University of California, San Francisco
Clinical Research Protocol

The effect of sodium-glucose cotransporter (SGLT) 2 inhibitor on cystine stone formation: A preliminary study

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University of California, San Francisco
Clinical Research Protocol
SGLT2-01

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Coordinating Center:	Single center

Approval:

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PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study subjects enrolled under my supervision as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

Protocol Number: SGLT2-01

Protocol Title: The effect of sodium-glucose cotransporter-2 (SGLT-2) inhibitor on cystine stone formation

Protocol Date: 8/17/2021

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LIST OF ABBREVIATIONS

AE	adverse event
CFR	Code of Federal Regulations
CRF	case report form
DM	Diabetes mellitus
DMC	Data Monitoring Committee
DSMB	Data Safety Monitoring Board
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act of 1996
ICF	informed consent form
ICH	International Conference on Harmonisation
ICP-OES	inductively coupled plasma optical emission spectroscopy
IEC	Independent Ethics Committee
IRB	Institutional Review Board
PI	Principal Investigator
SAE	serious adverse experience
SGLT	sodium-glucose cotransporter

PROTOCOL SYNOPSIS

TITLE	The effect of sodium-glucose cotransporter (SGLT) 2 inhibitor on cystine stone formation: A preliminary study
SPONSOR	Marshall Stoller, MD
FUNDING ORGANIZATION	
NUMBER OF SITES	1
RATIONALE	Cystinuria is an inherited autosomal recessive disorder of the kidney that is the result of an inability to reabsorb cystine from the urine. Supersaturation of cystine in the urine produces crystals that precipitate and form stones in the kidney, which can be a cause of obstruction, infection, and chronic kidney disease. Cystine stones constitute a major health challenge for affected individuals with cystinuria because of the frequent recurrence of painful symptoms and the current absence of effective, patient-accepting treatment. A mainstay of therapy is breaking or preventing the cystine bond on the molecular level such that cystine (which is formed from the joining of two cysteine amino acids and their corresponding sulfur atoms) cannot precipitate in the urine. It is hypothesized that a glucose molecule may be able to do this if introduced into the urine. SGLT-2 inhibitors are a class of drug that are FDA approved to treat diabetes mellitus (DM) and heart failure by inhibiting an enzyme in the kidney that allows for reabsorption of glucose from the urine. This effectively increases the concentration of glucose in the urine. Our hypothesis suggests that administration of this drug to patients with cystine will introduce sufficient glucose into the urine to prevent the formation of cystine stones. To date, there has been no published data on the effectiveness of this therapy for this indication, although the dosage and administration would be identical to that already approved by the FDA for the treatment of DM and heart failure.
STUDY DESIGN	This study will be a single center, proof of concept study designed to assess how daily 10 mg dapagliflozin affects formation of cystine in the urine in patients with cystinuria.
PRIMARY OBJECTIVE	Use mass spectrometry to determine the effect of SGLT-2 inhibitor therapy on quantitative sulfur as a surrogate for presence of cysteine in freshly voided urine in patients with cystinuria.
SECONDARY OBJECTIVES	None
NUMBER OF SUBJECTS	10
SUBJECT SELECTION	Inclusion Criteria: males and females age 18 or older; documented cystinuria on prior 24-hour urine collection and/or stone analysis;

CRITERIA	history of previous cystine kidney stones; and being able and willing to provide consent Exclusion Criteria: prior diagnosis of diabetes mellitus (type I or type II); current SGLT-2 inhibitor administration at the time of screening; SGLT-2 inhibitor administration within the last year prior to screening; vulnerable populations including incarceration status; anticipation of pregnancy during the study duration; unable to give informed consent; non-English primary language; pregnancy, lactation, or child- bearing age without birth control devices; and serious illness likely to cause death within the next 5 years.
TEST PRODUCT, DOSE, AND ROUTE OF ADMINISTRATION	Dapagliflozin 10 mg administered orally once daily for 4 weeks
CONTROL PRODUCT, DOSE AND ROUTE OF ADMINISTRATION	None
DURATION OF SUBJECT PARTICIPATION AND DURATION OF STUDY	Subjects will be on study for up to 6 weeks • Screening: 1 week to determine eligibility • Treatment: 4 weeks after the initial visit (Day 0) • Follow-up: 1 week after the last visit (Day 28)
CONCOMMITANT MEDICATIONS	**Allowed**: The participants will be allowed to continue any medications they are currently taking with the exception of those listed below under "prohibited". Importantly, patients will be allowed to continue potassium citrate as this is an important mainstay of cystinuria treatment as discontinuation may lead to clinically significant cystine stone formation. **Prohibited**: Medications commonly used to treat diabetes, such as but not limited to insulin, metformin, and glipizides. These medications may interfere with the action of SGLT-2 inhibitors by altering the manner in which the body metabolizes and regulates glucose. Medications commonly used to treat cystinuria: thiola, penicillamine, alpha lipoic acid
EFFICACY EVALUATIONS	Efficacy in this study will be determined by utilizing mass spectrometry to analyze for the presence of sulfur in the urine before and after initiation of therapy with SGLT-2 inhibitor in our patients with cystinuria. Urinary cystine levels on 24-hour urine collection will also be measured.
PRIMARY ENDPOINT	Quantitative urinary sulfur via inductively coupled plasma optical emission spectroscopy (ICP-OES) in freshly voided urine

OTHER EVALUATIONS	
SAFETY EVALUATIONS	After participants are enrolled at 1 week during the treatment period, a study coordinator will contact each participant to check compliance, tolerability, and side effects with SGLT-2 inhibitor therapy. Any concern for a potential adverse event will be reviewed by the study investigators. Patient clinical care will continue as per the current standard for the duration of this study. Tolerability and side effects will be reviewed at each of the two clinic visits. All reported adverse events will be reviewed by the study investigators. Depending on the severity of the adverse event, the participant may be advised to stop SGLT-2 inhibitor therapy intake if it is determined that SGLT-2 inhibitor therapy is the cause. Most common AEs noted include male and female genital yeast infections, UTI, increased urination, discomfort with urination.
PLANNED INTERIM ANALYSES	When approximately 50% of patients have completed the study through Visit 2, an interim analysis for safety will be conducted. Serious adverse events will be monitored on an ongoing basis throughout the study.
STATISTICS Primary Analysis Plan	The primary efficacy endpoint will be assessed by comparing the qualitative and quantitative differences between the mass spectrometry output data between the participants' freshly voided urine at the initial visit and at the follow up visit after initiation of SGLT-2 inhibitor therapy, specifically examining the quantitative sulfur content between the two samples as a surrogate for cysteine in the urine.
Rationale for Number of Subjects	This is a preliminary proof of concept study designed to show only that administration of SGLT-2 inhibitor medications alter the excretion of cystine in the urine of patients with documented cystinuria. If initial analyses are promising, further investigation will be undertaken to draw further meaningful, statistical conclusions on the efficacy of this medication.

1 BACKGROUND

Cystinuria is an inherited autosomal recessive disorder of the kidney that is the result of a defect in the dibasic amino acid transporter in the apical membrane of the renal proximal tubule and small intestine. The disease causes hyperexcretion of cystine, lysine, arginine, and ornithine into the urine. Of these amino acids, only cystine is poorly soluble in urine. Supersaturation of cystine in the urine produces crystals that precipitate and form calculi, which can be a cause of obstruction, infection, and chronic kidney disease.[1] Cystine stone disease has an estimated prevalence of up to 1 in 1,000 in some parts of Europe and 1 in 17,000 in the United States,[2] and comprises up to 2% of all kidney stone disease overall and up to 10% of stone disease in children.[3] As it is a rare disease, pharmaceutical treatments for cystinuria are considered "orphan drugs" based on the U.S. Orphan Drug Act of 1983, which was passed to encourage pharmaceutical companies to develop treatments for diseases with a small market.[4] However, cystine stones constitute a major health challenge for affected individuals because of the frequent recurrence of painful symptoms and the current absence of effective, patient-accepting treatment.[5, 6]

Two primary factors known to affect the solubility of cystine include are cystine concentration and pH.[7] Therefore, the current mainstays of therapy include aggressive hydration to 2.5 to 3 liters of fluid intake daily, lowering of dietary sodium intake, urinary alkalinization to pH >7.0, and thiol derivative medications to bind urinary cystine to reduce urinary cystine concentrations to less than 250 mg/l, the level above which cystine becomes insoluble in urine.[8] Thiol derivatives in current use including D-penicillamine and alpha-mercaptopropionylglycione increase the solubility of cystine by forming more soluble mixed amino acid-disulfide products. However, these medications are difficult to tolerate due to their side effect profiles and, therefore, compliance is poor.[9] Furthermore, the medications require regular monitoring of complete blood count, liver enzymes, and urinary protein excretion. In our own kidney stone clinic population, even when compliance is high, cystinuric patients taking D-penicillamine and alpha-mercaptopropionylglycione often continue to form recurrent cystine stones. It is not surprising that because cystinuria is a lifelong disease and current medical management is difficult to achieve and not very effective, these individuals have frequent stone recurrences and a higher risk of kidney loss compared to the average calcium oxalate stone former.[10] Therefore, additional well-tolerated and effective medical therapies to treat cystinuria are desperately needed.

1.1 Overview of Clinical Studies

Sodium-glucose cotransporter 2 (SGLT2), expressed in the proximal renal tubules, is responsible for the majority of the reabsorption of filtered glucose from the tubular lumen. Dapagliflozin is an inhibitor of SGLT2. By inhibiting SGLT2, dapagliflozin reduces reabsorption of filtered glucose and lowers the renal threshold for glucose, and thereby increases urinary glucose excretion. Dapagliflozin also reduces sodium reabsorption and increases the delivery of sodium to the distal tubule. This may influence several physiological functions including, but not restricted to, lowering both pre-and afterload of the heart and downregulation of sympathetic activity.

Increases in the amount of glucose excreted in the urine were observed in healthy subjects and in patients with type 2 diabetes mellitus following the administration of dapagliflozin. Dapagliflozin doses of 5 or 10 mg per day in patients with type 2 diabetes mellitus for 12 weeks resulted in excretion of approximately 70 grams of glucose in the urine per day at Week 12. A near maximum glucose excretion was observed at the dapagliflozin daily dose of 20 mg. This urinary glucose excretion with dapagliflozin also results in increases in urinary volume. After discontinuation of dapagliflozin, on average, the elevation in urinary glucose excretion approaches baseline by about 3 days for the 10 mg dose.

Following oral administration of dapagliflozin, the maximum plasma concentration (Cmax) is usually attained within 2 hours under fasting state. The Cmax and AUC values increase dose proportionally with increase in dapagliflozin dose in the therapeutic dose range. The absolute oral bioavailability of dapagliflozin following the administration of a 10 mg dose is 78%. Administration of dapagliflozin with a high-fat meal decreases its Cmax by up to 50% and prolongs Tmax by approximately 1 hour, but does not alter AUC as compared with the fasted state. These changes are not considered to be clinically meaningful and dapagliflozin can be administered with or without food.

Overview of Clinical Studies of FARXIGA for Type 2 Diabetes

FARXIGA has been studied as monotherapy, in combination with metformin, pioglitazone, sulfonylurea (glimepiride), sitagliptin (with or without metformin), metformin plus a sulfonylurea, or insulin (with or without other oral antidiabetic therapy), compared to a sulfonylurea (glipizide), and in combination with a GLP-1 receptor agonist (exenatide extended-release) added-on to metformin. FARXIGA has also been studied in patients with type 2 diabetes and moderate renal impairment.

Treatment with FARXIGA as monotherapy and in combination with metformin, glimepiride, pioglitazone, sitagliptin, or insulin produced statistically significant improvements in mean change from baseline at Week 24 in HbA1c compared to control. Reductions in HbA1c were seen across subgroups including gender, age, race, duration of disease, and baseline body mass index (BMI).

Monotherapy

A total of 840 treatment-naive patients with inadequately controlled type 2 diabetes participated in 2 placebo-controlled studies to evaluate the safety and efficacy of monotherapy with FARXIGA.

In 1 monotherapy study, a total of 558 treatment-naive patients with inadequately controlled diabetes participated in a 24-week study (NCT00528372). Following a 2-week diet and exercise placebo lead-in period, 485 patients with HbA1c ≥7% and ≤10% were randomized to FARXIGA 5 mg or FARXIGA 10 mg once daily in either the morning (QAM, main cohort) or evening (QPM), or placebo.

At Week 24, treatment with FARXIGA 10 mg QAM provided significant improvements in HbA1c and the fasting plasma glucose (FPG) compared with placebo.

Initial Combination Therapy with Metformin XR

A total of 1236 treatment-naive patients with inadequately controlled type 2 diabetes (HbA1c ≥7.5% and ≤12%) participated in 2 active-controlled studies of 24-week duration to evaluate initial therapy with FARXIGA 5 mg (NCT00643851) or 10 mg (NCT00859898) in combination with metformin extended-release (XR) formulation.

In 1 study, 638 patients randomized to 1 of 3 treatment arms following a 1-week lead-in period received: FARXIGA 10 mg plus metformin XR (up to 2000 mg per day), FARXIGA 10 mg plus placebo, or metformin XR (up to 2000 mg per day) plus placebo. Metformin XR dose was up-titrated weekly in 500 mg increments, as tolerated, with a median dose achieved of 2000 mg.

The combination treatment of FARXIGA 10 mg plus metformin XR provided statistically significant improvements in HbA1c and FPG compared with either of the monotherapy treatments and statistically significant reduction in body weight compared with metformin XR alone. FARXIGA 10 mg as monotherapy also provided statistically significant improvements in FPG and statistically significant reduction in body weight compared with metformin alone and was noninferior to metformin XR monotherapy in lowering HbA1c.

In a second study, 603 patients were randomized to 1 of 3 treatment arms following a 1-week lead-in period: FARXIGA 5 mg plus metformin XR (up to 2000 mg per day), FARXIGA 5 mg plus placebo, or metformin XR (up to 2000 mg per day) plus placebo. Metformin XR dose was up-titrated weekly in 500 mg increments, as tolerated, with a median dose achieved of 2000 mg.

The combination treatment of FARXIGA 5 mg plus metformin XR provided statistically significant improvements in HbA1c and FPG compared with either of the monotherapy treatments and statistically significant reduction in body weight compared with metformin XR alone.

Add-On to Metformin

A total of 546 patients with type 2 diabetes with inadequate glycemic control (HbA1c ≥7% and ≤10%)
participated in a 24-week, placebo-controlled study to evaluate FARXIGA in combination with metformin (NCT00528879). Patients on metformin at a dose of at least 1500 mg per day were randomized after completing a 2-week, single-blind, placebo lead-in period. Following the lead-in period, eligible patients were randomized to FARXIGA 5 mg, FARXIGA 10 mg, or placebo in addition to their current dose of metformin.

As add-on treatment to metformin, FARXIGA 10 mg provided statistically significant improvements in HbA1c and FPG, and statistically significant reduction in body weight compared with placebo at Week 24 (see Table 9 and Figure 3). Statistically significant ($p < 0.05$ for both doses) mean changes from baseline in systolic blood pressure relative to placebo plus metformin were −4.5 mmHg and −5.3 mmHg with FARXIGA 5 mg and 10 mg plus metformin, respectively.

Active Glipizide-Controlled Study Add-On to Metformin

A total of 816 patients with type 2 diabetes with inadequate glycemic control (HbA1c >6.5% and ≤10%) were randomized in a 52-week, glipizide-controlled, noninferiority study to evaluate FARXIGA as add-on therapy to metformin (NCT00660907). Patients on metformin at a dose of at least 1500 mg per day were randomized following a 2-week placebo lead-in period to glipizide or dapagliflozin (5 mg or 2.5 mg, respectively) and were up-titrated over 18 weeks to optimal glycemic effect (FPG <110 mg/dL, <6.1 mmol/L) or to the highest dose level (up to glipizide 20 mg and FARXIGA 10 mg) as tolerated by patients. Thereafter, doses were kept constant, except for down-titration to prevent hypoglycemia.

At the end of the titration period, 87% of patients treated with FARXIGA had been titrated to the maximum study dose (10 mg) versus 73% treated with glipizide (20 mg). FARXIGA led to a similar mean reduction in HbA1c from baseline at Week 52 (LOCF), compared with glipizide, thus demonstrating noninferiority (see Table 10). FARXIGA treatment led to a statistically significant mean reduction in body weight from baseline at Week 52 (LOCF) compared with a mean increase in body weight in the glipizide group. Statistically significant ($p<0.0001$) mean change from baseline in systolic blood pressure relative to glipizide plus metformin was −5.0 mmHg with FARXIGA plus metformin.

Add-On Combination Therapy with a Sulfonylurea

A total of 597 patients with type 2 diabetes and inadequate glycemic control (HbA1c ≥7% and ≤10%) were randomized in this 24-week, placebo-controlled study to evaluate FARXIGA in combination with glimepiride (a sulfonylurea) (NCT00680745). Patients on at least half the maximum recommended dose of glimepiride as monotherapy (4 mg) for at least 8 weeks lead-in were randomized to FARXIGA 5 mg, FARXIGA 10 mg, or placebo in addition to glimepiride 4 mg per day. Down-titration of glimepiride to 2 mg or 0 mg was allowed for hypoglycemia during the treatment period; no up-titration of glimepiride was allowed.

In combination with glimepiride, FARXIGA 10 mg provided statistically significant improvement in HbA1c, FPG, and 2-hour PPG, and statistically significant reduction in body weight compared with placebo plus glimepiride at Week 24 (see Table 11). Statistically significant ($p<0.05$ for both doses) mean changes from baseline in systolic blood pressure relative to placebo plus glimepiride were −2.8 mmHg and −3.8 mmHg with FARXIGA 5 mg and 10 mg plus glimepiride, respectively.

Add-on Combination Therapy with Metformin and a Sulfonylurea

A total of 218 patients with type 2 diabetes and inadequate glycemic control (HbA1c ≥7% and ≤10.5%) participated in a 24-week, placebo-controlled study to evaluate FARXIGA in combination with metformin and a sulfonylurea (NCT01392677). Patients on a stable dose of metformin (immediate-or extended-release formulations) ≥1500 mg/day plus maximum tolerated dose, which must be at least half the maximum dose, of a sulfonylurea

for at least 8 weeks prior to enrollment were randomized after an 8week placebo lead-in period to FARXIGA 10 mg or placebo. Dose-titration of FARXIGA or metformin

Add-On Combination Therapy with a Thiazolidinedione

A total of 420 patients with type 2 diabetes with inadequate glycemic control (HbA1c ≥7% and ≤10.5%) participated in a 24-week, placebo-controlled study to evaluate FARXIGA in combination with pioglitazone (a thiazolidinedione [TZD]) alone (NCT00683878). Patients on a stable dose of pioglitazone of 45 mg per day (or 30 mg per day, if 45 mg per day was not tolerated) for 12 weeks were randomized after a 2-week lead-in period to 5 or 10 mg of FARXIGA or placebo in addition to their current dose of pioglitazone. Dose titration of FARXIGA or pioglitazone was not permitted during the study.

In combination with pioglitazone, treatment with FARXIGA 10 mg provided statistically significant improvements in HbA1c, 2-hour PPG, FPG, the proportion of patients achieving HbA1c <7%, and a statistically significant reduction in body weight compared with the placebo plus pioglitazone treatment groups (see Table 11) at Week 24. A statistically significant ($p < 0.05$) mean change from baseline in systolic blood pressure relative to placebo in combination with pioglitazone was −4.5 mmHg with FARXIGA 10 mg in combination with pioglitazone.

Add-On Combination Therapy with a DPP4 Inhibitor

A total of 452 patients with type 2 diabetes who were drug naive, or who were treated at entry with metformin or a DPP4 inhibitor alone or in combination, and had inadequate glycemic control (HbA1c ≥7.0% and ≤10.0% at randomization), participated in a 24-week, placebo-controlled study to evaluate FARXIGA in combination with sitagliptin (a DPP4 inhibitor) with or without metformin (NCT00984867).

Eligible patients were stratified based on the presence or absence of background metformin (≥1500 mg per day), and within each stratum were randomized to either FARXIGA 10 mg plus sitagliptin 100 mg once daily, or placebo plus sitagliptin 100 mg once daily. Endpoints were tested for FARXIGA 10 mg versus placebo for the total study group (sitagliptin with and without metformin) and for each stratum (sitagliptin alone or sitagliptin with metformin). Thirty-seven percent (37%) of patients were drug naive, 32% were on metformin alone, 13% were on a DPP4 inhibitor alone, and 18% were on a DPP4 inhibitor plus metformin. Dose titration of FARXIGA, sitagliptin, or metformin was not permitted during the study.

In combination with sitagliptin (with or without metformin), FARXIGA 10 mg provided statistically significant improvements in HbA1c, FPG, and a statistically significant reduction in body weight compared with the placebo plus sitagliptin (with or without metformin) group at Week 24 (see Table 11). These improvements were also seen in the stratum of patients who received FARXIGA 10 mg plus sitagliptin alone (placebo-corrected mean change for HbA1c −0.56%; n=110)

compared with placebo plus sitagliptin alone (n=111), and the stratum of patients who received FARXIGA 10 mg plus sitagliptin and metformin (placebo-corrected mean change for HbA1c −0.40; n=113) compared with placebo plus sitagliptin with metformin (n=113).

Add-On Combination Therapy with Insulin

A total of 808 patients with type 2 diabetes who had inadequate glycemic control (HbA1c ≥7.5% and ≤10.5%) were randomized in a 24-week, placebo-controlled study to evaluate FARXIGA as add-on therapy to insulin (NCT00673231). Patients on a stable insulin regimen, with a mean dose of at least 30 IU of injectable insulin per day, for a period of at least 8 weeks prior to enrollment and on a maximum of 2 oral antidiabetic medications (OADs), including metformin, were randomized after completing a 2-week enrollment period to receive either FARXIGA 5 mg, FARXIGA 10 mg, or placebo in addition to their current dose of insulin and other OADs, if applicable. Patients were stratified according to the presence or absence of background OADs. Up-or down-titration of insulin was only permitted during the treatment phase in patients who failed to meet specific glycemic goals. Dose modifications of blinded study medication or OAD(s) were not allowed during the treatment phase, with the exception of decreasing OAD(s) where there were concerns over hypoglycemia after cessation of insulin therapy.

In this study, 50% of patients were on insulin monotherapy at baseline, while 50% were on 1 or 2 OADs in addition to insulin. At Week 24, FARXIGA 10 mg dose provided statistically significant improvement in HbA1c and reduction in mean insulin dose, and a statistically significant reduction in body weight compared with placebo in combination with insulin, with or without up to 2 OADs (see Table 11); the effect of FARXIGA on HbA1c was similar in patients treated with insulin alone and patients treated with insulin plus OAD. Statistically significant ($p<0.05$) mean change from baseline in systolic blood pressure relative to placebo in combination with insulin was −3.0 mmHg with FARXIGA 10 mg in combination with insulin.

At Week 24, FARXIGA 5 mg (−5.7 IU, difference from placebo) and 10 mg (−6.2 IU, difference from placebo) once daily resulted in a statistically significant reduction in mean daily insulin dose ($p<0.0001$ for both doses) compared to placebo in combination with insulin, and a statistically significantly higher proportion of patients on FARXIGA 10 mg (19.6%) reduced their insulin dose by at least 10% compared to placebo (11.0%).

Combination Therapy with Exenatide-Extended Release as Add-On to Metformin

A total of 694 adult patients with type 2 diabetes and inadequate glycemic control (HbA1c ≥8.0 and ≤12.0%) on metformin, were evaluated in a 28-week double-blind, active-controlled study to compare FARXIGA in combination with exenatide extended-release (a GLP-1 receptor agonist) to FARXIGA alone and exenatide extended-release alone, as add-on to metformin (NCT02229396). Patients on metformin at a dose of at least 1,500 mg per day were randomized following a 1-week placebo lead-in period to receive either FARXIGA 10 mg once daily (QD) in combination with exenatide

extended-release 2 mg once weekly (QW), FARXIGA 10 mg QD, or exenatide extended–release 2 mg QW.

At Week 28, FARXIGA in combination with exenatide extended-release provided statistically significantly greater reductions in HbA1c (-1.77%) compared to FARXIGA alone (-1.32%, p=0.001) and exenatide extended-release alone (-1.42%, p=0.012). FARXIGA in combination with exenatide extended-release provided statistically significantly greater reductions in FPG (-57.35 mg/dL) compared to FARXIGA alone (-44.72 mg/dL, p=0.006) and exenatide extended-release alone (-40.53, p <0.001).

2 STUDY RATIONALE

It is hypothesized that glucose can serve as an agent to reduce the bond between the sulfur molecules in a cystine molecule, therefore breaking the disulfide bond that is the target of other therapies and forming soluble compounds with cysteine amino acid constituents in the urine. SGLT-2 inhibitors are an existing therapy that has already been FDA approved for the treatment of diabetes through inhibition of sodium-glucose cotransporter proteins within the renal tubules, therefore leading to regulation of blood glucose through loss in the urine. This therapy therefore sharply increases the glucose concentration in the urine as will ideally provide the substrate for the reduction reaction necessary to break the disulfide bond in a safe and tolerable fashion with far fewer side effects and greater tolerability than existing therapies.

2.1 Risk / Benefit Assessment

Risks of treatment are relatively low in this therapy that has received FDA approval for treatment of diabetes mellitus and heart failure. See below for a list of incidence of common adverse events the most common of which were mild and include male and female genital yeast infections, UTI, increased urination, discomfort with urination. Potential benefit includes a novel and effective therapy for the debilitating disorder cystinuria.

3 STUDY OBJECTIVES

3.1 Primary Objective

The primary objective of this study is to use mass spectrometry to determine the effect of SGLT-2 inhibitor therapy on quantitative sulfur as a surrogate of cysteine in freshly voided urine in patients with cystinuria

4 STUDY DESIGN

4.1 Study Overview

This is a single center, proof of concept prospective cohort trial designed to assess the effect of daily oral administration of dapagliflozin 10 mg on cystine formation in freshly voided urine. Ten subjects are planned, each with previously diagnosed cystinuria and without current treatment except with potassium citrate medication.

Each subject will have an initial visit during which screening data will be reviewed to determine subject eligibility. Subjects who meet all inclusion criteria and none of the exclusion criteria will be entered into the study. Consent will be obtained as well as an initial examination and collection/analysis of freshly voided urine with mass spectrometry to serve as a baseline. The therapy under investigation, dapagliflozin 10 mg, will then be administered orally to each participant daily for four weeks, at which time each subject will return for a second visit for an analysis of freshly voided urine using mass spectrometry. The different urine collections will be compared and analyzed for differences with the prescribed therapy. No placebo will be used during this proof-of-concept study.

Total duration of subject participation with be up to six weeks. Total duration of the study is expected to be six weeks.

5 CRITERIA FOR EVALUATION

5.1 Primary Efficacy Endpoint

The primary endpoint will be assessed via analysis using mass spectrometry of freshly voided urine and examining the resultant signals which signify the presence or absence of cystine as well as unbound cysteine amino acids and other reduction byproducts through the reaction with glucose. These will be assessed after 4 weeks of treatment with daily oral dapagliflozin 10 mg.

5.2 Safety Evaluations

After the one-week treatment period, each participant will undergo a check of compliance, tolerability, and side effects with dapagliflozin therapy. Any concern for a potential adverse event will be reviewed by the study investigators. In addition, during the informed consent process, all potential side effects and drug interactions will be discussed. Enrolled participants will be provided the email address of the study coordinator and a 24-hour contact phone number of a physician if any adverse events occur during the study period. When 50% of participants have been in the study for 1 week, an interim analysis will be performed.

6 SUBJECT SELECTION

6.1 Study Population

Subjects 18 of age and older with a diagnosis of cystinuria who meet the inclusion and exclusion criteria will be eligible for participation in this study.

6.2 Inclusion Criteria

Male or female ≥ 18 years of age at Visit 1; Documentation of a cystinuria diagnosis as evidenced by one or more clinical features of the disease and one or more of the following criteria: (1) 24-hour urine collection in the past consistent with cystinuria or (2) Prior stone analysis after stone intervention proving cystine stone formation; Written informed consent (and assent when applicable) obtained from subject or subject's legal

representative and ability for subject to comply with the requirements of the study; Absence of exclusion criteria

6.3 Exclusion Criteria

Pregnant, breastfeeding, or unwilling to practice birth control during participation in the study; Presence of a condition or abnormality that in the opinion of the Investigator would compromise the safety of the patient or the quality of the data; Diagnosis of diabetes mellitus (type I or type II); Current SGLT-2 inhibitor administration at the time of screening or SGLT-2 inhibitor administration within the last year prior to screening; Vulnerable populations including incarceration status; Anticipation of pregnancy during the study duration; Unable to give informed consent; Non-English primary language; pregnancy, lactation, or child- bearing age without birth control devices; Serious illness likely to cause death within the next 5 years.

7 CONCURRENT MEDICATIONS

All subjects should be maintained on the same medications throughout the entire study period, as medically feasible, with no introduction of new chronic therapies. However, patients will who are on medications commonly used to treat diabetes, such as but not limited to insulin, metformin, and glipizides will not be allowed to continue these while on study. These medications may interfere with the action of SGLT-2 inhibitors by altering the manner in which the body metabolizes and regulates glucose. Participants will also be asked to stop any medications commonly used to treat cystinuria they may be taking for the duration of the study as well: thiola, penicillamine, alpha lipoic acid.

7.1 Allowed Medications and Treatments

All medications the participant is on are allowed with the exception of: insulin, metformin, and glipizides. These medications may interfere with the action of SGLT-2 inhibitors by altering the manner in which the body metabolizes and regulates glucose. Participants will also be asked to stop any medications commonly used to treat cystinuria they may be taking for the duration of the study as well: thiola, penicillamine, alpha lipoic acid.

8 STUDY TREATMENTS

8.1 Method of Assigning Subjects to Treatment Groups

All participants will be assigned one treatment which includes daily oral administration of dapagliflozin 10 mg for 1 week duration.

8.2 Blinding

This is a nonblinded proof of concept study.

8.3 Formulation of Test and Control Products

8.3.1 Formulation of Test Product

Dapagliflozin 10mg as prescribed by local pharmacy. No control product is being used.

8.4 Supply of Study Drug at the Site

Participants will be given a prescription for dapagliflozin 10mg to be taken orally daily for 4 weeks by the study PI or his assistant MD.

8.4.1 Dosage/Dosage Regimen

10mg of dapagliflozin will be taken orally once daily for 4 weeks. Participants will be encouraged to take the study tablet in the morning to facilitate consistent bioavailability between participants.

8.4.2 Dispensing

Dapagliflozin will be given to the participant with sufficient quantity for administration at home, with a 4 week supply (28 tablets). At the subsequent visit, remaining pills will be counted and counts recorded to reflect compliance for participants.

8.4.3 Administration Instructions

Dapagliflozin 10 mg is to be taken orally once daily every morning.

8.5 Study Drug Accountability

An accurate and current accounting of the dispensing and return of study drug for each subject will be maintained on an ongoing basis by a member of the study site staff. The number of study drug dispensed and returned by the subject will be recorded on the Investigational Drug Accountability Record. The study monitor will verify these documents throughout the course of the study.

8.6 Measures of Treatment Compliance

Participants will be asked to keep a diary noting the day and date they take their study drug and any adverse events. They will be asked to bring their participant diary to each study visit along with all used and unused study drug containers.

9 STUDY PROCEDURES AND GUIDELINES

A Schedule of Events representing the required testing procedures to be performed for the duration of the study is diagrammed in Appendix 1.

Prior to conducting any study-related activities, written informed consent and the Health Insurance Portability and Accountability Act (HIPAA) authorization must be signed and dated by the subject or subject's legal representative. If appropriate, assent must also be obtained prior to conducting any study-related activities.

9.1 Clinical Assessments

9.1.1 Concomitant Medications

All concomitant medication and concurrent therapies will be documented at Visit 1 and at Visits 28. Dose, route, unit frequency of administration, and indication for administration and dates of medication will be captured.

9.1.2 Demographics

Demographic information (date of birth, gender, race) will be recorded at Visit 1.

9.1.3 Medical History

Relevant medical history, including history of current disease, other pertinent respiratory history, and information regarding underlying diseases will be recorded at Visit 1.

9.1.4 Physical Examination

A complete physical examination will be performed by either the investigator or a sub-investigator who is a physician at the initial visit. Qualified staff (MD, NP, RN, and PA) may complete the abbreviated physical exam at all other visits. New abnormal physical exam findings must be documented and will be followed by a physician or other qualified staff at the next scheduled visit.

9.1.5 Vital Signs

Body temperature, blood pressure, pulse and respirations will be performed after resting for 5 minutes on each scheduled visit.

9.1.6 Adverse Events

Information regarding occurrence of adverse events will be captured throughout the study. Duration (start and stop dates), severity/grade, outcome, treatment and relation to study drug will be recorded on the case report form (CRF).

9.2 Clinical Laboratory Measurements

9.2.1 Urinalysis

Urine will be obtained and sent to each site's clinical laboratory for determination of color, specific gravity, pH, protein, glucose, ketones, and blood

Urine will be collected for ICP-OES assessment of sulfur.

10 EVALUATIONS BY VISIT

10.1 Visit 1 (Day 0)

1. Review the study with the subject (subject's legal representative) and obtain written informed consent and HIPAA authorization.
2. Assign the subject a unique screening number.
3. Record demographics data.
4. Record medical history, including a history of cystinuria, diagnosis date, and prior treatments.
5. Record concomitant medications.
6. Perform a complete physical examination.
7. Perform and record vital signs.
8. Perform and record results of blood pressure testing.
9. Schedule subject for Visit 2 in 28 days.

10. Collect urinalysis.
11. Collect freshly voided urine samples and obtain baseline analysis using mass spectrometry.
12. Prescribe study drug and provide a participant diary.

10.2 Visit 2 (Day 28)

1. Record any Adverse Experiences and/or Review subject diary for adverse experiences and dosing compliance.
2. Concomitant medications review.
3. Perform abbreviated physical examination.
4. Perform and record vital signs.
5. Collect urinalysis.
6. Collect freshly voided urine samples and obtain treatment analysis using mass spectrometry.

11 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

11.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical investigation of a patient administered a pharmaceutical product and that does not necessarily have a causal relationship with the treatment. An AE is therefore any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product. An unexpected AE is one of a type not identified in nature, severity, or frequency in the current Investigator's Brochure or of greater severity or frequency than expected based on the information in the Investigator's Brochure.

The Investigator will probe, via discussion with the subject, for the occurrence of AEs during each subject visit and record the information in the site's source documents. Adverse events will be recorded in the patient CRF. Adverse events will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study drug, or if unrelated, the cause.

AE Severity

The National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. The modified criteria can be found in the study manual. If the experience is not covered in the modified criteria, the guidelines shown in Table 1 below should be used to grade severity. It should be pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

Table 1. AE Severity Grading

Severity (Toxicity Grade)	Description
Mild (1)	Transient or mild discomfort; no limitation in activity; no medical intervention or therapy required. The subject may be aware of the sign or symptom but tolerates it reasonably well.
Moderate (2)	Mild to moderate limitation in activity, no or minimal medical intervention/therapy required.
Severe (3)	Marked limitation in activity, medical intervention/therapy required, hospitalizations possible.
Life-threatening (4)	The subject is at risk of death due to the adverse experience as it occurred. This does not refer to an experience that hypothetically might have caused death if it were more severe.

AE Relationship to Study Drug

The relationship of an AE to the study drug should be assessed using the following the guidelines in Table 2.

Table 2. AE Relationship to Study Drug

Relationship to Drug	Comment
Definitely	Previously known toxicity of agent; or an event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is not explained by any other reasonable hypothesis.
Probably	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is unlikely to be explained by the known characteristics of the subject's clinical state or by other interventions.
Possibly	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to that suspected drug; but that could readily have been produced by a number of other factors.
Unrelated	An event that can be determined with certainty to have no relationship to the study drug.

11.2 Serious Adverse Experiences (SAE)

An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:

- death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the subject or require intervention to prevent one of the outcomes listed.

Adverse Reactions: Dapagliflozin

The most common adverse reactions associated with dapagliflozin (5% or greater incidence) were female genital mycotic infections, nasopharyngitis, and urinary tract infections. Other reported adverse reactions include:

- back pain
- nausea
- influenza
- dyslipidemia
- constipation
- discomfort with urination
- pain in the extremity
- male genital mycotic infections
- hypotension
- ketoacidosis
- acute kidney injury
- Urosepsis and Pyelonephritis
- Hypoglycemia with Concomitant Use with Insulin and Insulin Secretagogues
- Necrotizing Fasciitis of the Perineum (Fournier's Gangrene)

11.2.1 Serious Adverse Experience Reporting

Study sites will document all SAEs that occur (whether or not related to study drug) per UCSF CHR Guidelines. The collection period for all SAEs will begin after informed consent is obtained and end after procedures for the final study visit have been completed.

In accordance with the standard operating procedures and policies of the local Institutional Review Board (IRB)/Independent Ethics Committee (IEC), the site investigator will report SAEs to the IRB/IEC.

11.3 Medical Monitoring

Marshall Stoller, MD should be contacted directly at these numbers to report medical concerns or questions regarding safety.

Phone: 415-353-8520
Pager: 415 443-9138

12 DISCONTINUATION AND REPLACEMENT OF SUBJECTS

12.1 Early Discontinuation of Study Drug

A subject may be discontinued from study treatment at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue. The following is a list of possible reasons for study treatment discontinuation:

- Subject withdrawal of consent

- Subject is not compliant with study procedures
- Adverse event that in the opinion of the investigator would be in the best interest of the subject to discontinue study treatment
- Protocol violation requiring discontinuation of study treatment
- Lost to follow-up
- Sponsor request for early termination of study
- Positive pregnancy test (females)

If a subject is withdrawn from treatment due to an adverse event, the subject will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized.

All subjects who discontinue study treatment should come in for an early discontinuation visit as soon as possible and then should be encouraged to complete all remaining scheduled visits and procedures.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents.

12.3 Withdrawal of Subjects from the Study

A subject may be withdrawn from the study at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents. As noted above, subjects who discontinue study treatment early (i.e., they withdraw prior to Visit 2) should have an early discontinuation visit.

12.4 Replacement of Subjects

Subjects who withdraw from the study treatment will be replaced.
Subjects who withdraw from the study will be replaced.

13 PROTOCOL VIOLATIONS

A protocol violation occurs when the subject or investigator fails to adhere to significant protocol requirements affecting the inclusion, exclusion, subject safety and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

- Failure to meet inclusion/exclusion criteria
- Use of a prohibited concomitant medication
- Failure to orally intake prescribed dosage on a daily basis

Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The Investigator will determine if a protocol violation will result in withdrawal of a subject.

When a protocol violation occurs, it will be discussed with the investigator and a Protocol Violation Form detailing the violation will be generated. This form will be signed by a Sponsor representative and the Investigator. A copy of the form will be filed in the site's regulatory binder and in the Sponsor's files.

14 STATISTICAL METHODS AND CONSIDERATIONS

Prior to the analysis of the final study data, a detailed Statistical Analysis Plan (SAP) will be written describing all analyses that will be performed. The SAP will contain any modifications to the analysis plan described below.

14.1 Data Sets Analyzed

Data sets from all participants enrolled in the study will be analyzed. The primary efficacy endpoint will be assessed by medical history and physical exam, in addition to mass spectrometry data obtained at each study visit.

14.2 Demographic and Baseline Characteristics

The following demographic variables at screening will be summarized: race, gender, age.

14.3 Analysis of Primary Endpoint

The primary endpoint will be analyzed through quantitative comparison between the baseline urine mass spectrometry analysis and the treatment urine mass spectrometry analysis.

14.4 Interim Analysis

When approximately 50% of patients have completed the study through Visit 2, an interim analysis for safety will be conducted. Serious adverse events will be monitored on an ongoing basis throughout the study.

14.5 Sample Size and Randomization

Sample size of ten was determined to be adequate for this proof-of-concept prospective cohort study. Future research will be considered with proper power and stronger statistical analysis if promising results from this study.

15 DATA COLLECTION, RETENTION AND MONITORING

15.1 Data Collection Instruments

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject treated with the study drug.

The Investigator is responsible for all information collected on subjects enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigator.

15.2 Data Management Procedures

The data will be entered into a validated database. The Data Management group will be responsible for data processing, in accordance with procedural documentation. Database lock will occur once quality assurance procedures have been completed.

All procedures for the handling and analysis of data will be conducted using good computing practices meeting FDA guidelines for the handling and analysis of data for clinical trials.

15.3 Data Quality Control and Reporting

After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis. The study database will be updated in accordance with the resolved queries. All changes to the study database will be documented.

15.4 Archival of Data

The database is safeguarded against unauthorized access by established security procedures; appropriate backup copies of the database and related software files will be maintained. Databases are backed up by the database administrator in conjunction with any updates or changes to the database.

At critical junctures of the protocol (e.g., production of interim reports and final reports), data for analysis is locked and cleaned per established procedures.

15.5 Availability and Retention of Investigational Records

The Investigator must make study data accessible to the monitor, other authorized representatives of the Sponsor (or designee), IRB/IEC, and Regulatory Agency (e.g., FDA) inspectors upon request. A file for each subject must be maintained that includes the signed Informed Consent, HIPAA Authorization and Assent Form and copies of all source documentation related to that subject. The Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.

All study documents (patient files, signed informed consent forms, etc.) must be kept secured for a period of two years following marketing of the investigational product or for two years after centers have been notified that the IND has been discontinued. There may

be other circumstances for which the Sponsor is required to maintain study records and, therefore, the Sponsor should be contacted prior to removing study records for any reason.

15.6 Monitoring

Monitoring visits will be conducted by representatives of the Sponsor according to the U.S. CFR Title 21 Parts 50, 56, and 312 and ICH Guidelines for GCP (E6). By signing this protocol, the Investigator grants permission to the Sponsor (or designee), and appropriate regulatory authorities to conduct on-site monitoring and/or auditing of all appropriate study documentation.

15.7 Subject Confidentiality

In order to maintain subject confidentiality, only a site number, subject number and subject initials will identify all study subjects on CRFs and other documentation submitted to the Sponsor. Additional subject confidentiality issues (if applicable) are covered in the Clinical Study Agreement.

The study investigator will work with the study team to ensure that participants' privacy and information is protected. They both have access to experienced clinical trials offices and expertise in conducting clinical research similar to the one proposed in this application, ensuring that safe and ethical practices will be followed.

Physical risk will be minimized by the study design since routine clinical care will not be altered with inclusion in this study. All health-system source/legacy data files will be stored at the UCSF local health system and will be accessed only by the programmers/analysts working on this study. Since confidential information will be collected from the medical charts, we will not directly link personal identifiers with the data collected nor will we use personal identifiers in any reports, materials, or presentations that emanate from this work.

All electronic files containing personal identifiers will be stored only on the UCSF site file servers located behind a firewall. Information transferred to the server for backup purposes will be done via secure ftp. Files may be transferred to other computers temporarily via the Internet to facilitate central upload. In these circumstances, the files will be protected by encryption. The UCSF site file servers are only accessible by network support specialists working in secure locations. The on-site file server will be electronically accessible only to study investigators through user password protection.

Access to study data is protected and participant to the same security protections as other confidential health system data. Computer passwords are changed on a regular basis. Individual identifiers such as names and medical record numbers will be removed from study data files in the data processing steps and will not be accessible to collaborators accessing the database for further study as described in this study application. The participating collaborators will maintain a data link from the study data but not to the personal identifiers (to permit reanalysis of the data files). In addition, all investigators and support staff involved with collection or entry of clinical data are HIPAA certified and have completed and are current with regard to IRB training. Investigators and project

staff will sign confidentiality pledges annually HIPAA training annually. Datasets shared with external collaborators will be stripped of any identifying information according to HIPAA policy.

16 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. All study records will be kept in a locked file cabinet and code sheets linking a patient's name to a patient identification number will be stored separately in another locked file cabinet. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the FDA. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

16.1 Protocol Amendments

Protocol amendments cannot be implemented without prior written IRB/IEC approval except as necessary to eliminate immediate safety hazards to patients. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRBs are notified within five working days.

16.2 Institutional Review Boards and Independent Ethics Committees

The protocol and consent form will be reviewed and approved by the IRB/IEC of each participating center prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB/IEC in accordance with the standard operating procedures and policies of the IRB/IEC, and the Investigator will keep the IRB/IEC informed as to the progress of the study. The Investigator will obtain assurance of IRB/IEC compliance with regulations.

Any documents that the IRB/IEC may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB/IEC. The IRB/IECs written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB/IEC approval except when necessary to eliminate immediate hazards to the patients or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB/IEC and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB/IEC must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

16.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations.

The Investigator will prepare the informed consent form, assent and HIPAA authorization and provide the documents to the Sponsor or designee for approval prior to submission to the IRB/IEC. The consent form generated by the Investigator must be acceptable to the Sponsor and be approved by the IRB/IEC. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonisation and will also comply with local regulations. The Investigator will send an IRB/IEC-approved copy of the Informed Consent Form to the Sponsor (or designee) for the study file.

A properly executed, written, informed consent will be obtained from each subject prior to entering the subject into the trial. Information should be given in both oral and written form and subjects (or their legal representatives) must be given ample opportunity to inquire about details of the study. If appropriate and required by the local IRB/IEC, assent from the subject will also be obtained. If a subject is unable to sign the informed consent form (ICF) and the HIPAA authorization, a legal representative may sign for the subject. A copy of the signed consent form (and assent) will be given to the subject or legal representative of the subject and the original will be maintained with the subject's records.

16.4 Publications

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the study Sponsor and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

16.5 Investigator Responsibilities

By signing the Agreement of Investigator form, the Investigator agrees to:

1. Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor (or designee), except when to protect the safety, rights or welfare of subjects.
2. Personally conduct or supervise the study (or investigation).
3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.

4. Report to the Sponsor or designee any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.
5. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
6. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the Sponsor (or designee).
7. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
8. Promptly report to the IRB and the Sponsor (or designee) all changes in the research activity and all unanticipated problems involving risks to subjects or others (to include amendments and IND safety reports).
9. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects.
10. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

APPENDIX 1.

	VISIT 1 (Day 0)	VISIT 2 (Day 28)
Informed Consent	**X**	
Medical History	**X**	
Complete Physical Exam	**X**	
Abbreviated Physical Exam		**X**
Height	**X**	**X**
Weight	**X**	**X**
Vital Signs	**X**	**X**
Urinalysis	**X**	**X**
Dispensing or Administration of Study Drug	**X**	
Counting of Returned Study Drug		**X**
Initiate Subject Diary	**X**	
Subject Diary Review		**X**
Concomitant Medication Review	**X**	**X**
Adverse Experiences		**X**
Mass Spectrometry Analysis	**X**	**X**

References

1. Chillaron J, Font-Llitjos M, Fort J et al: Pathophysiology and treatment of cystinuria. Nat Rev Nephrol 2010; 6: 424.
2. Cabello-Tomas ML, Garcia-Gomez AM, Guillen-Dominguez ML: Pilot screening programme for cystinuria in the Valencian community. Eur J Epidemiol 1999; 15: 681.
3. Milliner DS, Murphy ME: Urolithiasis in pediatric patients. Mayo Clin Proc 1993; 68:241.
4. Griggs RC, Batshaw M, Dunkle M et al: Clinical research for rare disease: opportunities, challenges, and solutions. Mol Genet Metab 2009; 96: 20.
5. Pareek G, Steele TH, Nakada SY: Urological intervention in patients with cystinuria is decreased with medical compliance. J Urol 2005; 174: 2250.
6. Parks JH, Asplin JR, Coe FL: Patient adherence to long-term medical treatment of kidney stones. J Urol 2001; 166: 2057.
7. Asplin DM, Asplin JR: The Interaction of thiol drugs and urine pH in the treatment of cystinuria. J Urol 2013;189: 2147.
8. Pak CY, Fuller CJ: Assessment of cystine solubility in urine and of heterogeneousnucleation. J Urol 1983;129: 1066.
9. Pietrow P, Auge BK, Weizer AZ et al: Durability of the medical management of cystinuria. J Urol 2003; 169:68.
10. Assimos DG, Leslie SW, Ng C et al: The impact of cystinuria on renal function. J Urol 2002; 168: 27.