinic Admission)

The following information will be gathered and the indicated procedures will be performed:

- Partial PE will be performed as described in Section 6.3.
- If screening was conducted more than 5 days prior to dosing, the inclusion and exclusion criteria shall be confirmed and a urine drug screen performed.
- All female subjects will receive urine pregnancy test.
- Pre-dose urine collection in 12 h batches will begin with the -12 h to 0 h batch for baseline analysis of UGE and creatinine (PD) as described in Section 6.6.2.3.
- Concomitant medications and adverse event information will be collected as appropriate.
- If the subject is still eligible based on the study inclusion and exclusion criteria, the subject will be admitted into the study.

7.2.3 Day 1 (Dosing Day): Pre-dose

- Vital signs will be recorded pre-dose.
- Pre-dose urine will be collected until 0 h (-12 h to 0 h batch) for baseline UGE and creatinine (PD) analysis as described in Section 6.6.2.3.
- Urine sample will be collected for pre-dose urinalysis as described in Section 6.6.2.2. If urine is dipstick positive for leukocyte esterase or nitrites, sample is to be sent for microscopic evaluation and culture. If screening is conducted more than 5 days prior to dosing, drug screening shall be repeated.
- Pre-dose blood will be drawn for hematology, and serum chemistry as detailed in Section 6.6.2.1.
- Pre-dose plasma samples will be drawn for PK analysis as detailed in Section 6.6.2.4.
- Pre-dose concomitant medications and adverse event information will be collected as appropriate.

7.2.4 Day 1: Dosing

- Subjects will receive a single dose of bexagliflozin tablets, 20 mg, after an overnight fast of at least 10 h with approximately 240 mL water as detailed in Section 5.3

7.2.5 Day 1: Post-Dose

- Vital signs will be recorded at 4 h post-oral dose
- Urine samples for PD (UGE and creatinine) analysis will be at specified time interval after oral administration of bexagliflozin, as described in Section 6.6.2.3.

- Blood samples for PK samples will be collected at 1, 2, 3, 4, 5, 6, 8, 10, 12, 16 h after oral administration of bexagliflozin.
- Post-dose concomitant medications and adverse event information will be collected as appropriate.

For subjects who are terminated from the study for any reason after dosing, all activities for the clinic day except for PK and PD sample collection shall be completed. The reason for termination shall be entered onto the case report form.

7.2.6 Day 2

- Urine samples for PD (UGE and creatinine) analysis will be collected at 24-36 h and 36-48 h after oral administration of bexagliflozin, as described in Section 6.6.2.3.
- Plasma samples will be drawn for PK analysis as detailed in Section 6.6.2.4 at 24 and 36 h post-dose.
- Post-dose concomitant medications and adverse event information will be collected as appropriate.

7.2.7 Day 3: Pre-dose

- Partial PE will be performed as described in Section 6.3.
- Vital signs will be recorded at 48 h post-dose (from day 1).
- Urine samples for PD (UGE and creatinine) analysis will be collected at specified time interval after oral administration of bexagliflozin, as described in Section 6.6.2.3.
- Plasma samples will be drawn for PK analysis
- Concomitant medications and adverse event information will be collected as appropriate.
- Urine will be collected for urinalysis as described in Section 6.6.2.2. If urine is dipstick positive for leukocyte esterase or nitrites, sample is to be sent for microscopic evaluation and culture.
- Blood will be drawn for hematology and serum chemistry as detailed in Section 6.6.2.1.

7.2.8 Day 3: Dosing

- After an overnight fast of at least 10 h, rifampin capsules, 600 mg ((2 x 300 mg capsules), will be administered with approximately 240 mL water prior to first meal of the day as detailed in Section 5.3

7.2.9 Day 3: Post-Dose

- Vital signs will be recorded at 4 h post-oral dose

- Post-dose concomitant medications and adverse event information will be collected as appropriate.

For subjects who are terminated from the study for any reason after dosing, all activities for the last clinic day except for PK and PD sample collection shall be completed. The reason for termination shall be entered onto the case report form.

7.2.10 Day 4-Day 5: Dosing

- After an overnight fast of at least 10 h, rifampin tablets, 600 mg, will be administered with approximately 240 mL water prior to first meal of the day as detailed in Section 5.3

7.2.11 Day 4-Day 5: Post-Dose

- Post-dose concomitant medications and adverse event information will be collected as appropriate.
- Pre-dose urine will be collected on Day 5 (-12 h to 0 h batch) for PD (UGE and creatinine) analysis as described in Section 6.6.2.3.

For subjects who are terminated from the study for any reason after dosing, all activities for the study termination day except for PK and PD sample collection shall be completed. The reason for termination shall be entered onto the case report form.

7.2.12 Day 6: Pre-dose

- Partial PE will be performed as described in Section 6.3.
- Vital signs will be recorded at pre-dose.
- 12-lead ECG will be taken in the supine position after at least 5 min of rest as described in Section 6.5.
- Pre-dose urine will be collected until 0 h (-12 h to 0 h batch) for PD (UGE and creatinine) analysis as described in Section 6.6.2.3.
- Urine sample will be collected for pre-dose urinalysis as described in Section 6.6.2.2. If urine is dipstick positive for leukocyte esterase or nitrites, sample is to be sent for microscopic evaluation and culture.
- Blood will be drawn for hematology and serum chemistry as detailed in Section 6.6.2.1.
- Pre-dose plasma samples will be drawn for PK analysis as detailed in Section 6.6.2.4.
- Pre-dose concomitant medications and adverse event information will be collected as appropriate.

7.2.13 Day 6: Dosing

- After an overnight fast of at least 10 h, rifampin capsules, 600 mg (2 x 300 mg capsules), and bexagliflozin tablets, 20 mg, will be administered with approximately 240 mL water prior to first meal of the day as detailed in Section 5.3

7.2.14 Day 6: Post-dose Activities

- Vital signs will be recorded at 4 h post-oral dose
- 12-lead ECG will be taken in the supine position after at least 5 min of rest at 4 h post-dose.
- Urine samples for PD (UGE and creatinine) analysis will be at specified time interval after oral administration of bexagliflozin, as described in Section 6.6.2.3.
- Blood samples for PK samples will be collected at 1, 2, 3, 4, 5, 6, 8, 10, 12, 16 h after oral administration of bexagliflozin.
- Post-dose concomitant medications and adverse event information will be collected as appropriate.

For subjects who are terminated from the study for any reason after dosing, all activities for the clinic day except for PK and PD sample collection shall be completed. The reason for termination shall be entered onto the case report form.

7.2.15 Day 7 (Dosing):

- After an overnight fast of at least 10 h, rifampin capsules, 600 mg (2 x 300 mg capsules), will be administered with approximately 240 mL water prior to first meal of the day as detailed in Section 5.3

7.2.16 Day 7: Post-dose

- Plasma samples will be drawn for PK analysis as detailed in Section 6.6.2.4 at 24 and 36 h post-dose of bexagliflozin on Day 6.
- Urine samples for PD (UGE and creatinine) analysis will be collected at 24-36 h and 36-48 h after oral administration of bexagliflozin, as described in Section 6.6.2.3.
- Post-dose concomitant medications and adverse event information will be collected as appropriate.

For subjects who are terminated from the study for any reason after dosing, all activities for the last clinic day except for PK and PD sample collection shall be completed. The reason for termination shall be entered onto the case report form.

7.2.17 Day 8

The following information will be gathered and the indicated procedures will be performed post-dose:

- Complete PE will be performed as described in Section 6.3
- Vital signs will be recorded 48 h post-dose of bexagliflozin (from Day 6)
- Plasma samples will be drawn for PK analysis as detailed in Section 6.6.2.4 at 48 h post-dose (from Day 6)
- Urine samples for PD (UGE and creatinine) analysis will be collected at 36-48 h post dose (from Day 6) as described in Section 6.6.2.3.
- Urine will be collected for urinalysis as described in Section 6.6.2.2.
- Blood will be drawn for hematology and serum chemistry as detailed in Section 6.6.2.1.
- Post-dose concomitant medications and adverse event information will be collected as appropriate.
- Subject will be discharged after completion of all activities

For subjects who are terminated from the study for any reason after dosing, all activities for the study termination day except for PK and PD sample collection shall be completed. The reason for termination shall be entered onto the case report form.

7.3 Study Activities for Study 3

7.3.1 Screening (Day -21 to Day -1)

During the screening period, the following information will be gathered and the indicated procedures will be performed:

- Content of informed consent will be explained to the subject and the signed informed consent collected.
- Medical history and demographic information will be obtained as described in Section 6.2.
- Physical examination will be conducted, including height and weight measurements as described in Section 6.3.
- Vital signs will be measured, including pulse, temperature, respiratory rate, and blood pressure taken in the sitting position after at least 5 min of rest as described in Section 6.4.
- 12-lead ECG will be taken in the supine position after at least 5 min of rest as described in Section 6.5.

- Clean-catch, mid-stream urine will be collected for urinalysis as described in Section 6.6.2.2. If urine is dipstick positive for leukocyte esterase or nitrites, sample is to be sent for microscopic evaluation and culture.
- Urine screen will be conducted for drug abuse and cotinine. If the drug screen is conducted more than 5 days pre-dose, the drug screen shall be repeated prior to dosing. Drug abuse testing includes amphetamines, barbiturates, cocaine metabolites, opiates, benzodiazepines, cotinine, and cannabinoids.
- All female subjects will receive urine pregnancy test.
- Blood will be drawn for hematology, serum chemistry, and serology as detailed in Section 6.6.2.1.
- Inclusion/exclusion criteria will be evaluated based on the information collected at the screening examination.

7.3.2 Day 0 (Clinic Admission)

The following information will be gathered and the indicated procedures will be performed:

- Partial PE will be performed as described in Section 6.3.
- If screening was conducted more than 5 days prior to dosing, the inclusion and exclusion criteria shall be confirmed and a urine drug screen performed.
- All female subjects will receive urine pregnancy test.
- Subjects will void their bladders at 12 h prior to dosing and begin the collection of the -12 h to 0 h batch for baseline analysis of UGE and creatinine (PD) as described in Section 6.6.2.3.
- Concomitant medications and adverse event information will be collected as appropriate.
- If the subject is still eligible based on the study inclusion and exclusion criteria, the subject will be admitted into the study.

7.3.3 Day 1 (Dosing Day): Pre-dose

The following information will be gathered and the indicated procedures will be performed pre-dose:

- Vital signs will be recorded pre-dose.
- Pre-dose urine will be collected until 0 h (-12 h to 0 h batch) for baseline UGE and creatinine (PD) analysis as described in Section 6.6.2.3.
- Urine sample will be collected for pre-dose urinalysis as described in Section 6.6.2.2. If urine is dipstick positive for leukocyte esterase or nitrites, sample is to be sent for microscopic evaluation and culture. If screening is conducted more than 5 days prior to dosing, drug screening shall be repeated.

- Pre-dose blood will be drawn for hematology, and serum chemistry as detailed in Section 6.6.2.1.
- Pre-dose plasma samples will be drawn for PK analysis as detailed in Section 6.6.2.4.
- Pre-dose concomitant medications and adverse event information will be collected as appropriate.

7.3.4 Day 1 (Dosing Day): Dosing

- Bexagliflozin tablets, 20 mg, will be administered ~30 minutes after breakfast (standard low-fat meal), as detailed in Section 5.3.

7.3.5 Day 1: Post-dose

- Vital signs will be recorded at 4 h post-oral dose
- Urine samples for PD (UGE and creatinine) analysis will be at specified time interval after oral administration of bexagliflozin, as described in Section 6.6.2.3.
- Blood samples for PK samples will be collected at 1, 2, 3, 4, 5, 6, 8, 10, 12, 16 h after oral administration of bexagliflozin.
- Post-dose concomitant medications and adverse event information will be collected as appropriate.

For subjects who are terminated from the study for any reason after dosing, all activities for the clinic day except for PK and PD sample collection shall be completed. The reason for termination shall be entered onto the case report form.

7.3.6 Day 2

- Urine samples for PD (UGE and creatinine) analysis will be collected at specified time interval after oral administration of bexagliflozin, as described in Section 6.6.2.3.
- Plasma samples will be drawn for PK analysis as detailed in Section 6.6.2.4 at 24 and 36 h post-dose.
- Post-dose concomitant medications and adverse event information will be collected as appropriate.

7.3.7 Day 3

- Vital signs will be recorded at 48 h post-dose (from day 1).
- Urine samples for PD (UGE and creatinine) analysis will be collected at specified time interval after oral administration of bexagliflozin, pre-dose urine will also be collected until 0 h (-12 h to 0 h batch), as described in Section 6.6.2.3.
- Plasma samples will be drawn for PK analysis as detailed in Section 6.6.2.4 at 48 h post-dose

- Post-dose concomitant medications and adverse event information will be collected as appropriate.

7.3.8 Day 4 (Dosing Day): Pre-dose

- Partial PE will be performed as described in Section 6.3.
- Vital signs will be recorded at pre-dose.
- 12-lead ECG will be taken in the supine position after at least 5 min of rest as described in Section 6.5.
- Pre-dose urine will be collected until 0 h (-12 h to 0 h batch) for PD (UGE and creatinine) analysis as described in Section 6.6.2.3.
- Urine sample will be collected for pre-dose urinalysis as described in Section 6.6.2.2. If urine is dipstick positive for leukocyte esterase or nitrites, sample is to be sent for microscopic evaluation and culture.
- Blood will be drawn for hematology and serum chemistry as detailed in Section 6.6.2.1.
- Pre-dose plasma samples will be drawn for PK analysis as detailed in Section 6.6.2.4.
- Pre-dose concomitant medications and adverse event information will be collected as appropriate.

7.3.9 Day 4 (Dosing Day): Dosing

- About 30 min after breakfast, subjects will take one verapamil tablet, 120 mg, and one hour later one bexagliflozin tablet, 20 mg will be taken with approximately 240 mL water as detailed in Section 5.3.

7.3.10 Day 4 (Dosing Day): Post-dose

- Vital signs will be recorded at 4 h post-oral dose
- 12-lead ECG will be taken at 4 h post-dose in the supine position after at least 5 min of rest.
- Urine samples for PD (UGE and creatinine) will be collected at specified time interval after oral administration of bexagliflozin, as described in Section 6.6.2.3.
- Blood samples for PK analysis will be collected as detailed in Section 6.6.2.4 at 1, 2, 3, 4, 5, 6, 8, 10, 12, 16 h after oral administration of bexagliflozin
- Post-dose concomitant medications and adverse event information will be collected as appropriate.

For subjects who are terminated from the study for any reason after dosing, all activities for the last clinic day except for PK and PD sample collection shall be completed. The reason for termination shall be entered onto the case report form.

7.3.11 Day 5

- Plasma samples will be drawn for PK analysis as detailed in Section 6.6.2.4 at 24 and 36 h post-dose of bexagliflozin on Day 4.
- Urine samples for PD (UGE and creatinine) analysis will be collected at 24-36 h and 36-48 h after oral administration of bexagliflozin, as described in Section 6.6.2.3.
- Post-dose concomitant medications and adverse event information will be collected as appropriate.

For subjects who are terminated from the study for any reason after dosing, all activities for the last clinic day except for PK and PD sample collection shall be completed. The reason for termination shall be entered onto the case report form.

7.3.12 Day 6

The following information will be gathered and the indicated procedures will be performed post-dose:

- Complete PE will be performed as described in Section 6.3
- Vital signs will be recorded 48 h post-dose of bexagliflozin (from Day 4)
- Plasma samples will be drawn for PK analysis as detailed in Section 6.6.2.4 at 48 h post-dose (from Day 4)
- Urine samples for PD (UGE and creatinine) analysis will be collected at 36-48 h post dose (from Day 4) as described in Section 6.6.2.3.
- Urine will be collected for urinalysis as described in Section 6.6.2.2.
- Blood will be drawn for hematology and serum chemistry as detailed in Section 6.6.2.1.
- Post-dose concomitant medications and adverse event information will be collected as appropriate.
- Subject will be discharged after completion of all activities

For subjects who are terminated from the study for any reason after dosing, all activities for the study termination day except for PK and PD sample collection shall be completed. The reason for termination shall be entered onto the case report form.

7.4 Early Termination or Follow-up Procedures

Subjects who have withdrawn consent and have received investigational product should have a follow-up examination if feasible and clinically indicated, including a complete physical examination, vital signs, and clinical laboratory tests (hematology, and serum chemistry). The sponsor shall be notified in the event that a subject withdraws or has been withdrawn from the study.

8 QUALITY CONTROL AND ASSURANCE

The clinical research facility shall be monitored by the study monitor to ensure correct performance of the study procedures and assure that the study will be conducted according to the protocol and relevant regulatory requirements. CRF entries shall be verified with the source documentation.

Quality control principles shall be applied throughout the performance of this study by following the standard operating procedure (SOPs) of the contract research organization (CRO) and the sponsor. Review procedures shall be implemented at the CRO for all documents that are generated in relation to the study.

9 PLANNED STATISTICAL METHODS

9.1 General Considerations

The statistical evaluation of PK parameters will be conducted by the designated CRO. A detailed Statistical and Analytical Plan will be generated prior to any PK statistical analysis of the data. Statistical analysis will be performed using Statistical Analysis Software SAS for Windows® (SAS, USA). Non-compartmental analysis will be performed using Phoenix®WinNonlin® 6.4 (Certara, USA).

9.2 Determination of Sample Size

The sample size for this study is not based upon formal statistical consideration. The sample size is considered adequate to characterize the PK of bexagliflozin to assess the potential effect of probenecid, rifampin or verapamil on bexagliflozin.

9.3 Analysis Populations

9.3.1 Safety Population

The Safety Population will include all subjects who received at least one dose of study drug. Subjects will be analyzed according to the treatment received.

9.3.2 PK Population

The PK Population will include all subjects who receive at least one dose of study drug and who have sufficient plasma bexagliflozin and metabolite (where applicable) measurements to derive PK parameters following dosing. The PK Population will be used to summarize the PK parameters. Subjects will be analyzed according to the treatment received.

9.3.3 PD Population

The PD Population will include all randomized subjects who receive at least one dose of investigational product and have pre-dose urine collection and postdose 0-12 and 12-24 hour post dose urine samples from which to calculate UGE measurement following dosing for at least one study day. The PD Population will be used to summarize the PD parameters. Subjects will be analyzed according to the treatment received.

9.4 Demographics and Baseline Characteristics

Baseline characteristics will be summarized for all subjects in the Safety and PK Populations. Descriptive statistics will be performed.

9.5 Pharmacokinetic Analysis

9.5.1 Calculation of Pharmacokinetic Variables

A non-compartmental analysis will be used to calculate the PK parameters of bexagliflozin in all three studies and its 3'-*O*-glucuronide (EGT0002149; in study 1 and 2) after each subject is dosed with bexagliflozin using the software Phoenix® WinNonlin® 6.4 (Certara, USA). From the plasma concentration data, the following PK parameters will be estimated for each subject where feasible.

C_{max} Maximum observed plasma concentration

T_{max} Time of maximum observed plasma concentration

λ_z Terminal elimination phase rate constant

$T_{1/2}$ Apparent terminal elimination half-life

CL/F Apparent oral clearance

V_z/F Apparent volume of distribution

AUC_{0-t} Area under the plasma concentration-time curve from Time 0 to Time t (time of last quantifiable plasma concentration)

$AUC_{0-\infty}$ Area under the plasma concentration-time curve from Time 0 to infinity

AUC_{extr} % of $AUC_{0-\infty}$ due to extrapolation from T_{last} to infinity

M/P AUC Ratio Metabolite-to-parent ratio for AUC (AUC_{0-t} and $AUC_{0-\infty}$)

M/P C_{max} Ratio Metabolite-to-parent ratio for C_{max}

C_{max} and T_{max} will be obtained directly from experimental observations. If multiple maxima occur at equal concentrations, the first temporal value will be taken as the C_{max} and T_{max}.

The apparent terminal elimination half-life, $T_{1/2}$, where determinable, will be calculated as the natural log of 2 divided by the terminal phase rate constant, λ_z. The number of data points included in the regression will be determined by visual inspection, but a minimum of three data points in the terminal phase, excluding C_{max}, is required to estimate λ_z. In order for the selection to take place the adjusted r^2 value reported in Phoenix®WinNonlin® must be ≥ 0.7.

AUC_{0-t} and $AUC_{0-\infty}$ will be calculated using the linear trapezoidal linear interpolation method, using actual elapsed time values. If the actual collection time is unknown the nominal collection time may be used for the purposes of PK parameter estimation. For the purpose of calculating AUC, all missing values will be treated as missing in the PK analysis and excluded from analysis except when they occur at pre-dose where they will be set to zero. All values that were below the limit of quantitation (BLOQ) prior to T_{max} will be set to zero. BLOQ values that occur after T_{max} will be set to missing. When ≥ 2 consecutive plasma concentrations BLOQ are encountered after T_{max}, these and all subsequent values will be excluded from the analysis.

$AUC_{0-\infty}$ will be calculated according to the following equation:

$AUC_{0-\infty} = AUC_{last} + (C_{last} / \lambda_z)$, where C_{last} is the last temporal quantifiable plasma concentration corresponding to T_{last}.

The proportion of $AUC_{0-\infty}$ due to extrapolation (AUC_{extr}) will be calculated and expressed as a percentage. $AUC_{0-\infty}$ values will be considered unreliable estimates if the AUC_{extr} is greater than 20%.

CL/F will be calculated as Dose/$AUC_{0-\infty}$.

Vz/F will be calculated as Dose/($\lambda_z \times AUC_{0-\infty}$).

$T_{½}$ will be calculated as $0.693/\lambda_z$.

Descriptive statistics for the plasma concentrations of bexagliflozin and/or EGT0002149 by Treatment, and Timepoint will be provided. A listing of plasma concentrations by Subject Number, Treatment Period, Timepoint and Sex/Gender will also be provided.

9.5.2 Statistical Analysis of Pharmacokinetic Variables

To assess the effect of probenecid, rifampin and verapamil on the PK of bexagliflozin in all three studies and EGT0002149 (Study 1 and Study 2 only), an analyses of variance (ANOVA) using a linear mixed-effects model will be fitted to the natural logarithmic transformation of PK parameters of bexagliflozin (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$). The linear mixed-effects model will include subject as a random effect, and treatment as fixed effects. The 90% confidence intervals will be constructed for the ratio of geometric means of PK parameters (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$) when bexagliflozin is dosed in combination with interacting drug (probenecid, rifampin or verapamil) versus when dosed alone, with 80-125% defined as the lack of interaction boundaries.

Descriptive statistics for the PK parameters C_{max}, T_{max}, $AUC_{0-\infty}$, AUC_{0-t}, AUC_{extr}, M/P C_{max} and AUC Ratio (AUC_{0-t} and $AUC_{0-\infty}$) (for Study 1 and Study 2), CL/F, V_z/F, λ_z, and $T_{1/2}$ will be tabulated by treatment. Means, standard deviations, medians, ranges (min, max) and geometric means and coefficients of variation will be presented for all PK parameters with the exception of T_{max}. Medians and ranges will be presented for T_{max}.

A listing of derived PK parameters of bexagliflozin and/or EGT0002149 by Subject ID, Period and Sex will be provided.

9.6 Pharmacodynamic Analysis

The amount of urinary glucose excretion (UGE), urinary creatinine, and creatinine normalized UGE (nUGE) will be determined as a PD parameter at baseline and up to 48 h post-dose. Descriptive statistics will be used to describe any differences in these PD parameters between treatments. The drug-drug interaction effect between bexagliflozin and interaction drugs (probenecid, rifampin and verapamil) will be evaluated by comparing the mean cumulative UGE and creatinine normalized UGE (nUGE) between subjects taking bexagliflozin alone and bexagliflozin and interaction drug in combination.

The PD parameter, UGE and UGE normalized by urinary creatinine, including UGE_{t1-t2} over specified time interval, and total 24-hour, 48-hour UGE, will be determined. UGE_{t1-t2} (mg) will be derived from urine volume (V_{t1-t2}, mL) × glucose concentration (mg/dL) / 100. UGE and nUGE will be listed and summarized by treatment arm using descriptive statistics.

9.7 Safety Analysis

Safety data will include AEs, PE results, vital signs, ECG results, and clinical lab results, including serum chemistry, hematology, and urinalysis. Observed data will be described as counts and percentages for discrete variables and estimation of means, standard deviations (SDs), medians, inter-quartile range, minimum and maximum for continuous metrics. All subjects in the Safety Population will be included in the safety analyses. All safety data will be presented in by-subject listings and included in the clinical trial report.

9.7.1 Adverse Events

Adverse events will be mapped to preferred term and body system using the Medical Dictionary for Regulatory Activities (MedDRA). The number and percentage of subjects reporting adverse events will be determined by relationship to treatment and by severity of the event. Drug-related adverse events will be considered those to be possibly related to bexagliflozin or interacting drug administration.

Adverse event listings will be provided for the following subsets:

- all treatment emergent AEs (TEAEs).
- all TEAEs at least possibly related to bexagliflozin or interacting drug.
- serious TEAEs (if any).
- TEAEs leading to study discontinuation (if any).

AEs are dosing emergent if they occur on or after bexagliflozin or interacting drug administration. TEAEs will be considered at least possibly related to bexagliflozin or interacting drug based on the investigator's assessment. Only TEAEs will be tabulated in summary tables. If the AE(s) onset date-time or date occurs after the first dose up until right before the next dose, the AE(s) will be assigned to the first treatment. Any AE(s) that occur after the first dose of interacting drug up until the follow-up visit will be assigned to the second treatment (combination treatment).

Tabulations will display TEAEs by severity and relationship to bexagliflozin or interacting drug.

Tabulations will display TEAEs by severity and relationship to bexagliflozin or interacting drug.

9.7.2 Hypoglycemia

Hypoglycemia as defined in Section 6.7.3.4 will be presented in listings and summarized.

9.7.3 Clinical and Laboratory Events and Analyses

Clinical and laboratory metrics are measured at baseline (pre-dose on Day 1) and during the treatment periods (Appendix 1). These variables include vital signs (blood pressure, respiration, temperature), clinical laboratory results (see Section 6.6 for a complete list), and ECGs.

Serum chemistry, hematology, and urinalysis (quantitative parameters) data will be summarized for each treatment period. Summaries for change from baseline will be presented for these laboratory tests.

ECG results will be summarized as changes from baseline in intervals. Abnormalities as well as changes from previous assessment will be listed.

9.7.4 Concomitant Medications

Concomitant medications will be coded using the World Health Organization Drug Dictionary (WHO-DD). A by-subject listing of concomitant medications will include all medications taken during the study.

10 ADMINISTRATIVE CONSIDERATIONS

10.1 Investigators and Study Administrative Structure

Information regarding key personnel involved in the conduct of the study, including names and contact details of participating investigators, monitors, clinical laboratories, and technical departments and/or institutions, as well as information on members of additional study committees, will be found in the study files of the investigational site.

10.2 Institutional Review Board (IRB) or Independent Ethics Committee (IEC) Approval

The study protocol, informed consent document, relevant supporting information, and all types of subject recruitment or advertisement information shall be submitted to the IRB for review and shall be approved by the sponsor and the IRB before the study is initiated. Any amendments or addenda to the protocol shall also be approved by the IRB prior to implementing changes in the study. The investigator is responsible for keeping the IRB informed of the progress of the study and of any changes made to the protocol as deemed appropriate. The investigator shall also keep the IRB informed of any reportable SAEs occurring to subjects under their supervision following the local IRB requirements.

10.3 Ethical Conduct of the Study

The procedures set out in this protocol pertaining to the conduct, evaluation, and documentation of this study are designed to ensure that the sponsor and investigator follow Good Clinical Practice (GCP) Guidelines and the guiding principles detailed in the Declaration of Helsinki. The study will also be carried out in keeping with applicable local laws and regulations. An inspection by the sponsor representatives and/or their designee and/or healthy authority or other authorized regulatory authorities representatives may occur at any time. The investigator shall agree to the inspection of study-related records by the regulatory authority/sponsor representatives, and shall allow direct access to source documents to the regulatory authority/sponsor representatives.

The investigator is responsible for complying with the protocol and all appropriate regulations and guidelines governing global clinical research. Additionally, he/she is responsible for ensuring that all participating staff members are adequately trained and competent to perform his/her assigned tasks.

Modifications to the study protocol will not be implemented by either the sponsor or the investigator without agreement by both parties. However, the investigator may implement a deviation from or a change of the protocol to eliminate any immediate hazards to the trial subjects without prior IRB or sponsor approval/favorable opinion. As soon as possible, the implemented deviation or change, the reasons for it, and if appropriate, the proposed protocol amendment shall be submitted to the head of the investigational site, IRB (via the head of the investigational site)/sponsor.

Any deviations from the protocol shall be fully explained and documented by the investigator. The circumstances, action taken, and impact of the deviation on the trial shall be communicated by the principal investigator to the designated medical monitor. Any subsequent actions will be assessed by the designated medical monitor and documented.

10.4 Subject Information and Consent

The investigator will draft the informed consent form based on the protocol. The sponsor will review the investigator's draft informed consent form prior to submission to the IRB and the final IRB approved document shall be provided to the sponsor for regulatory purposes.

Prior to the beginning of the study, the investigator shall have received from the IRB the written approval or favorable opinion of the informed consent form and any other written information to be provided to subjects. The written approval of the IRB together with the approved subject information and informed consent forms shall be filed. The informed consent form shall contain all elements required by the Federal Drug Administration under 21 Code of Federal Regulations Part 50 and the ICH GCP Guidelines (E6), in addition to, any other elements required by regulations or institutional policy.

Written informed consent shall be obtained before any study-specific procedure takes place. Participation in the study and date of informed consent given by the subject shall be documented appropriately in the subject's files. A copy of the signed informed consent form shall be provided to the subject. If applicable, it will be provided in a certified translation in the language understood by the subject, if not English. Signed consent forms shall remain in each subject's study file and shall be available for verification by study monitors at any time.

10.5 Subject Confidentiality

The information obtained during the conduct of this clinical study is confidential, and disclosure to third parties other than those noted below is prohibited. Information obtained during the conduct of this study will be used by the sponsor in connection with the development of the investigational product. The study investigator is obliged to provide the sponsor with complete test results and all data developed in this study. Subject-specific information may be provided to other appropriate medical personnel only with the subject's permission. To ensure compliance with current ICH guidelines, data generated by this study shall be available for inspection upon request by representatives of national and local health authorities, the sponsor, and the IRB for each study site.

Subject names and other identifiers, such as photographs, audio, or videotapes, may not be disclosed in any publication without prior written authorization from the subject.

10.6 Study Monitoring

An authorized sponsor representative will conduct site visits to inspect study data, subjects' medical records, and CRFs in accordance with ICH guidelines, GCPs, and the respective national and local government regulations and guidelines.

The investigator will permit authorized representatives of the sponsor and the respective national or local health authorities, other authorized regulatory authorities, and IRB to inspect facilities and records relevant to this study.

10.7 Case Report Forms and Study Records

For each subject consented, a case report form (CRF), in paper or electronic format, will be supplied and maintained by the CRO staff and signed by the investigator or authorized designee to indicate that he/she has reviewed and agrees with the entered data. This also applies to those subjects who fail to complete the trial. The reason a subject is withdrawn shall be recorded in the case report form.

Entries made in the CRF shall be verifiable against source documents. Source documents are defined as all medical records, medical notes, laboratory results, ECG traces and any additional document other than the CRF that has original subject information contained within it.

All CRFs and source documents shall be completed following GCPs and the CRO's standard operating procedures.

10.8 Protocol Violations/Deviations

It is important to conduct the study according to the protocol. Protocol deviations will not be prospectively granted by the sponsor. If deviations occur, such as a visit or sampling window being missed, the investigator shall decide whether to proceed, for example, whether or not to complete the visit or sample collection outside of the protocol-defined window. The sponsor's medical monitor shall be notified immediately when protocol deviations are discovered so that a decision about whether to keep the subject in the study can be made.

Only when an emergency occurs that requires a departure from the protocol for an individual subject will there be such a departure without the sponsor's pre-approval. The nature and reasons for the protocol deviation will be recorded in the subject's CRF, and the principal investigator shall notify the Sponsor.

Protocol violations shall be reported in the final study report.

10.9 Access to Source Documentation

Authorized sponsor representatives will conduct site visits to inspect study data, subjects' medical records, and CRFs in accordance with ICH guidelines, GCPs, and the respective local and national government regulations and guidelines.

The investigator will permit authorized representatives of the sponsor and the respective national or local health authorities, other authorized regulatory authorities, and IRB to inspect facilities and records relevant to this study.

The center will be visited at regular intervals by a monitor to ensure compliance with the study protocol, GCP, and legal aspects. This will include on-site checking of the CRFs for completeness and clarity, cross-checking with source documents, and clarification of administrative matters.

All CRF data will be entered into a clinical database. Following the correction of any errors, the clinical database will be locked.

10.10 Retention of Data

The study file and all source data shall be retained until notification is given by the sponsor for destruction.

If the investigator withdraws from the trial and relinquishes his/her responsibility for the maintenance and retention of records, he/she shall notify the sponsor in writing so that arrangements can be made to properly store the trial materials.

10.11 Publication and Disclosure Policy

All data and results and all intellectual property rights in the data and results derived from the study will be the property of the sponsor, who may utilize the data in various ways, such as for submission to government regulatory authorities or disclosure to other investigators.

No publication or disclosure of study results will be permitted except as specified in a separate, written agreement between Theracos Sub, LLC and the investigator. If results of this study are reported in medical journals or at meetings, all subjects' identities will remain confidential.

11 REFERENCE LIST

(FDA), F.a.D.A. (2012). Guidance for Industry Drug Interaction Studies — Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations. 1-79.

Baneyx, G., Parrott, N., Meille, C., Iliadis, A., and Lave, T. (2014). Physiologically based pharmacokinetic modeling of CYP3A4 induction by rifampicin in human: influence of time between substrate and inducer administration. European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences *56*, 1-15.

Chen, J., and Raymond, K. (2006). Roles of rifampicin in drug-drug interactions: underlying molecular mechanisms involving the nuclear pregnane X receptor. Ann Clin Microbiol Antimicrob *5*, 3.

Cunningham, R.F., Israili, Z.H., and Dayton, P.G. (1981). Clinical pharmacokinetics of probenecid. Clinical pharmacokinetics *6*, 135-151.

Hartter, S., Sennewald, R., Nehmiz, G., and Reilly, P. (2013). Oral bioavailability of dabigatran etexilate (Pradaxa((R))) after co-medication with verapamil in healthy subjects. British journal of clinical pharmacology *75*, 1053-1062.

Macha, S., Sennewald, R., Rose, P., Schoene, K., Pinnetti, S., Woerle, H.J., and Broedl, U.C. (2013). Lack of clinically relevant drug-drug interaction between empagliflozin, a sodium glucose cotransporter 2 inhibitor, and verapamil, ramipril, or digoxin in healthy volunteers. Clinical therapeutics *35*, 226-235.

Miners, J.O., Bowalgaha, K., Elliot, D.J., Baranczewski, P., and Knights, K.M. (2011). Characterization of niflumic acid as a selective inhibitor of human liver microsomal UDP-glucuronosyltransferase 1A9: application to the reaction phenotyping of acetaminophen glucuronidation. Drug metabolism and disposition: the biological fate of chemicals *39*, 644-652.

Pui, K., Gow, P.J., and Dalbeth, N. (2013). Efficacy and tolerability of probenecid as urate-lowering therapy in gout; clinical experience in high-prevalence population. The Journal of rheumatology *40*, 872-876.

Soars, M.G., Ring, B.J., and Wrighton, S.A. (2003). The effect of incubation conditions on the enzyme kinetics of udp-glucuronosyltransferases. Drug metabolism and disposition: the biological fate of chemicals *31*, 762-767.

Uchaipichat, V., Mackenzie, P.I., Guo, X.H., Gardner-Stephen, D., Galetin, A., Houston, J.B., and Miners, J.O. (2004). Human udp-glucuronosyltransferases: isoform selectivity and kinetics of 4-methylumbelliferone and 1-naphthol glucuronidation, effects of organic solvents, and inhibition by diclofenac and probenecid. Drug metabolism and disposition: the biological fate of chemicals *32*, 413-423.

Washburn, W.N. (2009). Evolution of sodium glucose co-transporter 2 inhibitors as anti-diabetic agents. Expert opinion on therapeutic patents *19*, 1485-1499.

Yamashita, F., Sasa, Y., Yoshida, S., Hisaka, A., Asai, Y., Kitano, H., Hashida, M., and Suzuki, H. (2013). Modeling of rifampicin-induced CYP3A4 activation dynamics for the prediction of clinical drug-drug interactions from in vitro data. PloS one *8*, e70330.

Zhang, W., Welihinda, A., Mechanic, J., Ding, H., Zhu, L., Lu, Y., Deng, Z., Sheng, Z., Lv, B., Chen, Y.*, et al.* (2011). EGT1442, a potent and selective SGLT2 inhibitor, attenuates blood glucose and HbA1c levels in db/db mice and prolongs the survival of stroke-prone rats. Pharmacological Research *63*, 284-293.

Appendix 1 Schedule of Events

A. SCHEDULE OF EVENTS FOR STUDY 1 (BEXAGLIFLOZIN AND PROBENECID):

	Screening		Treatment Period						
Study activity	**D -21 to -1**	**D0**	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**
Medical history and ICF	X								
Screening for I/E criteria[1]	X	X							
Physical exam[2]	X	X			X		X		X
Demographics	X								
Admission and discharge		X							X
Dispense investigational study drug bexagliflozin			X				X		
Dispense interaction drug probenecid					X	X	X	X	
Vital signs[3]	X		X		X		X		X
ECG[4]	X						X		
Urinalysis[5]	X		X		X		X		X
Blood draw for clinical lab tests[6]	X		X		X		X		X
Blood sample for PK[7]			X	X	X		X	X	X
Urine collection[8]		X	X	X	X	X	X	X	X
Urine pregnancy test	X	X							
Adverse event and concomitant medication		X	X	X	X	X	X	X	X
Study termination[9]									X

1. If screening is conducted more than 5 days prior to dosing, subject eligibility criteria must be confirmed on Day 0.
2. Weight and height will be recorded as part of the physical examination. Height will be recorded once at screening only. A complete physical exam (PE) will be performed at screening and last day of clinic prior to discharge. A partial PE will be performed on Day 0, Day 3, Day 5, and Day 6.
3. Vital signs include: pulse, body temperature, respiratory rate, systolic and diastolic blood pressure. On Day 1, and Day 5, vital signs will be determined at pre- and at 4 h post-oral dose, and 48 h post dose (Day 3), and on the last day of clinic (Day 7) prior to discharge and when clinically indicated.

4. 12-lead ECG will be conducted after 5 min resting. ECG data will be recorded at screening, on Day 5, at pre- and at 4 h post-oral dose and when clinically indicated.
5. Clean sample to be collected at each visit. If urine is dipstick positive for leukocyte esterase or nitrites, sample is to be sent for microscopic evaluation and culture. Urine drug screen will be performed on screening visit only. If screening is conducted more than 5 days prior to dosing, drug screening shall be repeated.
6. Blood for clinical chemistry and hematology will be drawn after a minimum of 10 h fasting prior to breakfast. Infectious disease testing will be conducted at screening only.
7. Plasma samples for PK will be collected at pre-dose and at 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 36, and 48 h after oral administration of bexagliflozin on Day 1 and Day 5.
8. Urine samples for PD (UGE) analysis are described in Section 6.6.2.3.
9. If early termination occurs, activities scheduled for the last day of clinic prior to discharge should be conducted. Reasons for all withdrawals shall be recorded on the CRF.

B. SCHEDULE OF EVENTS FOR STUDY 2 (BEXAGLIFLOZIN AND RIFAMPIN):

	Screening		Treatment Period							
Study activity	**D -21 to -1**	**D0**	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**
Medical history and ICF	X									
Screening for I/E criteria[1]	X	X								
Physical exam[2]	X	X			X			X		X
Demographics	X									
Admission and discharge		X								X
Dispense investigational study drug bexagliflozin			X					X		
Dispense interaction drug rifampin					X	X	X	X	X	
Vital signs[3]	X		X		X			X		X
ECG[4]	X							X		
Urinalysis[5]	X		X		X			X		X
Blood draw for clinical lab tests[6]	X		X		X			X		X
Blood sample for PK[7]			X	X	X			X	X	X
Urine collection[8]		X	X	X	X		X	X	X	X
Urine pregnancy test	X	X								
Adverse event and concomitant medication		X	X	X	X	X	X	X	X	X
Study termination[9]										X

1. If screening is conducted more than 5 days prior to dosing, subject eligibility criteria must be confirmed on Day 0.
2. Weight and height will be recorded as part of the physical examination. Height will be recorded once at screening only. A complete physical exam (PE) will be performed at screening and last day of clinic (Day 8) prior to discharge. A partial PE will be performed on Day 0, Day 3 and Day 6.
3. Vital signs include: pulse, body temperature, respiratory rate, systolic and diastolic blood pressure. On Day 1, and Day 6, vital signs will be determined at pre- and at 4 h post-oral dose, and 48 h post dose (Day 3), and on the last day of clinic (Day 8) prior to discharge and when clinically indicated.
4. 12-lead ECG will be conducted after 5 min resting. ECG data will be recorded at screening, on Day 6 at pre- and at 4 h post-oral dose, and when clinically indicated.

5. Clean sample to be collected at each visit. If urine is dipstick positive for leukocyte esterase or nitrites, sample is to be sent for microscopic evaluation and culture. Urine drug screen will be performed on screening visit only. If screening is conducted more than 5 days prior to dosing, drug screening shall be repeated.
6. Blood for clinical chemistry and hematology will be drawn after a minimum of 10 h fasting prior to breakfast. Infectious disease testing will be conducted at screening only.
7. Plasma samples for PK will be collected at pre-dose and at 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 36, and 48 h after oral administration of bexagliflozin on Day 1 and Day 6.
8. Urine samples for PD (UGE) analysis are described in Section 6.6.2.3.
9. If early termination occurs, activities scheduled for the last day of clinic prior to discharge should be conducted. Reasons for all withdrawals shall be recorded on the CRF.

C. SCHEDULE OF EVENTS FOR STUDY 3 (BEXAGLIFLOZIN AND VERAPAMIL):

	Screening		Treatment Period					
Study activity	**D -21 to -1**	**D0**	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**
Medical history and ICF	X							
Screening for I/E criteria[1]	X	X						
Physical exam[2]	X	X				X		X
Demographics	X							
Admission and discharge		X						X
Dispense investigational study drug bexagliflozin			X			X		
Dispense interaction drug verapamil						X		
Vital signs[3]	X		X		X	X		X
ECG[4]	X					X		
Urinalysis[5]	X		X			X		X
Blood draw for clinical lab tests[6]	X		X			X		X
Blood sample for PK[7]			X	X	X	X	X	X
Urine collection[8]		X	X	X	X	X	X	X
Urine pregnancy test	X	X						
Adverse event and concomitant medication		X	X	X	X	X	X	X
Study termination[9]								X

1. If screening is conducted more than 5 days prior to dosing, subject eligibility criteria must be confirmed on Day 0.
2. Weight and height will be recorded as part of the physical examination. Height will be recorded once at screening only. A complete physical exam (PE) will be performed at screening and last day of clinic prior to discharge. A partial PE will be performed on Day 0, and Day 4.
3. Vital signs include: pulse, body temperature, respiratory rate, systolic and diastolic blood pressure. On Day 1, and Day 4, vital signs will be determined at pre- and at 4 h post-oral dose, and 48 h post dose (Day 3), and on the last day of clinic (Day 6) prior to discharge and when clinically indicated.
4. 12-lead ECG will be conducted after 5 min resting. ECG data will be recorded at screening, on Day 4, at pre- and at 4 h post-oral dose and when clinically indicated.

5. Clean sample to be collected at each visit. If urine is dipstick positive for leukocyte esterase or nitrites, sample is to be sent for microscopic evaluation and culture. Urine drug screen will be performed on screening visit only. If screening is conducted more than 5 days prior to dosing, drug screening shall be repeated.
6. Blood for clinical chemistry and hematology will be drawn after a minimum of 10 h fasting prior to breakfast. Infectious disease testing will be conducted at screening only.
7. Plasma samples for PK will be collected at pre-dose and at 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 36, and 48 h after oral administration of bexagliflozin on Day 1 and Day 4.
8. Urine samples for PD (UGE) analysis are described in Section 6.6.2.3.
9. If early termination occurs, activities scheduled for the last day of clinic prior to discharge should be conducted. Reasons for all withdrawals shall be recorded on the CRF.

Appendix 2 Sponsor Signatures

Study Title: A Phase 1, Open-label, Non-randomized, Fixed-sequence Study to Evaluate the Effects of Probenecid, Rifampin, and Verapamil on the Pharmacokinetics and Pharmacodynamics of Bexagliflozin in Healthy Subjects

Study Number: THR-1442-C-454

Final Date: 28 August 2017

This clinical study protocol was subject to critical review and has been approved by the sponsor. The following personnel contributed to writing and/or approving this protocol:

Signed: Xiaoyan Li Date: Aug 28, 2017

Xiao-Yan Li, Ph.D.
Sponsor Study Representative
Massachusetts General Hospital

Signed: Yuan-di Halvorsen Date: 28 August 2017

Yuan-Di Halvorsen, Ph.D.
Sponsor Study Representative
Massachusetts General Hospital

Signed: Mason W Freeman Date: 28 Aug 2017

Mason W. Freeman, M.D.
Medical Monitor
Massachusetts General Hospital
Consultant for Theracos Sub, LLC

Appendix 3 Investigator's Signature

Study Title:	A Phase 1, Open-label, Non-randomized, Fixed-sequence Study to Evaluate the Effects of Probenecid, Rifampin, and Verapamil on the Pharmacokinetics and Pharmacodynamics of Bexagliflozin in Healthy Subjects
Study Number:	THR-1442-C-454
Final Date:	28 August 2017

I have read the protocol described above. I agree to comply with all applicable regulations and to conduct the study as described in the protocol.

Signed: _____ Date: 29 Aug 2017

Melanie Fein, M.D, CPI, DABFM
Principal Investigator/Medical Director
Covance, Clinical Research Unit