Clinical Trial Protocol: THR-1442-C-453

Study Title:	A phase 1, open-label, randomized, three-period, crossover study to evaluate pharmacokinetic interaction between bexagliflozin tablets and metformin, glimepiride, or sitagliptin in healthy subjects
Study Number:	THR-1442-C-453
Study Phase:	1
Product Name:	Bexagliflozin tablets
IND Number:	103822
Indication:	Type 2 Diabetes Mellitus
Investigators:	Single center
Sponsor:	Theracos Sub, LLC
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	Date
Original Protocol:	01 September 2016

Confidentiality Statement

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SYNOPSIS

Sponsor:

Theracos Sub, LLC

Name of Finished Product:

Bexagliflozin tablets

Name of Active Ingredient:

Bexagliflozin

Name of Inactive Ingredients:

Polyethylene oxide, glyceryl behenate, lactose monohydrate, micronized poloxamer 188, microcrystalline cellulose, colloidal silicon dioxide, and magnesium stearate (vegetable grade). The tablets are film-coated with Opadry II Blue with no markings on the tablets.

Study Title:

A phase 1, open-label, randomized, three-period, crossover study to evaluate pharmacokinetic interaction between bexagliflozin tablets and metformin, glimepiride, or sitagliptin in healthy subjects

Study Number:

THR-1442-C-453

Study Phase: 1

Primary Objective:

To evaluate the drug-drug interaction of co-administration of bexagliflozin with commonly prescribed oral hypoglycemic agents (OHAs) metformin, glimepiride, or sitagliptin on the pharmacokinetics (PK) of bexagliflozin and each OHA

Secondary Objective:

To evaluate the safety, tolerability, and pharmacodynamics (PD) of bexagliflozin when it is co-administered with commonly prescribed OHAs

Study Design:

In this study, a total of 54 healthy subjects will be enrolled and assigned to one of three groups of eighteen. Each group will participate in one of three open-label, randomized, three-period, three-treatment crossover studies:

Group 1: Bexagliflozin/metformin drug-drug interaction (DDI)

Group 2: Bexagliflozin/glimepiride DDI

Group 3: Bexagliflozin/sitagliptin DDI

Within each study, subjects will be randomized to one of six treatment sequences in an equal ratio. Each subject will receive a single dose of bexagliflozin tablet, 20 mg, alone, a single dose of an OHA (1000 mg metformin, 4 mg glimepiride or 100 mg sitagliptin) alone, and the combination of both (bexagliflozin tablet and OHA) alternately in a crossover fashion, with three treatment periods separated by a washout period of at least 7 days.

To prevent hypoglycemia, subjects assigned to Group 2 (bexagliflozin/glimepiride DDI) will receive 240 mL of a 20% glucose solution in water with study medication at the time of dosing, as well as 60 mL of a 20% glucose solution in water every 15 min for 4 hours post-dose.

For each treatment period in Group 1 (bexagliflozin/metformin DDI) and Group 2 (bexagliflozin/glimepiride DDI), subjects will be admitted to the clinic on the day before dosing and will stay in the clinic until 48 h post-dose. For Group 3 (bexagliflozin/sitagliptin DDI), subjects will be admitted to the clinic on the day before dosing and will stay in the clinic until 72 h post-dose.

For all Groups, blood samples for PK analysis will be collected in each period prior to dosing (pre-dose) and at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 36, and 48 h post-dose. For Group 3, PK blood samples will also be collected at 60 and 72 h post-dose.

Urine samples for PD analysis will be collected in 12 h intervals. For all Groups, urine samples will be collected pre-dose (-12 to 0 h) and post-dose at 0 to 12 h, 12 to 24 h, 24 to 36 h, and 36 to 48 h. For Group 3, additional samples at 48 to 60 h and 60 to 72 h post-dose will be collected.

Clinical laboratory tests and safety monitoring will be conducted during all treatment periods.

Study Population:

Approximately 54 healthy male and female subjects are planned to be enrolled.

Diagnosis and Main Criteria for Inclusion

1. Male and female subjects who are between 18 to 65 years of age, inclusive, in good health based on medical history, physical examination (PE), electrocardiogram (ECG) and routine laboratory tests.
2. Subjects with body-mass index (BMI) between 18.0 kg/m^2 and 32.0 kg/m^2, inclusive.
3. Subjects who are non-smokers for at least 3 months prior to screening.
4. Subjects with adequate venous access at multiple sites in both arms.
5. Subjects who are willing and able to be confined to the clinical research facility as required by the protocol.
6. Subjects who have the ability to comprehend and who are willing to provide written informed consent in accordance with institutional and regulatory guidelines.

Test Product, Dose, and Mode of Administration:

Bexagliflozin tablets, 20 mg, single dose per treatment period, oral administration

Reference Therapy, Dose, and Mode of Administration:

Metformin, 1000 mg, single dose per treatment period, oral administration
Glimepiride, 4 mg, single dose per treatment period, oral administration
Sitagliptin, 100 mg, single dose per treatment period, oral administration

Duration of Treatment:

After subjects are screened and randomized, the duration of the study will be up to 22 days from the day of initial investigational product administration. There will be 3 treatments separated by a washout period of at least 7 days.

Pharmacokinetic Assessment:

The following PK parameters of bexagliflozin and the OHAs will be determined when feasible after each subject is dosed with bexagliflozin, an OHA or a combination of both:

C_{max} Maximum observed plasma concentration

T_{max} Time of maximum observed plasma concentration

λ_z Terminal elimination phase rate constant

$T_{½}$ 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

Pharmacodynamic Assessment:

Urinary glucose excretion (UGE) will be derived from the urine samples collected in 12-hour batches in study subjects administered bexagliflozin or OHA alone or bexagliflozin with an OHA in combination. UGE_{t1-t2} (mg) will be derived from urine volume (V_{t1-t2}, mL) x glucose concentration (mg/dL)/100.

Safety Assessments:

- Vital signs
- Clinical laboratory tests including blood chemistry and hematology parameters
- Urinalysis
- Adverse events
- Concomitant medication use

Statistical Methods:

Statistical analysis will be performed using Statistical Analysis Software SAS for Windows® (SAS Institute Inc., USA). PK parameters of bexagliflozin will be calculated by non-compartmental analysis (NCA) of plasma concentration-time data. Non-compartmental analysis will be performed using Phoenix® WinNonlin® 6.4 (Certara, USA). To assess the effect of OHAs metformin, glimepiride or sitagliptin on the PK of bexagliflozin and vice versa, 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, period, and sequence as fixed effects. The 90% confidence intervals will be constructed for the (bexagliflozin and OHA in combination) to (bexagliflozin alone) or (OHA alone) ratio of the least squares (LS) geometric means of PK parameters (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$), 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}, 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 exception of T_{max}. Medians and ranges will be presented for T_{max}.

Descriptive statistics on the PD parameters will be also performed. The effect of OHA alone, bexagliflozin alone and OHA and bexagliflozin in combination on the cumulative UGE of healthy subjects will be compared.

Date of Original Protocol: 01 September 2016

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)
BMI	body mass index
BLOQ	below level of quantification
BP	blood pressure
C_{max}	maximum plasma drug concentration
C_{last}	concentration corresponding to T_{last}
CL/F	apparent oral clearance
CRF	case report form
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
K_2EDTA	potassium ethylenediaminetetraacetic acid
LS	least squares
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
PK	pharmacokinetic
SAE	serious adverse event

SD	standard deviation
SFU	sulfonylurea
SGLT2	sodium glucose cotransporter 2
SOP	standard operating procedure
TEAE	treatment emergent adverse event
$T_{1/2}$	terminal elimination half life
T_{last}	time of last measurable concentration
T_{max}	time to maximum plasma concentration
T2DM	type 2 diabetes mellitus
UGE	urine glucose excretion
ULN	upper limit of normal
UTI	urinary tract infection
V_z/F	apparent volume of distribution
WHO-DD	World Health Organization Drug Dictionary
WOCBP	women of child-bearing potential

Definitions of Terms

Study	The THR-1442-C-453 study refers to all activities within the protocol. Within THR-1442-C-453, there are 3 open-label, randomized, three-period, three-treatment crossover studies. Each of the 3 studies will enroll a Group of 18 subjects (see Group below).
Group	The 54 subjects in the THR-1442-C-453 study will be assigned to one of 3 groups of 18 subjects. Each Group will be treated with bexagliflozin and one of the three oral hypoglycemic agents (OHAs), metformin (Group 1), sitagliptin (Group 2), or glimepiride (Group 3).
Sequence	Each Group of 18 subjects will be randomized to participate in 1 of 6 treatment sequences with 3 subjects in each sequence. Each sequence will have a different order of 3 possible treatments: bexagliflozin alone, OHA alone, or bexagliflozin and OHA in combination.
Period	Each Sequence will have 3 treatment periods. During each Period, subjects will receive 1 of 3 possible treatments (bexagliflozin alone, OHA alone, or bexagliflozin and OHA in combination).

1 INTRODUCTION

Diabetes mellitus, a metabolic disease characterized by prolonged hyperglycemia, is one of the leading causes of morbidity and mortality worldwide, affecting an estimated 387 million people as of 2014 (IDF, 2014). More than 95% of people with diabetes have type 2 diabetes mellitus (T2DM) (IDF, 2014).

Several classes of oral agents are available for treating T2DM, including biguanides, sulfonylureas (SFU), or dipeptidyl peptidase-4 (DPP-4) inhibitors. Metformin (a biguanide), sitagliptin (an SFU) and glimepiride (a DDP-4 inhibitor) are 3 commonly prescribed oral hypoglycemic agents (OHAs) with different mechanism of actions (Tahrani et al., 2016). Metformin is the first-line treatment for T2DM that works by increasing insulin sensitivity of body tissues and decreasing glucose production by the liver. Sitagliptin causes elevation of circulating incretins, which results in enhanced nutrient-induced insulin secretion. Glimepiride increases secretion of insulin by the pancreas.

Sodium glucose cotransporter 2 (SGLT2) inhibitors are a new class of OHAs that targets the renal Na+/glucose transport protein, SGLT2. SGLT2 inhibitors increase excretion of glucose in the urine and thereby lower fasting and post-prandial glucose levels. SGLT2 inhibitors increase urinary glucose excretion without increasing insulin secretion, causing weight gain, or inducing hypoglycemia (Nauck, 2014).

Bexagliflozin is a candidate OHA that is a potent and highly specific inhibitor of SGLT2. (Zhang et al., 2011). Bexagliflozin has been shown to cause dose-dependent increases in urinary glucose excretion (UGE) in humans, rats, dogs and monkeys and to reduce HbA1c in animal models of T2DM and in diabetic subjects. Detailed information regarding bexagliflozin clinical studies and potential risks for study subjects are provided in the Investigator's Brochure.

Bexagliflozin is cleared predominantly by metabolism to an inactive metabolite, bexagliflozin 3-O-glucuronide by uridine diphosphate glucuronosyltransferase isoform 1A9 (UGT1A9) pathway.

The aim of the study is to evaluate potential drug-drug interaction (DDI) effects on the pharmacokinetics (PK) and pharmacodynamics (PD) of co-administration of bexagliflozin with three commonly prescribed OHAs, i.e., metformin, glimepiride, or sitagliptin.

2 STUDY OBJECTIVES

2.1 Primary Objective

The primary objective of this study is to evaluate whether drug-drug interactions following co-administration of bexagliflozin with commonly prescribed OHAs metformin, glimepiride, or sitagliptin result in any change in the PK of bexagliflozin or each of the OHAs.

2.2 Secondary Objective

The secondary objective of this study is to evaluate the safety, tolerability, and PD of bexagliflozin following single dose administration of the drug in combination with commonly prescribed OHAs.

3 INVESTIGATIONAL PLAN

3.1 Overall Study Design and Plan

In this study, a total of 54 healthy subjects will be enrolled and assigned to three groups of eighteen. Each group will participate in one of three open-label, three treatment period, crossover studies:

Group 1: Bexagliflozin/metformin drug-drug interaction (DDI)
Group 2: Bexagliflozin/glimepiride DDI
Group 3: Bexagliflozin/sitagliptin DDI

For each Group, every subject will receive a single dose of bexagliflozin tablet, 20 mg, alone, a single dose of an OHA (1000 mg metformin, 4 mg glimepiride or 100 mg sitagliptin) alone, and the combination of both (bexagliflozin tablet and OHA) alternately in a crossover fashion, in three treatment periods separated by a washout period of at least 7 days. Within each Group, subjects will be randomized to one of six treatment sequences in an equal ratio as shown in Table 1.

Table 1. Treatment Sequences

Treatment Sequence	Treatment Period 1	Treatment Period 2	Treatment Period 3
1	Bexa alone[1]	OHA alone[2]	Bexa + OHA
2	Bexa alone	Bexa + OHA	OHA alone
3	OHA alone	Bexa alone	Bexa + OHA
4	OHA alone	Bexa + OHA	Bexa alone
5	Bexa + OHA	OHA alone	Bexa alone
6	Bexa + OHA	Bexa alone	OHA alone

[1] Bexa: bexagliflozin; OHA: oral hypoglycemic agent

To prevent hypoglycemia, subjects assigned to Group 2 will receive a 20% oral glucose solution with study medication at the time of dosing, as well as a 20% glucose solution every 15 min for 4 hours post-dose.

During each treatment period for Group 1 (bexagliflozin/metformin DDI) and Group 2 (bexagliflozin/glimepiride DDI), subjects will be admitted to the clinic on the day before dosing and will stay in the clinic until 48 h post-dose. For Group 3 (bexagliflozin/sitagliptin DDI), subjects will stay in the clinic until 72 h post-dose.

For Group 1 and 2, blood samples for PK analysis will be collected in each period prior to dosing (pre-dose) and at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 36, and 48 h post-dose. For Group 3, PK blood samples will also be collected at 60 and 72 h post-dose. Plasma

concentrations of bexagliflozin and OHAs will be determined by validated liquid chromatography tandem mass spectrometry (LC-MS/MS) assays.

Urine samples for PD analysis will be collected in 12 h intervals. For Group 1 and 2, urine samples will be collected pre-dose (-12 to 0 h) and post-dose at 0 to 12 h, 12 to 24 h, 24 to 36 h, and 36 to 48 h. For Group 3, additional samples at 48 to 60 h and 60 to 72 h post-dose will be collected.

Clinical laboratory tests and safety monitoring will be conducted during all treatment periods 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

Each of the 3 Groups within THR-1442-C-453 will participate in a randomized, three-period, three-treatment crossover study. This design was chosen to properly separate treatment effects from period effects.

Three commonly prescribed OHAs, metformin, sitagliptin and glimepiride, were chosen for the THR-1442-C-453 study.

Sitagliptin does not undergo extensive metabolism. After oral exposure, the parent drug comprises the majority of plasma and urinary drug concentrations (Vincent et al., 2007). Sitagliptin metabolites were detected in small amounts and *in vitro* experiments found that CYP3A4 and, to a lesser extent, CYP2C8, were the major CYP isozymes responsible for the limited metabolism (Vincent et al., 2007). Metformin also does not undergo significant metabolism, and both metformin and sitagliptin are eliminated unchanged in urine via tubular secretion and glomerular filtration (Pentikainen et al., 1979). Glimepiride is mainly metabolized by CYP2C9 and the metabolites are excreted in urine (Langtry and Balfour, 1998).

The metabolic pathway for bexagliflozin is different from that of the 3 OHAs (metformin, sitagliptin, and glimepiride), which suggests a low potential for metabolic drug-drug interaction between bexagliflozin and the OHAs being tested. *In vitro* metabolism studies demonstrated that bexagliflozin is not a potent inhibitor or inducer of CYP450 enzymes. UGT1A9 is principally responsible for the glucuronidation of bexagliflozin. In the urine, unchanged parent drug accounted for less than 2% of the dose.

3.2.2 Rationale for Dose Selection

Bexagliflozin produces a dose-dependent, saturable increase in UGE in healthy volunteers and diabetic subjects. Population pharmacodynamic modeling has indicated that bexagliflozin doses of 20 mg result in 90% of the maximal UGE. In a long term treatment study, daily administration of 20 mg bexagliflozin was found to reduce HbA1c by 0.79% compared to placebo at week 24. In addition, adverse events (AEs), particularly those involving urinary tract infection (UTI) and genital mycotic infection (GMI), were found to be

similar between placebo and active agent cohorts. To evaluate the drug-drug interaction of co-administration of bexagliflozin with metformin, glimepiride, or sitagliptin, bexagliflozin tablets, 20 mg, which is intended to be the commercial product, will be administered in this trial.

The dosages for the OHAs are equivalent to the highest single tablet dose available for each OHA (1000 mg metformin, 4 mg glimepiride or 100 mg sitagliptin), and are within recommended daily dose ranges.

3.2.3 Rationale for PK and PD Sampling Time Points

The $T_{½}$ of bexagliflozin is 7.80 to 9.71 h, and $T_{½}$ of metformin and glimepiride are 4-9 h and 5-9 h, respectively. Based on the biological half-lives of the study drugs, the PK sampling time points for Group 1 (bexagliflozin/metformin DDI) and Group 2 (bexagliflozin/ glimepiride DDI) are pre-dose, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 36, and 48 h post-dose. For Group 3 (bexagliflozin/sitagliptin DDI), two additional PK sampling time points, at 60 and 72 h post-dose, will be added as the $T_{½}$ for sitagliptin is 12.4 h.

Urine samples for PD analysis will be collected in 12 h intervals from -12 h pre-dose to 48 h post dose (-12 to 0 h, 0 to 12 h, 12 to 24 h, 24 to 36 h, and 36 to 48 h) for Groups 1 and 2, and additional samples at 48 to 60 h and 60 to 72 h post-dose will be collected for Group 3.

3.3 Study Duration and Dates

Subjects will be screened within 28 days of the planned initiation of investigational product dosing. Eligible subjects who consent to the study will be assigned to 1 of 3 Groups. Subjects in each Group will be randomly assigned to receive 1 of 6 treatment regimens in a crossover fashion with three treatment periods separated by a washout period of at least 7 days. Up to 2 additional washout days will be allowed.

For each treatment period in Group 1 (bexagliflozin/metformin DDI) and Group 2 (bexagliflozin/glimepiride DDI), subjects will be admitted to the clinic on the day before dosing (day 0 for treatment period 1, day 7 for treatment period 2 and day 14 for treatment period 3) and will stay in the clinic until 48 h post-dose. For Group 3 (bexagliflozin/sitagliptin DDI), subjects will be admitted on the day before dosing (day 0 for treatment period 1, day 7 for treatment period 2 and day 14 for treatment period 3), but will stay in the clinic until 72 h post-dose.

The duration of the overall study from screening until study termination is estimated to be a maximum of 50 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

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

4.2 Inclusion Criteria

Each subject must meet the following criteria to be enrolled in this study.

1. Male and female subjects who are between 18 to 65 years of age, inclusive, in good health based on medical history, physical examination (PE), electrocardiogram (ECG) and routine laboratory tests.
2. Subjects with body-mass index (BMI) between 18.0 kg/m^2 and 32.0 kg/m^2, inclusive.
3. Subjects who are non-smokers for at least 3 months prior to screening.
4. Subjects with adequate venous access at multiple sites in both arms.
5. Subjects who are willing and able to be confined to the clinical research facility as required by the protocol.
6. Subjects who have the ability to comprehend and who are willing to provide written informed consent in accordance with institutional and regulatory guidelines.

4.3 Exclusion Criteria

Subjects who meet any of the following criteria will be excluded from the study.

1. Subjects who are determined by the investigator or sub-investigator to be unsuitable for participating in the study based on medical conditions or factors that would influence adherence to study activities.
2. Subjects with a clinically significant history of allergy to drugs or latex.
3. Subjects with a history of alcohol or drug dependence in the last 12 months.
4. Subjects who have donated 400 mL of whole blood within 56 days, 200 mL of whole blood within one month, or donated blood components within 14 days of screening.
5. Subjects who have used prescription or over-the-counter (OTC) drugs within 14 days prior to the first dose.
6. Subjects who have used vitamin preparations within 7 days or supplements (including St. John's Wort and ginseng) within 14 days prior to the first dose.
7. Subjects who have undergone strenuous physical activity within 72 hours prior to dosing in each period.
8. Subjects who have been treated with 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.

9. Subjects who had previously received EGT0001474 or bexagliflozin, or any other SGLT2 inhibitors within 3 months from screening or have participated in previous bexagliflozin clinical trials., regardless of whether they received bexagliflozin or placebo in those trials.
10. Subjects who had previously received OHAs, including metformin, sitagliptin, glimepiride or drugs of the same class (i.e. biguanides, DPP-4 inhibitors or sulfonylureas), within 3 months of screening.
11. Subjects whose screening ECG 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.
12. Subjects whose sitting blood pressure is above 140/90 mmHg at screening. If the sitting blood pressure at screening is above 140/90 mmHg, one repeat measurement is allowed and the subject may be randomized if the repeat screening blood pressure is 140/90 +/-5 mmHg at the discretion of the Investigator.
13. Subjects who have a positive result of hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV) antibody, urinary drug or urinary cotinine test.
14. Subjects with known human immunodeficiency virus (HIV) infection.
15. Subjects who have had a febrile illness within 5 days prior to the first dose of investigational drug.
16. Subjects vaccinated (with the exception of the flu vaccine) within 30 days prior to the first dose of investigational drug.
17. Subjects with estimated glomerular filtration rate (eGFR) < 90 mL/min/1.73 m^2 or a history of kidney transplant.
18. Male subjects who do not 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.
19. Female subjects of childbearing potential who are not willing to use an adequate method of contraception and to not become pregnant for the duration of the study. Female subjects who are surgically sterile (hysterectomy, oophorectomy) or postmenopausal (absence of menses greater than 12 months and age > 45 years) are eligible if they test negative on the urine pregnancy test.

5 STUDY TREATMENTS

5.1 Description of Treatments

5.1.1 Investigational products

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 Oral Hypoglycemic Agents

Three OHAs that are frequently prescribed in patients with T2DM are included in the study.

5.1.2.1 Metformin

Metformin is a biguanide, which decreases hepatic glucose production and may improve peripheral glucose uptake and utilization. After oral administration, metformin is absorbed from the gastrointestinal tract. Metformin is excreted unchanged in the urine and does not undergo hepatic metabolism or biliary excretion.

5.1.2.2 Glimepiride

Glimepiride is a 2nd generation SFU, which stimulates glucose-independent insulin release from the pancreas. After oral administration, glimepiride is absorbed from the gastrointestinal tract. Glimepiride is metabolized mostly by hepatic P450 enzyme CYP2C9.

5.1.2.3 Sitagliptin

Sitagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor. After oral administration, sitagliptin is absorbed from the gastrointestinal tract. Sitagliptin is metabolized by hepatic P450 enzyme CYP3A4 and CYP2C8.

5.2 Treatments Administered

Subjects who consent to be in the study will be assigned to 1 of 3 groups. Each subject will receive a single oral dose of bexagliflozin tablet, 20 mg, alone, a single dose of one other OHA alone, and the combination of both (bexagliflozin tablet and OHA) alternately in a crossover fashion in three treatment periods separated by a washout period of at least 7 days:

Group 1: Bexagliflozin tablet, 20 mg, and metformin, 1000 mg
Group 2: Bexagliflozin tablet, 20 mg, and glimepiride, 4 mg
Group 3: Bexagliflozin tablet, 20 mg, and sitagliptin, 100 mg

5.3 Selection and Timing of Dose for Each Subject

Dosing order with bexagliflozin alone, OHA alone, or bexagliflozin in combination with OHA will be based on randomized assignment. Bexagliflozin and OHAs will be administered with 240 mL of liquid (water for Group 1 and 3, 20% glucose solution for Group 2). If for some reason, a portion of the liquid is not consumed, the reason and the amount of remaining liquid will be recorded.

5.3.1 Group 1: Bexagliflozin and Metformin

- **Bexagliflozin alone**: After an overnight fast of at least 10 hours, a bexagliflozin tablet, 20 mg, will be administered before the first meal of the day with 240 mL of water.
- **Metformin alone**: After an overnight fast of at least 10 hours, metformin, 1000 mg, will be administered before the first meal of the day with 240 mL of water.
- **Co-administration of bexagliflozin and metformin**: After an overnight fast of at least 10 hours, a bexagliflozin tablet, 20 mg, and metformin, 1000 mg, will be administered before the first meal of the day with 240 mL of water.

5.3.2 Group 2: Bexagliflozin and Glimepiride

- **Bexagliflozin alone**: After an overnight fast of at least 10 hours, a bexagliflozin tablet, 20 mg, will be administered before the first meal of the day with 240 mL 20% glucose solution in water. Subjects will be administered 60 mL of 20% glucose solution in water every 15 minutes during the first 4 hours following administration of bexagliflozin.
- **Glimepiride alone**: After an overnight fast of at least 10 hours, glimepiride, 4 mg, will be administered before the first meal of the day with 240 mL of 20% glucose solution in water. Subjects will be administered 60 mL of 20% glucose solution in water every 15 minutes during the first 4 hours following administration of glimepiride.
- **Co-administration of bexagliflozin and glimepiride**: After an overnight fast of at least 10 hours, a bexagliflozin tablet, 20 mg, and glimepiride, 4 mg, will be administered before the first meal of the day with 240 mL of 20% glucose solution in water. Subjects will be administered 60 mL of 20% glucose solution in water every 15 minutes during the first 4 hours following administration of bexagliflozin and glimepiride.

5.3.3 Group 3: Bexagliflozin and Sitagliptin

- **Bexagliflozin alone**: After an overnight fast of at least 10 hours, a bexagliflozin tablet, 20 mg, will be administered before the first meal of the day with 240 mL of water.
- **Sitagliptin alone**: After an overnight fast of at least 10 hours, sitagliptin, 100 mg, will be administered before the first meal of the day with 240 mL of water.

- **Co-administration of bexagliflozin and sitagliptin**: After an overnight fast of at least 10 hours, a bexagliflozin tablet, 20 mg, and sitagliptin, 100 mg, will be administered before the first meal of the day with 240 mL of water.

5.4 Method of Assigning Subjects to Treatment Groups and Sequences

A total of 54 healthy subjects will be enrolled and assigned to one of three groups of eighteen. Each group of eighteen will be randomized to 1 of 6 possible treatment sequences. Subjects who discontinue the study early will not be replaced.

5.5 Blinding

This is an open-label study.

5.6 Concomitant Therapy

The participants are not allowed to take any prescription or non-prescription drugs, or dietary supplements at any time during the 14 days prior to first drug administration and for the duration of all three study periods. Vitamins can be taken up to 7 days prior to first study drug administration. No other concomitant medications are permitted with the exception of those required for treatment of an AE. Subjects may receive any medications for AEs that are necessary to control or minimize the likelihood of more serious adverse events in the investigators' judgment.

Concomitant medications administered at the time of randomization and 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 must be recorded. This documentation should continue until the end of the last study period. Medications that a subject receives after entering the study and prior to randomization must be recorded in the CRF.

5.7 Restrictions

5.7.1 Prior Therapy

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

5.7.2 Fluid and Food Intake

An overnight fast of at least 10 h prior to dosing is required. Water is permitted during the fasting period up to 1 hour prior to and after the administration of each investigational

product administration. Adequate hydration is encouraged during each study period. Food can be consumed 4 h after dosing.

- Bexagliflozin will be taken before the first meal of the day with 240 mL of water, except during Group 2 (bexagliflozin/glimepiride DDI) when bexagliflozin will be administered with 240 mL 20% glucose solution in water and 60 mL 20% glucose solution in water every 15 minutes during the first 4 hours following administration of bexagliflozin.
- Metformin will be taken before the first meal of the day with 240 mL of water.
- Glimepiride will be taken before the first meal of the day with 240 mL 20% glucose solution in water and 60 mL of 20% glucose solution in water will be provided every 15 minutes during the first 4 hours following administration of glimepiride.
- Sitagliptin will be taken before the first meal of the day with 240 mL of water.

5.7.3 Subject Activity Restrictions

Light physical activity is permitted. Subjects should not perform strenuous activity which could result in elevations of muscle creatine kinase levels. Smoking during the study is prohibited.

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 checks followed by hand mouth inspection.

5.9 Packaging and Labeling

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

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.

5.10 Storage and Accountability

Drug supplies should be stored at controlled room temperature (15° to 30°C) in a secure area with access limited to authorized personnel. The sponsor will perform an ongoing inventory of study products. The responsible pharmacist must keep a careful inventory of drug shipments received and the number of tablets dispensed per study subject. A full reconciliation of drug inventory will be performed at the end of the study and the results of this inventory must be recorded in the Drug Accountability Form. Empty and partially used bottles may be discarded after use according to the study sites' 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 in the clinical study, written informed consent will be obtained from the subjects according to the regulatory and legal requirements. As part of this procedure, the investigator or designee must 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 should 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 must be informed that he/she is free to withdraw from the study at any time. He/she will receive all information that is required by federal regulations and International Council on Harmonisation (ICH) guidelines.

The informed consent document must be signed and dated. One copy will be given to the subjects, 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 when it was obtained must 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, surgical history and timeframe of the history relative to study screening, if applicable.
- Clinically significant history of allergy including drugs and latex.
- History of smoking in the last 3 months, and alcohol or drug dependence, or abuse in the last 12 months.
- Any blood donation within 56 days or blood component donation within 14 days.
- Use of any medications including OTC drugs, or dietary supplements in the last 14 days. Use of any vitamins in the last 7 days.
- History of vaccination (except the flu vaccine) within 30 days prior to the first dose of study medication.
- History of diagnosis with HIV, hepatitis B or hepatitis C.
- Use of any investigational drug in the previous 30 days or 7 half-lives, whichever time frame is longer.
- Prior exposure to bexagliflozin (or EGT0001474), or any other SGLT2 inhibitors in the last 3 months.

- Prior exposure to metformin, sitagliptin, glimepiride, or drugs of the same class (biguanides, DPP-4 inhibitors, or sulfonylureas) in the last 3 months.

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 period 3 prior to discharge. Partial PEs will be performed at scheduled visits.

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 temperature, will be measured at the scheduled visits described in Appendix 1.

Vital signs should be measured prior to blood draws.

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 measures will be obtained using a calibrated sphygmomanometer. Devices designed to measure BP from the finger or wrist may not be used.

6.5 Electrocardiography

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

This procedure should 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 should 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 needs to ascertain if this is a clinically significant change from the screening ECG for that individual subject. This determination, however, does not necessarily need to 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 an abnormal change from baseline for that subject, this is considered an AE.

6.6 Clinical Laboratory Tests

6.6.1 Laboratory Parameters

Subjects will be in a seated or in a supine position during blood collection. Clinical blood chemistry and hematology tests will be performed at the scheduled visits (Appendix 1). Blood samples should be drawn after overnight fasting prior to breakfast. The details of the required laboratory tests are listed in Table 2.

Table 2. 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 and Electrolytes		5.0 (serum)
Albumin	Calcium	
Alanine aminotransferase (ALT)	Magnesium	
Aspartate aminotransferase (AST)	Phosphorus	
Blood urea nitrogen	Potassium	
Glucose	Sodium	
Bicarbonate	Total bilirubin	
Creatinine	Direct bilirubin	
Chloride	Uric acid	
Total protein		
Serum Lipids		3.5 (serum)
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 (in 12-h batches)		All (urine)
Glucose		
Creatinine		
Urine Drug Screen		10 (urine)
Amphetamines	Opiates	
Barbiturates	Benzodiazepines	
Cocaine Metabolites	Cannabinoids	
Cotinine		
Urine Pregnancy Test (Female only)		5 (urine)
Infectious Disease Testing		5.0 (serum)
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 per schedule outlined in Section 7 and in Appendix 1. Dipstick urinalysis will be conducted. Microscopy will be obtained if the subject has a positive result on any of the dipstick tests that require microscopic follow-up to clarify their significance. 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

Pre-dose urine samples must be collected from -12 to 0 h for baseline measurement. Subjects will empty their bladders prior to dosing. Post-dose urine will be collected without preservative in four (4) batches for Groups 1 and 2 at 0 to 12 h, 12 to 24 h, 24 to 36 h, and 36 to 48 h collections. Post-dose urine will be collected in six (6) batches for Group 3 at 0 to 12 h, 12 to 24 h, 24 to 36 h, and 36 to 48 h, 48 to 60 h and 60 to 72 h. Urine must be refrigerated at 2-8^{o}C during collection. After collection, the total volume of each batch and collection time will be recorded. A 20 mL aliquot will be prepared from well mixed urine collections. The samples will be analyzed for urinary glucose (for PD analysis) and creatinine.

6.6.2.4 Plasma Sample Collection for PK

Whole venous blood samples of 5 mL will be collected from a peripheral vein in each period at pre-dose and at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 36, and 48 h post-dose for Group 1 and 2. PK blood samples will be collected at pre-dose and at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 36, 48, 60 and 72 h post-dose for Group 3. Blood samples will be collected in tubes containing potassium ethylenediaminetetraacetic acid (K_2EDTA) and stored on ice until centrifuged under refrigeration for at least 10 min at 3,000 rpm. After centrifugation, plasma will be removed, divided into 2 aliquots of approximately 1 mL, frozen and stored at or below -20°C. Plasma should be processed and frozen within 2 h of blood collection. Processed frozen plasma samples will be transferred on dry ice to the analytical laboratory and will be stored at or below -20^{o}C until analysis.

6.7 Adverse Events Assessments

Adverse Event (AE*)*: Any untoward medical occurrence in 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, for example), symptom, or disease temporally associated with the use of

an investigational product, whether or not it is considered related 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.

Clinical Laboratory Changes: It is the investigator's responsibility to review the results of all laboratory tests as they become available. For each abnormal laboratory test result, the investigator needs to ascertain if this is a clinically significant change from baseline 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 an abnormal 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 laboratory utilized 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: The site and database should ask for the causality relative to the study compound. 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 collection will begin on the first clinical admission day (Day 0). 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 must be recorded on the source documents and CRFs provided by the sponsor.

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

6.7.2 Immediately Reportable Adverse Events

The investigator must report any serious SAE, by telephone, email, or fax, to Theracos or its representative immediately after the investigator becomes aware of the event. An IRAE form should 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) should be reported to Theracos within 3 working days. The IRAE form should be completed and sent by email or fax or overnight courier to the sponsor.

Subjects experiencing an SAE should 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 Pregnancy

Women of childbearing potential (WOCBP) who are sexually active must use an effective method of birth control during the course of the study, in a manner such that risk of failure is minimized.

Before enrolling WOCBP in this clinical trial, investigators must review guidelines about study participation for WOCBP. The topics should generally include:

1. General information.
2. Informed consent form.
3. Pregnancy prevention information.
4. Drug interactions with hormonal contraceptives.
5. Contraceptives in current use.
6. Guidelines for the follow-up of a reported pregnancy.

Prior to study enrollment, WOCBP must be advised of the importance of avoiding pregnancy during trial participation and the potential risk factors for an unintentional pregnancy. The subject must sign an informed consent form stating the above-mentioned risk factors and that the consequences were discussed with her.

During the study, all WOCBP should be instructed to contact the investigator immediately if they suspect they might be pregnant (e.g., missed or late menstrual cycle).

If a subject or investigator suspects that the subject may be pregnant prior to investigational product administration, the investigational product administration must be withheld until the results of serum pregnancy tests are available. If the pregnancy is confirmed, the subject must not receive the investigational product and must not remain in or be enrolled in the study.

The investigator must notify the Medical Monitor within 3 working days of the receipt of information that any female subject who has become pregnant.

The investigator must record the event on the Pregnancy Surveillance Form and forward it to the sponsor's clinical or designated personnel.

6.7.4 Follow-up of Adverse Events

6.7.4.1 Follow-up of Non-serious Adverse Events

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

6.7.4.2 Follow-up of Post-Study Serious Adverse Events

SAEs that are identified on the last scheduled contact must be recorded on the AE CRF page and reported to Theracos according to the reporting procedures outlined in Section 6.7.1. These may include unresolved previously reported SAEs, or new SAEs. The investigator should follow these subjects until the events are resolved, or the subject is lost to follow-up. Resolution means the subject has returned to the baseline state of health, or the investigator does not expect any further improvement or worsening of the subject's condition. The investigator should continue to report any significant follow-up information to Theracos until the event has been resolved.

Any new SAEs reported by the subject to the investigator that occur after the last scheduled contact, and are determined by the investigator to be reasonably associated with the use of the investigational product, should 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 the defined study period (i.e., up to last scheduled contact). The investigator should follow subjects with SAEs identified after the last scheduled contact until the events are resolved, or the subject is lost to follow-up. The investigator should continue to report any significant follow-up information to the Sponsor until the event has been resolved. This study requires that subjects be actively monitored for SAEs for at least 14 days after discharge from the study.

6.7.4.3 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 should be stopped and the event should be reported as an adverse event within the CRF if the enzyme elevation is confirmed or worsening. Potential contributors to hepatic enzyme elevation should be evaluated by the investigator. The investigator is encouraged to consult with the Medical Monitor regarding ongoing diagnostic workup.

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

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

6.7.4.4 Hypoglycemia

Hypoglycemia will be recorded under 5 categories:

- Critical hypoglycemia: An event requiring assistance of another person to actively administer carbohydrate, glucagons, 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 should 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 with diabetes 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).

6.8 Concomitant Medication Assessments

A concomitant medication is any medication the subject enters the trial taking and is expected to continue taking for some portion of the trial, as well as any medication the subject takes during the course of the trial. All prescription and over-the-counter medications, including vitamins and herbal supplements, that subjects receive during the trial must be documented on the CRF. This documentation should continue until the subjects are discharged.

Concomitant medications will 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 will be produced. A listing of concomitant medications will include all medications taken by any subjects during the course of the study.

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

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

The investigator must 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 will 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 will be informed immediately. If there is a medical reason for withdrawal, the volunteer should remain under the supervision of the medical investigator until satisfactory health returns.

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

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

The investigator may withdraw a subject from the THR-1442-C-453 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, ECG and clinical laboratory tests if clinically indicated 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 Screening (Days -28 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 must be repeated prior to dosing. Drug abuse testing includes amphetamines, barbiturates, cocaine metabolites, opiates, benzodiazepines, 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 Period 1

7.2.1 Day 0 (Clinic Admission)

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

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

- If the subject is still eligible based on the study inclusion and exclusion criteria, the subject will be admitted and randomized into the study.
- Concomitant medications and adverse event information will be collected as appropriate.
- Pre-dose urine collection in 12 h batches will begin with the -12 h to 0 h batch for baseline analysis of creatinine and for UGE (PD) analysis as described in Section 6.6.2.3.

7.2.2 Day 1 (Dosing Day): Pre-dose Activities

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

- Vital signs will be recorded pre-dose.
- 12-lead ECG will be taken in the supine position after at least 5 min of rest pre-dose.
- Pre-dose urine will be collected until 0 h (-12 h to 0 h batch) for baseline 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.
- 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.

7.2.3 Day 1 (Dosing Day): Dosing

- For Group 1 subjects, after an overnight fast of at least 10 h, bexagliflozin tablets, 20 mg, alone, metformin, 1000 mg, alone or bexagliflozin and metformin in combination will be administered prior to first meal of the day as detailed in Section 5.3.1.
- For Group 2 subjects, after an overnight fast of at least 10 h, bexagliflozin tablets, 20 mg, alone, glimepiride, 4 mg, alone or bexagliflozin and glimepiride in combination will be administered prior to first meal of the day with glucose solution as detailed in Section 5.3.2.
- For Group 3 subjects, after an overnight fast of at least 10 h, bexagliflozin tablets, 20 mg, alone, sitagliptin, 100 mg, alone or bexagliflozin and sitagliptin in combination will be administered prior to first meal of the day as detailed in Section 5.3.3.

7.2.4 Day 1: Post-dose Activities

- Vital signs will be recorded at 4 h post-dose.
- 12-lead ECG will be taken in the supine position after at least 5 min of rest at 4 h post-dose.
- Plasma samples will be drawn for PK analysis as detailed in Section 6.6.2.4 at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16 h post-dose.
- Urine for analysis of creatinine and UGE will be collected in 12 h batches at 0 to 12 h and 12 to 24 h post-dose as described in Section 6.6.2.3.
- Post-dose concomitant medications and adverse event information will be collected as appropriate.

7.2.5 Day 2: Post-dose Activities

- Plasma samples will be drawn for PK analysis as detailed in Section 6.6.2.4 at 24 and 36 h post-dose.
- Urine for analysis of creatinine and UGE will be collected in 12 h batches 12 to 24 h, 24 to 36 h and 36 to 48 h as described in Section 6.6.2.3.
- Post-dose concomitant medications and adverse event information will be collected as appropriate.

7.2.6 Day 3: Post-dose Activities

- Plasma samples will be drawn for PK analysis as detailed in Section 6.6.2.4 at 48 h post-dose for all Groups and additionally at 60 h post-dose for Group 3.
- Urine for analysis of creatinine and UGE will be collected in 12 h batches at 36 to 48 h post-dose for all Groups and additionally at 48 to 60 h and 60 to 72 h post-dose for Group 3 as described in Section 6.6.2.3.
- Post-dose concomitant medications and adverse event information will be collected as appropriate.
- For Group 1 and 2, vital signs will be recorded at 48 h post-dose.
- For Group 1 and 2, urine will be collected for urinalysis as described in Section 6.6.2.2.
- For Group 1 and 2, blood will be drawn for hematology and serum chemistry as detailed in Section 6.6.2.1.
- For Group 1 and 2, the subject will be discharged after completion of all activities and scheduled for admission for Period 2.

7.2.7 Day 4 (Group 3 Only): Post-dose Activities

- Vital signs will be recorded at 72 h post-dose.
- Plasma samples will be drawn for PK analysis as detailed in Section 6.6.2.4 at 72 h post-dose.
- Urine for analysis of creatinine and UGE will be collected in 12 h batches at 60 to 72 h post-dose as described in Section 6.6.2.3.
- Post-dose 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.
- Blood will be drawn for hematology and serum chemistry as detailed in Section 6.6.2.1.
- Subject will be discharged after completion of all activities and scheduled for admission for Period 2.

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 must be completed. The reason for termination must be entered onto the case report form.

7.3 Period 2

7.3.1 Day 7 (Clinic Admission)

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

- All female subjects will receive urine pregnancy test.
- If the subject is still eligible based on the study inclusion and exclusion criteria, the subject will be admitted.
- Concomitant medications and adverse event information will be collected as appropriate.
- Pre-dose urine collection in 12 h batches will begin with the -12 h to 0 h batch for baseline analysis of creatinine and for UGE (PD) analysis as described in Section 6.6.2.3.

7.3.2 Day 8 (Dosing Day): Pre-dose Activities

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

- Vital signs will be recorded pre-dose.
- 12-lead ECG will be taken in the supine position after at least 5 min of rest pre-dose.
- Pre-dose urine will be collected until 0 h (-12 h to 0 h batch) for baseline 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.
- 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.

7.3.3 Day 8 (Dosing Day): Dosing

- For Group 1 subjects, after an overnight fast of at least 10 h, bexagliflozin tablets, 20 mg, alone, metformin, 1000 mg, alone or bexagliflozin and metformin in combination will be administered prior to first meal of the day as detailed in Section 5.3.1.
- For Group 2 subjects, after an overnight fast of at least 10 h, bexagliflozin tablets, 20 mg, alone, glimepiride, 4 mg, alone or bexagliflozin and glimepiride in combination will be administered prior to first meal of the day with glucose solution as detailed in Section 5.3.2.
- For Group 3 subjects, after an overnight fast of at least 10 h, bexagliflozin tablets, 20 mg, alone, sitagliptin, 100 mg, alone or bexagliflozin and sitagliptin in combination will be administered prior to first meal of the day as detailed in Section 5.3.3.

7.3.4 Day 8 (Dosing Day): Post-dose Activities

- Vital signs will be recorded at 4 h post-dose.
- 12-lead ECG will be taken in the supine position after at least 5 min of rest at 4 h post-dose.
- Plasma samples will be drawn for PK analysis as detailed in Section 6.6.2.4 at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16 h post-dose.
- Urine for analysis of creatinine and UGE will be collected in 12 h batches at 0 to 12 h and 12 to 24 h post-dose as described in Section 6.6.2.3.
- Post-dose concomitant medications and adverse event information will be collected as appropriate.

7.3.5 Day 9: Post-dose Activities

- Plasma samples will be drawn for PK analysis as detailed in Section 6.6.2.4 at 24 and 36 h post-dose.

- Urine for analysis of creatinine and UGE will be collected in 12 h batches 12 to 24 h, 24 to 36 h and 36 to 48 h as described in Section 6.6.2.3.
- Post-dose concomitant medications and adverse event information will be collected as appropriate.

7.3.6 Day 10: Post-dose Activities

- Plasma samples will be drawn for PK analysis as detailed in Section 6.6.2.4 at 48 h post-dose for all Groups and additionally at 60 h post-dose for Group 3.
- Urine for analysis of creatinine and UGE will be collected in 12 h batches at 36 to 48 h post-dose for all Groups and additionally at 48 to 60 h and 60 to 72 h post-dose for Group 3 as described in Section 6.6.2.3.
- Post-dose concomitant medications and adverse event information will be collected as appropriate.
- For Group 1 and 2, vital signs will be recorded at 48 h post-dose.
- For Group 1 and 2, urine will be collected for urinalysis as described in Section 6.6.2.2.
- For Group 1 and 2, blood will be drawn for hematology and serum chemistry as detailed in Section 6.6.2.1.
- For Group 1 and 2, the subject will be discharged after completion of all activities and scheduled for admission for Period 3.

7.3.7 Day 11 (Group 3 Only): Post-dose Activities

- Vital signs will be recorded at 72 h post-dose.
- Plasma samples will be drawn for PK analysis as detailed in Section 6.6.2.4 at 72 h post-dose.
- Urine for analysis of creatinine and UGE will be collected in 12 h batches at 60 to 72 h post-dose as described in Section 6.6.2.3.
- Post-dose 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.
- Blood will be drawn for hematology and serum chemistry as detailed in Section 6.6.2.1.
- Subject will be discharged after completion of all activities and scheduled for admission for Period 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 must be completed. The reason for termination must be entered onto the case report form.

7.4 Period 3

7.4.1 Day 14 (Clinic Admission)

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

- All female subjects will receive urine pregnancy test.
- If the subject is still eligible based on the study inclusion and exclusion criteria, the subject will be admitted.
- Concomitant medications and adverse event information will be collected as appropriate.
- Pre-dose urine collection in 12 h batches will begin with the -12 h to 0 h batch for baseline analysis of creatinine and for UGE (PD) analysis as described in Section 6.6.2.3.

7.4.2 Day 15 (Dosing Day): Pre-dose Activities

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

- Vital signs will be recorded pre-dose.
- 12-lead ECG will be taken in the supine position after at least 5 min of rest pre-dose.
- Pre-dose urine will be collected until 0 h (-12 h to 0 h batch) for baseline UGE 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.
- 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.

7.4.3 Day 15 (Dosing Day): Dosing

- For Group 1 subjects, after an overnight fast of at least 10 h, bexagliflozin tablets, 20 mg, alone, metformin, 1000 mg, alone or bexagliflozin and metformin in combination will be administered prior to first meal of the day as detailed in Section 5.3.1.
- For Group 2 subjects, after an overnight fast of at least 10 h, bexagliflozin tablets, 20 mg, alone, glimepiride, 4 mg, alone or bexagliflozin and glimepiride in combination will be administered prior to first meal of the day with glucose solution as detailed in Section 5.3.2.

- For Group 3 subjects, after an overnight fast of at least 10 h, bexagliflozin tablets, 20 mg, alone, sitagliptin, 100 mg, alone or bexagliflozin and sitagliptin in combination will be administered prior to first meal of the day as detailed in Section 5.3.3.

7.4.4 Day 15 (Dosing Day): Post-dose Activities

- Vital signs will be recorded at 4 h post-dose.
- 12-lead ECG will be taken in the supine position after at least 5 min of rest at 4 h post-dose.
- Plasma samples will be drawn for PK analysis as detailed in Section 6.6.2.4 at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16 h post-dose.
- Urine for analysis of creatinine and UGE will be collected in 12 h batches at 0 to 12 h and 12 to 24 h post-dose as described in Section 6.6.2.3.
- Post-dose concomitant medications and adverse event information will be collected as appropriate.

7.4.5 Day 16: Post-dose Activities

- Plasma samples will be drawn for PK analysis as detailed in Section 6.6.2.4 at 24 and 36 h post-dose.
- Urine for analysis of creatinine and UGE will be collected in 12 h batches 12 to 24 h, 24 to 36 h and 36 to 48 h as described in Section 6.6.2.3.
- Post-dose concomitant medications and adverse event information will be collected as appropriate.

7.4.6 Day 17: Post-dose Activities

- Plasma samples will be drawn for PK analysis as detailed in Section 6.6.2.4 at 48 h post-dose for all Groups and additionally at 60 h post-dose for Group 3.
- Urine for analysis of creatinine and UGE will be collected in 12 h batches at 36 to 48 h post-dose for all Groups and additionally at 48 to 60 h and 60 to 72 h post-dose for Group 3 as described in Section 6.6.2.3.
- Post-dose concomitant medications and adverse event information will be collected as appropriate.
- For Group 1 and 2, vital signs will be recorded at 48 h post-dose.
- For Group 1 and 2, urine will be collected for urinalysis as described in Section 6.6.2.2.
- For Group 1 and 2, blood will be drawn for hematology and serum chemistry as detailed in Section 6.6.2.1.

- For Group 1 and 2, a physical examination will be conducted as described in Section 6.3.
- For Group 1 and 2, a 12-lead ECG will be taken in the supine position after at least 5 min of rest as described in Section 6.5.
- For Group 1 and 2, the subject will be discharged after completion of all activities.

7.4.7 Day 18 (Group 3 Only): Post-dose Activities

- Vital signs will be recorded at 72 h post-dose.
- Plasma samples will be drawn for PK analysis as detailed in Section 6.6.2.4 at 72 h post-dose.
- Urine for analysis of creatinine and UGE will be collected in 12 h batches at 60 to 72 h post-dose as described in Section 6.6.2.3.
- Post-dose 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.
- Blood will be drawn for hematology and serum chemistry as detailed in Section 6.6.2.1.
- Physical examination will be conducted as described in Section 6.3.
- 12-lead ECG will be taken in the supine position after at least 5 min of rest as described in Section 6.5.
- 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 must be completed. The reason for termination must be entered onto the case report form.

7.5 Early Termination or Follow-up Procedures

Subjects who have completed study activities or have withdrawn consent and have received investigational product should have a follow-up examination if clinically indicated, including a complete physical examination, vital signs, ECG, and clinical laboratory tests (hematology, and serum chemistry). The sponsor must 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 will 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 will be verified with the source documentation.

Quality control principles will 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 will 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, PD or statistical analysis of the data. Statistical analysis will be performed using Statistical Analysis Software SAS for Windows® (SAS Institute Inc., 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 and OHAs, to assess potential drug-drug interactions, and to provide safety and tolerability data on bexagliflozin when administered alone and in combination with OHAs.

9.3 Analysis Populations

9.3.1 Safety Population

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

9.3.2 PK Population

The PK Population will include all randomized subjects who receive at least one dose of investigational product and who have sufficient plasma bexagliflozin and OHA measurements to derive PK parameters following dosing. The PK Population will be used to summarize the PK parameters.

9.3.3 PD Population

The PD Population will include all randomized subjects who receive at least one dose of investigational product and will have had at least 2 batches of 12 h urine collection from which to calculate UGE measurement following dosing. The PD Population will be used to summarize the PD parameters.

9.4 Demographics and Baseline Characteristics

Baseline characteristics will be summarized for all subjects in the Safety, PK and PD 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, metformin, glimepiride and sitagliptin individually and the combination of bexagliflozin with each tested OHA using the software Phoenix® WinNonlin® 6.4 (Certara, USA). From the plasma bexagliflozin concentration-time 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

$T_{½}$: 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

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/λ_z.

Descriptive statistics for the plasma concentrations of bexagliflozin, metformin, glimepiride and sitagliptin by Treatment and Timepoint will be provided. A listing of plasma concentrations by Subject Number, Treatment Period and Timepoint will also be provided.

9.5.2 Statistical Analysis of Pharmacokinetic Variables

To assess the effect of OHAs metformin, glimepiride or sitagliptin on the PK of bexagliflozin and vice versa, 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, period, and sequence as fixed effects. The 90% confidence intervals will be constructed for the (bexagliflozin and OHA in combination) to (bexagliflozin alone) or (OHA alone) ratio of the least squares (LS) geometric means of PK parameters (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$), 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}, 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 by Subject ID and Treatment Period will be provided.

9.6 Pharmacodynamic Analysis

Urinary glucose excretion will be determined as a PD parameter at baseline and up to 72 h post-dose. The drug-drug interaction effect between bexagliflozin and OHAs will be evaluated by comparing the mean cumulative UGE between subjects taking bexagliflozin or OHA alone and bexagliflozin and OHA in combination.

Descriptive statistics on the PD parameters will be performed. The effect of an OHA on the cumulative UGE between subjects administered bexagliflozin alone or in combination with an OHA will be compared. The PD parameters, UGE and UGE normalized by urinary creatinine, including UGE_{t1-t2}, in 12-hour and 24 –hour increments, and total 24-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 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 administration.

Adverse event listings will be provided for the following subsets:

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

AEs are dosing emergent if they occur on or after bexagliflozin administration. TEAEs will be considered at least possibly related to bexagliflozin 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 second dose up until the follow-up visit will be assigned to the second treatment.

Tabulations will display TEAEs by severity and relationship to bexagliflozin.

9.7.2 Hypoglycemia

Hypoglycemia as defined in Section 6.7.4.4 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 measurement of each period) 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 must be submitted to the IRB/IEC for review and must be approved by the sponsor and the IRB/IEC before the study is initiated. Any amendments or addenda to the protocol must also be approved by the IRB/IEC prior to implementing changes in the study. The investigator is responsible for keeping the IRB/IEC informed of the progress of the study and of any changes made to the protocol as deemed appropriate. The investigator must also keep the IRB/IEC informed of any SAEs occurring to subjects under their supervision.

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 must agree to the inspection of study-related records by the regulatory authority/sponsor representatives, and must 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/IEC 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 should be submitted to the head of the investigational site, IRB (via the head of the investigational site)/sponsor.

Any deviations from the protocol must be fully explained and documented by the investigator. The circumstances, action taken, and impact of the deviation on the trial must 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 and CRO's draft informed consent form. The sponsor will review the investigator's draft informed consent form prior to submission to the IRB/IEC and the final IRB/IEC approved document must be provided to the sponsor for regulatory purposes.

Prior to the beginning of the study, the investigator must have received from the IEC or 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/IEC together with the approved subject information and informed consent forms must be filed. The informed consent form must 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 must be obtained before any study-specific procedure takes place. Participation in the study and date of informed consent given by the subject should be documented appropriately in the subject's files. A copy of the signed informed consent form must 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 must remain in each subject's study file and must 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 must be available for inspection upon request by representatives of national and local health authorities, the sponsor, and the IRB/IEC 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/IEC 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 must be recorded in the case report form.

Entries made in the CRF must 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 should 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 must 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 must 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 must notify the Sponsor.

Protocol violations must 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/IEC 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 should 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 must 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

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Appendix 1 Schedule of Events

A. Schedule of Events For Study 1: Bexagliflozin and Metformin Drug-Drug Interaction

Study activity	Screening	Period 1					Period 2					Period 3				
	D -28 to -1	D0	D1 pre-dose	D1 post-dose	D2	D3	D7[1]	D8 pre-dose	D8 post-dose	D9	D10	D14[1]	D15 pre-dose	D15 post-dose	D16	D17
Informed consent form	X															
Screening for I/E criteria	X	X					X					X				
Physical examination[2]	X	X														X
Medical history and demographics	X															
Randomization		X														
Admission/discharge		X				X	X				X	X				X
Vital signs[3]	X		X	X		X		X	X		X		X	X		X
ECG[4]	X		X	X				X	X				X	X		X
Urinalysis[5]	X		X			X		X			X		X			X
Blood draw for clinical lab tests[6]	X		X			X		X			X		X			X
Blood sample for PK[7]			X	X	X	X		X	X	X	X		X	X	X	X
Urine collection for PD[8]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urine Pregnancy Test	X	X					X					X				
Adverse event and concomitant medication		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Study completion																X

1 The admission day for Period 2 and 3 will be 4-6 days after discharge from the previous Period.

2 A complete physical examination will be performed at screening and day 17 prior to discharge. A partial physical exam will be performed on admission day of Period 1.

3 On days 1, 8 and 15, vital signs will be determined at pre-dose and at 4 h and 48 h (day 3, 10 and 17) post-dose.

4 ECG data will be recorded at screening, on days 1, 8 and 15 at pre-dose and at 4 h post-dose, at the study termination visit and when clinically indicated.

5 Urine samples will be collected for urinalysis at screening, pre-dose and prior to discharge. If urine is dipstick positive for leukocyte esterase or nitrites, sample will be sent for microscopic evaluation and culture. Urine drug screen will only be performed at screening, and if it is conducted more than 5 days prior to dosing, screening shall be repeated.

6 Blood samples will be drawn for clinical laboratory tests at screening, pre-dose and prior to discharge. Clinical laboratory tests are listed in Table 2.

7 Plasma samples for PK analysis will be collected as described in Section 6.6.2.4.

8 Urine collection for the evaluation of urinary glucose and creatinine are described in Section 6.6.2.3.

B. SCHEDULE OF EVENTS FOR STUDY 2: BEXAGLIFLOZIN AND GLIMEPIRIDE DRUG-DRUG INTERACTION

	Screening	Period 1					Period 2					Period 3				
Study activity	**D -28 to -1**	**D0**	**D1 pre-dose**	**D1 post-dose**	**D2**	**D3**	**D7[1]**	**D8 pre-dose**	**D8 post-dose**	**D9**	**D10**	**D14[1]**	**D15 pre-dose**	**D15 post-dose**	**D16**	**D17**
Informed consent form	X															
Screening for I/E criteria	X	X					X					X				
Physical examination[2]	X	X														X
Medical history and demographics	X															
Randomization		X														
Admission/discharge		X				X	X				X	X				X
Vital signs[3]	X		X	X		X		X	X		X		X	X		X
ECG[4]	X		X	X				X	X				X	X		X
Urinalysis[5]	X		X			X		X			X		X			X
Blood draw for clinical lab tests[6]	X		X			X		X			X		X			X
Blood sample for PK[7]			X	X	X	X		X	X	X	X		X	X	X	X
Urine collection for PD[8]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urine Pregnancy Test	X	X					X					X				
Adverse event and concomitant medication		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Study completion																X

1 The admission day for Period 2 and 3 will be 4-6 days after discharge from the previous Period.

2 A complete physical examination will be performed at screening and day 17 prior to discharge. A partial physical exam will be performed on admission day of Period 1.

3 On days 1, 8 and 15, vital signs will be determined at pre-dose and at 4 h and 48 h (day 3, 10 and 17) post-dose.

4 ECG data will be recorded at screening, on days 1, 8 and 15 at pre-dose and at 4 h post-dose, at the study termination visit and when clinically indicated.

5 Urine samples will be collected for urinalysis at screening, pre-dose and prior to discharge. If urine is dipstick positive for leukocyte esterase or nitrites, sample will be sent for microscopic evaluation and culture. Urine drug screen will only be performed at screening, and if it is conducted more than 5 days prior to dosing, screening shall be repeated.

6 Blood samples will be drawn for clinical laboratory tests at screening, pre-dose and prior to discharge. Clinical laboratory tests are listed in Table 2.

7 Plasma samples for PK analysis will be collected as described in Section 6.6.2.4.

8 Urine collection for the evaluation of urinary glucose and creatinine are described in Section 6.6.2.3.

C. SCHEDULE OF EVENTS FOR STUDY 3: BEXAGLIFLOZIN AND SITAGLIPTIN DRUG-DRUG INTERACTION

	Screening	Period 1						Period 2						Period 3					
Study activity	**D -28 to -1**	**D0**	**D1 pre-dose**	**D1 post-dose**	**D2**	**D3**	**D4**	**D7[1]**	**D8 pre-dose**	**D8 post-dose**	**D9**	**D10**	**D11**	**D14[1]**	**D15 pre-dose**	**D15 post-dose**	**D16**	**D17**	**D18**
Informed consent form	X																		
Screening for I/E criteria	X	X						X						X					
Physical examination[2]	X	X																	X
Medical history and demographics	X																		
Randomization		X																	
Admission/discharge		X					X	X					X	X					X
Vital signs[3]	X		X	X			X		X	X			X		X	X			X
ECG[4]	X		X	X					X	X					X	X			X
Urinalysis[5]	X		X				X		X				X		X				X
Blood draw for clinical lab tests[6]	X		X				X		X				X		X				X
Blood sample for PK[7]			X	X	X	X	X		X	X	X	X	X		X	X	X	X	X
Urine collection for PD[8]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urine Pregnancy Test	X	X						X						X					
Adverse event and concomitant medication		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Study completion																			X

1 The admission day for Period 2 and 3 will be 4-6 days after discharge from the previous Period.
2 A complete physical examination will be performed at screening and day 17 prior to discharge. A partial physical exam will be performed on admission day of Period 1.
3 On days 1, 8 and 15, vital signs will be determined at pre-dose and at 4 h and 72 h (day 4, 11 and 18) post-dose.
4 ECG data will be recorded at screening, on days 1, 8 and 15 at pre-dose and at 4 h post-dose, at the study termination visit and when clinically indicated.
5 Urine samples will be collected for urinalysis at screening, pre-dose and prior to discharge. If urine is dipstick positive for leukocyte esterase or nitrites, sample will be sent for microscopic evaluation and culture. Urine drug screen will only be performed at screening, and if it is conducted more than 5 days prior to dosing, screening shall be repeated.
6 Blood samples will be drawn for clinical laboratory tests at screening, pre-dose and prior to discharge. Clinical laboratory tests are listed in Table 2.
7 Plasma samples for PK analysis will be collected as described in Section 6.6.2.4.
8 Urine collection for the evaluation of urinary glucose and creatinine are described in Section 6.6.2.3.

Appendix 2 Sponsor Signatures

Study Title: A phase 1, open-label, randomized, three-period, crossover study to evaluate pharmacokinetic interaction between bexagliflozin tablets and metformin, glimepiride, or sitagliptin in healthy subjects

Study Number: THR-1442-C-453

Final Date: 01 September 2016

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: _____ Date: 01 September 2016
Annie L. Conery, Ph.D.
Sponsor Study Representative
Massachusetts General Hospital

Signed: _____ Date: 01 September 2016
Yuan-Di Halvorsen, Ph.D.
Sponsor Study Representative
Massachusetts General Hospital

Signed: _____ Date: 02 September 2016
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, randomized, three-period, crossover study to evaluate pharmacokinetic interaction between bexagliflozin tablets and metformin, glimepiride, or sitagliptin in healthy subjects
Study Number:	THR-1442-C-453
Final Date:	01 September 2016

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: 18 Oct 2016

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